STUDY ON LIFE CYCLE OF LITCHI FRUIT BORER(S) AND THEIR MANAGEMENT

Thesis

Submitted to

NAGALAND UNIVERSITY

in partial fulfilment of requirements for the Degree

of

Doctor of Philosophy

in

Entomology

by

PASAM MAHESWARA REDDY

Admn. No. Ph-312/20 Regn. No. PhD/ENT/00430 (2020-2023)

Department of Entomology

School of Agricultural Sciences,

Nagaland University, Medziphema Campus – 797106

Nagaland

2024

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Nagaland

2024

Dedicated to my Beloved

Parents & Teachers

DECLARATION

I, PASAM MAHESWARA REDDY, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

This is being submitted to Nagaland University for the degree of Doctor of Philosophy in Entomology.

Date:
Place: SAS: Medziphema (**PASAM MAHESWARA REDDY)**

………………………………
Supervisor

NAGALAND UNIVERSITY
Medziphema campus
School of Agricultural Sciences
Medziphema – 797 106, Nagaland

Dr. Imti Naro L.
Professor
Department of Entomology

CERTIFICATE – I

This is to certify that the thesis entitled **"Study on life cycle of litchi fruit borer(s) and their management"** submitted to Nagaland University in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Entomology is the record of research work carried out by Mr. PASAM MAHESWARA REDDY Registration No. PhD/ENT/00430 under my personal supervision and guidance.

The results of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

Date :
Place : SAS: Medziphema

……………………………...…
Dr. Imtinaro L.
Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences
Medziphema – 797 106, Nagaland

CERTIFICATE – II

VIVA VOCE ON THESIS OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY

This is to certify that the thesis entitled **"Study on life cycle of litchi fruit borer(s) and their management"** submitted by P. Maheswara Reddy, Admission No. Ph-312/20, Registration No. Ph.D./ENT/00430, to the NAGALAND UNIVERSITY in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Entomology has been examined by the Advisory Board and External examiner on …………………….

The performance of the student has been found **Satisfactory/Unsatisfactory.**

Member	Signature
1. Dr. Imtinaro L. (Supervisor)	…………………….
2. ………………………. (External Examiner)	…………………….
3. Dean, SAS, NU (Pro-Vice-chancellor Nominee)	…………………….
4. Dr. Pankaj Neog	……………………
5. Dr. Hijam Shila Devi	..….……………….
6. Dr. Susanta Banik	….…………………
7. Dr. C.S. Maiti	...………………..…….

Head
Department of Entomology

Dean
School of Agricultural Sciences

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Date:

Place: SAS: Medziphema (***PASAM MAHESWARA REDDY)***

LIST OF ABBREVIATIONS/ SYMBOLS

a.i.	:	Active ingredient
@	:	At the rate
%	:	Per cent
BLAST	:	Basic Local Alignment Search Tool
CD	:	Critical Difference
cm	:	Centimeter
°C	:	Degree Celsius
DAS	:	Days after spraying
DAF	:	Days after fruit set
Dept.	:	Department
DNA	:	Deoxyribose Nucleic Acid
E	:	East
EC	:	Emulsifiable Concentrate
et al.	:	and other
g	:	Gram
hr.	:	Hour
ha	:	Hectare
i.e.,	:	That is
K_2P	:	Kimura-2 parameter model
kg	:	Kilogram
lit.	:	Liter
m	:	Meter
mg	:	Milligram
Max	:	Maximum
MEGA	:	Molecular Evolutionary Genetics Analysis
Min	:	Minimum
min.	:	Minute

µl	:	Micro liter
mm	:	Millimeter
m.s.l.	:	Mean Sea Level
MT	:	Metric ton
MUSCLE	:	Multiple Sequence Comparison by Log-Expectation
NCBI	:	National Centre for Biotechnology Information
N	:	North
ng	:	Nano gram
nm	:	Nanometer
No.	:	Number
NSKE	:	Neem Seed Kernel Extract
NRCL	:	National Research Centre for Litchi
NU	:	Nagaland University
PCR	:	Polymerase Chain Reaction
PPM	:	Parts Per Million
PTC	:	Pre-treatment count
RCBD	:	Randomized Complete Block Design
rpm	:	rotations per minute
RH	:	Relative Humidity
ROC	:	Reduction Over Control
SAS	:	School of Agricultural Sciences
SC	:	Soluble Concentrate
Sec.	:	Second
S.E.m	:	Standard Error Mean
Sp.	:	Species
t	:	ton
Ver.	:	Version

viz.,	:	namely
WP	:	Wettable Powder

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ABSTRACT

The present investigation entitled "study on life cycle of litchi fruit borer(s) and their management was conducted during April, 2021 - June, 2023 at Experimental Research Block, Dept. of Horticulture, School of Agricultural Sciences, Medziphema campus, Nagaland University, and farmers farm, Medziphema, Nagaland. The study reveals that, a total of five identified species *i.e*., *Conogethes punctiferalis* (Guenée), *Conopomorpha sinensis* (Bradley) and *Deudorix epijarbus* (Moore), *Cryptophlebia ombrodelta* (Lower) and *Thaumatotibia zophophanes* (Turner) were recorded out of 565 specimens collected and reared on litchi fruits. Of these species, *T. zophophanes* was the first record from India feeding on litchi fruits. *C. sinensis* was found to be predominant with 46.37 per cent followed by *C. ombrodelta* with 32.03 per cent. Other species *i.e*., *D. epijarbus*, *T. zophophanes* and *C. punctiferalis* recorded 10.61, 7.05, and 3.89 per cent, respectively. An illustrated key was prepared for families and species of fruit borers of litchi based on the morphological and genital characters of adults.

The phylogenetic analysis reveals that, there was a strong AT bias (70. 54%). The overall transition/transversion bias is R = 2.01. The intraspecific genetic divergence ranged from 0.00% to 0.14% with overall mean of 0.13%. A minimum intraspecific nucleotide divergence of 0.00% was found in all the species except *T. zophophanes* (0.01%), while a maximum intraspecific nucleotide divergence of 0.04% was found with *C. punctiferalis* and *C. ombrodelta*. Further, the phylogenetic tree analysis showed that, all the species were placed in their respective clades *i.e*., Crambidae, Gracillariidae, Lycaenidae and Tortricidae.

The biology of *C. sinensis* was studied under laboratory condition. Eggs are laid singly, yellowish orange, flattened and scale like. During the larval period, the larva moulted four times and thus having five larval instars. The first larval instar is transparent, milky white in colour. The second and third instar larva is creamy white and thick creamy white in colour, respectively. The fourth and fifth instar larva is yellowish cream and light green in colour. The pupa is slender, yellowish in colour with prominent eyes, well developed maxillary palpi, antennae, proboscis and legs. Adult smaller in size, greyish brown moth with a yellowish-brown wing apex. The duration of developmental stages such as egg, larval, pre-pupal, pupal, male and female adult period lasts for 3-5, 8-14, 1-3, 4-7, 4-7, and 7-11 days, respectively. The total life cycle from egg to adult stage last for 20-36 days in male, whereas 23-40 days in female. Further, the morphometric observations were also made for various life stages.

In case of seasonal incidence, it was observed that litchi fruit borer, *C. sinensis* infestation gradually increased and reaches its peak during the last week of May to first week of June and then decreases gradually. Also, it was found that temperature has a significant impact on the pest larval activity, whereas, relative humidity and rainfall has little influence on the activity of the pest species. A total of four natural enemies were recorded on litchi fruit borer, *C. sinensis*. Among these, one was a predator, *Cheilomenes sexmaculata* (Fabricius). While, the other three were hymenopteran parasitoids. To study the efficacy, a total of eight treatments *viz*., neem seed kernel extract 4% @ 400ml/lit, *B. thuringiensis* var. *kurstaki* @ 50gm/lit, spinosad 45 SC @ 4.5 ml/lit, diflubenzuron 25 WP @ 3 gm/lit, novaluron 10 EC @ 1.5 ml/lit, neem oil 0.2% @ 20 ml/lit, kamdhenu keet niyantak 5% @ 500 ml/lit and untreated check were evaluated against the litchi fruit borer, *C. sinensis*. It was found that spinosad 45 SC @ 4.5ml/10lit. was found much effective in reducing the fruit borer infestation followed by *B. thuringiensis* var. *kurstaki* @ 50gm/10 lit. Whereas, neem oil 0.2% @ 20ml/10 lit. was found least effective compared to other treatments.

Keywords: Litchi fruit borer(s), *Conopomorpha sinensis*, phylogenetic analysis, life cycle, seasonal incidence, natural enemies and management.

CHAPTER I

INTRODUCTION

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is one of the most important subtropical fruit trees of the family Sapindaceae. It is known as queen of fruits due to its attractive deep pink/red colour and flavored juicy aril. Litchi appears to be native of Guangdong and Fujian provinces, near South-Eastern China (Morton, 1987) and Northern Vietnam, from where it was introduced into India during the 18th century in the North Eastern Region (Tripura) and over the period of time to eastern states and percolated in the northern states (Ghosh, 2000; Rai *et al*., 2000; Sahni *et al*., 2020).

Litchi has spread to most of the tropical and subtropical world. The spread of litchi to other parts of the world was rather slow probably due to its soil, climatic requirements and short life span of its seed (Anonymous, 1978, 1981; Cull and Paxton, 1983; Chapman, 1984). The major litchi growing countries are China, Australia, Thailand, Taiwan, India, Vietnam, parts of Africa and at higher elevations in Mexico, Central and South America. Among these, China, India, Taiwan, Thailand and Vietnam are the top five litchi producing countries in the world. Global production of litchi is more than two million tons, of which, Asian countries contribute more than 95% of the area and production share (Nath *et al*., 2018).

India and China account for more than 90% of the world's litchi production. India enjoys a prominent position in the litchi map of the world both in terms of production and productivity. The increasing demand for litchi in both domestic and international markets has been the major driver behind the growth of litchi production in India. The spread and growth of litchi has attained a Pan-India phenomenon, recording about 3.8% growth annually over the last decade (Nath *et al*., 2018). In India, 686.4 thousand metric tons of litchi fruits are produced annually from 92.3 thousand hectares area with productivity of 7.4

MT/ha. National average productivity of litchi is 6.1 t/ha (Anonymous, 2021).

Litchi is an extremely environmentally sensitive tree that requires specific climate conditions. It requires warm temperatures for its growth and fruit production. The optimal temperature range is 20-30°C during the day and 15-20 °C at night. It requires high humidity for its growth and fruit production. The optimal relative humidity is 70-90%. The ideal rainfall range is 1000-2000 mm per year. It grows well in deep, well drained, fertile and slightly acidic soils having P^H between 5.0 to 6.5. Litchi can be grown up to an altitude of 800 m. (m.s.l.) (Singh and Jawanda, 1962; Singh *et al*., 2011).

The production of litchi is mainly confined to Bihar (40%), West Bengal (16%), Jharkhand (10%), Assam (8.2%), Chhattisgarh (6.4%), Uttarakhand (5.2%) and to a smaller extent in Punjab, Odisha and Tripura (Sahni *et al*., 2020). The North Eastern Region comprises of eight states *viz*., Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. In Nagaland, litchi production accounts over 3.94 thousand MT (Anonymous, 2021). Nagaland has a good potentiality of producing litchi especially in the foothills of 4-12°C temperature for a month or more. The foothills and midhills of Dimapur, Mokokchung, Wokha, Peren, Kohima and Zunhebeto districts are also congenial for litchi cultivation (Marboh *et al*., 2019).

Litchi is consumed as fresh fruit, pulp and various processed products like squash, wine *etc*. The translucent, flavored aril or edible flesh of the litchi is popular as a table fruit in India. Whereas, in China and Japan it is preferred in dried or canned state. Litchi is an excellent source of vitamin C and B-complex vitamins *viz*., thiamin, niacin and folates and minerals like potassium and copper (Singh *et al*., 2012).

The major reason for slow spread of litchi cultivation are mainly biotic and abiotic factors. Among these, biotic factors are the major constraints for the successful production and productivity of litchi. Of several biotic factors, pests and diseases are the major constraints. Unlike agricultural crops, litchi is grown as monoculture and the pest occurring are entirely different and complex in nature. Earlier, only two pest species *viz*., erineum mite, *Aceria litchi* (Keifer) and bark eating caterpillar, *Inderbela* spp. were reported causing serious damage to litchi trees (Butani, 1977). According to Huang *et al*. (2005a, 2005b), in China there are 193 species of litchi pests under 11 orders and 57 families. The majority belonging to the Lepidoptera and Coleoptera, followed by Hemiptera.

Yet, about a dozen species are major pests which requires regular control. They include fruit borers, defoliators, sucking bugs, bark eating caterpillars, stem borers and erinose mites. Among these, the most important fruit borers include *Conopomorpha sinensis* Bradley, *Conopomorpha litchiella* Bradley, *Conopomorpha cramerella* Meyer and *Conogethes punctiferalis* (Guenee), *Cryptophlebia ombrodelta* (Lower), *Duedorix epijarbus* (Moore) and *Gatesclarkeana* sp. Among various sucking pests, litchi stink bug, *Tessarotoma javanica* (Thunberg) and *Tessaratoma papillosa* (Drury) are economically important. The losses by the bugs ranges from 70-90%. In sever attack, it leads to heavy fruit dropping and ultimately total damage to the litchi crop (Srivastava *et al*., 2019). Among the defoliators, leaf webber, *Dudua aprobola* (Meyrick) and leaf roller, *Statherotis leucaspis* Meyrick is reported as a serious pest in Bihar (Srivastava *et al*., 2015, 2021). Randhawa *et al*. (2015) reported 81% damage to the new growth/flush in Gurdaspur district. Besides, Litchi looper, *Perixeria illepidaria* Guenée and bag worm, *Eumeta crameri* Westwood were reported as emerging pets of litchi (Hameed *et al*., 2001; Kumar *et al*., 2013).

However, the most important constraint in successful production of litchi is managing fruit borers. They are the major pests of litchi fruits, feeds internally in humid and orchard conditions at the time of fruit ripening reducing the marketable yields (Srivastava *et al*., 2018). Fruit borers (*C. sinensis* and *C. litchiella*) cause severe losses to fruit as well as young shoots causing up to 24-48% and 7-70%, respectively (Li *et al*., 2014; Srivastava *et al*., 2019). The maximum fruit damage occurs during April-July puncturing the peduncle of fruits causing severe loss through early fruit drop. Singh and Kaur (2015) reported castor capsule borer, *C. punctiferalis* feeding on litchi fruits for the first time in Hoshiarpur and Gurdaspur districts of Punjab during May and June. Approximately 10% damage was reported by *C. punctiferalis* even in the well managed orchards. Kumar *et al*. (2011) reported the occurrence of *C. cramerella* in severe form in Bihar during 2009-10.

Due to the cryptic feeding behavior and overlapping generations right from initial to maturity, fruit borers are much difficult to manage (Upadhyay *et al*., 2020). Being the internal feeders, it is very problematic managing the fruit borers. The management of fruit borer complex hence, warrants the integration of alternative methods such as use of pheromone traps, biocontrol agents, organic products (*viz*. neem oil, cow urine, cow dung, panchagavya *etc*.), removal and destruction of dropped fruits and wild host such as *Eugenia jambolana* and *Cassia tora* from orchards, prophylactic spray of neem-based insecticides and need based application of chemical insecticides. Minimal use of pesticides in litchi are more relevant because of greater hazards of pesticide residues in the fruits.

Besides, the major constraint in formulating any management strategies against the borers is the difficulty in identification of the correct species. The classification of closely related lepidopteran species based on morphological characters alone presents several difficulties and the risk of inaccuracy because of the function of certain attributes differs in different environments, leading to the prevalence of several biotypes (Linares *et al*., 2009). Recently molecular marker techniques have been facilitated for the assessment of genetic diversity, improving the accuracy of genotyping, classification, inventorying and molecular phylogenetic studies (Silva *et al*., 2010). In recent days, mitochondrial and nuclear genes, such as elongation factor-1 α gene and wingless gene, have been used widely for the molecular study of butterflies and moths (Kandul *et al*., 2004; Wahlberg *et al*., 2003).

Several studies have used mtDNA sequences to study the phylogenetic relationships of certain groups of lepidopterans (Murray and Prowell, 2005). Of the several mitochondrial genes, the cytochrome c oxidase subunit I (COI) gene has been widely used to identify various organisms (Herbert *et al*., 2003). DNA barcoding sequences like cytochrome c oxidase subunit I (COI) gene, provide a highly reliable method for species identification, helps in revealing the cryptic species, assists in creating phylogenetic trees that illustrates the evolutionary history of insect groups. It also helps in studying the population genetics and evolutionary ecology. Identifying the source of insect pests and understanding their genetic diversity can aid in the development of more effective pests control strategies (Win *et al*., 2015). It allows the researchers to track the origins and movement of pest's populations. Considering the above facts, a research proposal entitled "Study on life cycle of litchi fruit borer(s) and their management" proposed with the following objectives:

1. Identification of litchi fruit borer(s)
2. To study the life cycle of litchi fruit borer(s)
3. To study the seasonal incidence of litchi fruit borer(s) and their natural enemies
4. Efficacy study of various insecticides and biopesticides against litchi fruit borer(s)

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The available literature on litchi fruit borers with respect to identification through morphological and genital characters of the adults, taxonomic keys, DNA barcoding, life cycle studies, seasonal incidence, natural enemies and their management were compiled and presented in this chapter under the following headings.

2.1. Identification of litchi fruit borer(s)

2.1.1. Morphological and genital characterization of litchi fruit borer(s)

Bradley (1953) studied the morphological and genital characters of the genus *Cryptophlebia* Walsingham. He described fifteen species *viz*., *Cryptophlebia carpophagoides* Clarke, *Cryptophlebia distorta* (Hampson), *Cryptophlebia illepida* (Butler), *Cryptophlebia iridosoma* (Meyrick), *Cryptophlebia lasiandra* (Meyrick), *C. ombrodelta*, *Cryptophlebia pallifimbriana* Bradley, *Cryptophlebia peltastica* (Meyrick), *Cryptophlebia phaeacma* (Meyrick), *Cryptophlebia repletana* (Walker), *Cryptophlebia rhynchias* (Meyrick), *Cryptophlebia strepsibathra* (Meyrick), *Cryptophlebia toxogramma* (Meyrick), *Cryptophlebia williamsi* Bradley and a new species, *Cryptophlebia vitiensis* Bradley sp. nov. based on the morphological and genital characters.

Likewise, Zimmerman (1978) reviewed and described the genus *Cryptophlebia* Walsingham from Hawaii. Based on the morphological and genital characters, he described two species *viz*., *C. ombrodelta* and *C. illepida*. Stempffer (1967) revised 123 genera of African Lycaenidae. Further, he provided the descriptions, adult habitus, genital and wing venation photographs of each genus. Bradley (1986) clarified that, generally cited cocoa pod borer, *Acrocercops cramerella* as the *C. cramerella* a major pest of cocoa based on male and female genital characters from South-East Asia. He described three new species *viz*., *Conopomorpha oceanica* sp. nov., *C. sinensis* sp. nov., and *C. litchiella* sp. nov. based on the male and female genital characters. Further, he provided the illustrations of adult habitus, male and female genitalia. Similarly, Brown *et al*. (2002) described a new species of *Crocidosema* Zeller *i.e*., *Crocidosema litchivora* sp. nov., based on male and female genital characters, feeding on litchi from Florida.

Rentel (2013) described morphological and genital characters of economically important tortricid moths such as *Cydia pomonella* (Linnaeus), *Thaumatotibia leucotreta* (Meyrick), *Thaumatotibia batrachopa* (Meyrick), *Grapholita molesta* (Busck), *C. peltastica*, *Epichoristodes acerbella* (Walker) and *Lozotaenia capensana* (Walker) attacking sub-tropical fruit crops in South Africa. Horak and Komai (2016) described morphological and genital characters of the genus *Cryptophlebia* Walsingham and *Thaumatotibia* Zacher from Australia. Four species of *Cryptophlebia* namely *C. ombrodelta*, *C. iridosoma*, *C. rhynchias* and *C. pallifimbriana* were redescribed and three new species *viz*., *Cryptophlebia wraggae* sp. nov., *Cryptophlebia caulicola* sp. nov., and *Cryptophlebia stigmata* sp. nov. were described. The genus *Thaumatotibia* was represented with two species *viz*., *Thaumatotibia aclyta* (Turner) and *Thaumatotibia zophophanes* (Turner) were redescribed and the new species *Thaumatotibia maculata*, sp. nov. was described.

Sohn *et al*. (2016) described morphological and genital characters of *C. ombrodelta* from Korea for the first time. Further, they provided the illustrations of adult habitus, male and female genitalia. Similarly, Shashank *et al*. (2018) described a new species, *Conogethes sahyadriensis* Shashank, Kammar, Mally and Chakravarthy feeding on cardamom from India based on morphological and genital characters, and genetic data. Chaovalit *et al*. (2019) described seven species of the genus *Conogethes* (Guenée) namely *C. punctiferalis*, *Conogethes* *parvipunctalis* Inoue and Yamanaka, *Conogethes pinicolalis* Inoue and Yamanaka, *Conogethes pluto* (Butler), *Conogethes evaxalis* (Walker), *Conogethes haemactalis* (Snellen), and a new species *Conogethes tenuialalis* Chaovalit and Yoshiyasu, sp. nov. occurring in Thailand based on specimens preserved in Thailand and Japan.

Nagaharish *et al*. (2017) studied morphological and genital characters of *C. punctiferalis* feeding on guava, mango and pomegranate from Karnataka. Reddy *et al*. (2020) studied taxonomy of six species of Spilomelinae fauna occurring on economically important fruit crops of zone 1, 2 and 3 of Karnataka. Wherein, they studied the morphological and genital characters of *C. punctiferalis* occurring on mango, guava and pomegranate. Reddy and Shashank (2022) described three new species of tribe Grapholitini (Lepidoptera: Tortricidae: Olethreutinae) from India *viz*., *Acanthoclita bengaluruensis* Reddy and Shashank, Sp. nov., *Grapholita constricta* Reddy and Shashank sp. nov. and *Thaumatotibia ramamurthyi* Shashank and Reddy, sp. nov. They also provided descriptions and photographic illustrations of adult habitus and genitalia.

Pasam *et al*. (2023) studied morphological and genital characters of 27 species of Spilomelinae occurring on agriculturally important crops from Karnataka. Wherein, they studied three species of the genus *Conogethes* Meyrick *viz*., *C. punctiferalis* and *C. sahyadriensis* feeding on castor and cardamom, respectively and an unidentified species feeding on sorghum earheads.

2.1.2. To prepare an illustrated key to families, genera and species of litchi fruit borer(s)

Literature pertaining to an illustrated key to the families, genera and species of litchi fruit borers is very narrow. Hence, literature related to other lepidopterans were presented here.

Hampson (1896) provided key to families, sub families, genera and species of Pyralidae in his fauna of British India, Ceylon and Burma based on morphological characters. Similarly, Diakonoff (1938) prepared a key to 53 genera of Tortricidae occurring in Indo-Malayan and Papua. Zimmerman (1978) prepared a key for the economically important species of Hawaiian tortricids. Later, Arora (2000) prepared key for identification of economically important species of Indian Pyralidae.

Timm *et al*. (2007) prepared a key for economically important species of Tortricidae *viz*., *C. peltastica*, *T. leucotreta* and *T. batrachopa* occurring on tropical and subtropical fruits in South Africa based on larval and pupal characters. Shankaramurthy *et al*. (2015) prepared a key to sub families of agriculturally important Pyraloidea fauna (Lepidoptera) of India based on morphological and genital characters.

Horak and Komai (2016) prepared an illustrated key to the seven species of the genus *Cryptophlebia* Walsingham *viz*., *C. caulicola* sp. nov., *C. iridosoma*, *C. ombrodelta*, *C. pallifimbriana*, *C. rhynchias*, *C. stigmata* sp. nov., and *C. wraggae* sp. nov., and for two species of the genus *Thaumatotibia* Zacher such as *T. aclyta* and *T. zophophanes* from Australia.

Chaovalit *et al*. (2019) prepared a key to seven species of the genus *Conogethes* (Guenée) namely *C. punctiferalis*, *C. parvipunctalis*, *C. pinicolalis*, *C. pluto*, *C. evaxalis*, *C. haemactalis*, and *C. tenuialalis* occurring in Thailand based on external morphology, male and female genitalia. Pasam *et al*. (2023) provided a key to 27 species of Spilomelinae (Crambidae) occurring on agriculturally important crops in Karnataka.

2.1.3. DNA barcoding of litchi fruit borer(s)

Timm *et al*. (2006) analyzed the population genetic structure of two closely related tortricid species of economic importance of macadamia and litchi from South Africa. The results revealed that, gene diversity was high within the both species *viz*., *T. batrachopa* (H=0.2219) with significant genetic differentiation among populations (G_{st}=0.358) and *C. peltastica* (H=0.1906) with significant genetic differentiation among populations (G_{st}=0.4124). Further, they concluded that the population genetic structure of both species is influenced by their limited ability to disperse.

Timm *et al*. (2007) evaluated *mtCOI* barcoding for accurate identification of three economically important tortricid species of litchi *viz*., *C. peltastica*, *T. leucotreta*, and *T. batrachopa* from South Africa. They revealed that *T. leucotreta* and *T. batrachopa* are closely related to each other rather *C. peltastica*. Further, the average K_2P distances between the three species were calculated as 0.12 (*T. leucotreta* and *T. batrachopa*), 0.14 (*T. leucotreta* and *C. peltastica*) and 0.16 (*C. peltastica* and *T. batrachopa*).

Armstrong (2010) compared DNA barcoding of different populations of *Conogethes* (Guenée), and revealed that Australian and Asian specimens formed separate clades divergent by 6%. The barcode data successfully distinguished *C. punctiferalis* and *C. pluto*, but unexpectedly revealed divergence between the Asian and Australian populations. Morphologically, these were determined to be the same species and distinct from other closely related species found on the east coast of Australia such as *C. haemactalis*, *Conogethes semifascialis* Walker, and *Conogethes tharsalea* Walker.

Mally and Nuss (2011) screened and reported 1,600 sequences of Pyraloidea representing 430 species of 230 genera and 708 unidentified specimens. The genus *Conogethes* has 138 barcode sequences and about 19 species from six countries *viz*., Australia, Papua New Guinea, Cambodia, China, Indonesia and Nepal, but these sequences are not available in the public domain ((Ratnasingham and Hebert, 2007). Similarly, Jing *et al*. (2014) investigated relationship between fruit-feeding type (*C. punctiferalis*) and pinaceae-feeding type (*C. pinicolalis*) based on three genes of mitochondrial cytochrome oxidase subunits I, II and cytochrome b. The results of combined analysis of mitochondrial DNA sequences from three genes and morphological data represented powerful evidence that *C. pinicolalis* and *C. punctiferalis* are significantly different.

Shashank *et al.* (2014) studied the cryptic species of the genus *Conogethes* occurring on two different hosts namely castor and cardamom through morphological studies and DNA barcoding using cytochrome oxidase I gene. They revealed that, *Conogethes punctiferalis* occurring on castor showed high haplotype diversity (0.817±0.073) and nucleotide diversity (0.0285±0.002). Further, the topologies of neighbor-joining trees indicate that *Conogethes* sp. breeding on castor belongs to *C. punctiferalis* while, those on cardamom are of a different clade. Also, genetic analysis revealed significant genetic differentiations among the two sampled populations reflecting limited gene flow.

Jayanthi Mala *et al*. (2017) studied the molecular identification of litchi fruit borers using the nucleospin tissue kit method (MACHEREY-NAGEL). Molecular identification was performed using the mitochondrial cytochrome oxidase I (COI) and nuclear elongation factor 1 alpha (Ef1α) genes. Molecular analyses confirmed the species as *Conopomorpha sinensis*, as the samples showed 99% (CO1) and 100% (Ef1α) similarly to *C. sinensis*, rather *C. cramerella*.

Srivastava *et al*. (2018) followed molecular approaches to identify various borer species occurring on litchi. They utilized the partial cytochrome oxidase I (COI) sequences to understand the phylogenetic relationship among borer complex. The phylogenetic analysis showed that the borer specimens used in this study clustered in to distinct species-groups designated as *C. sinensis*, *C. litchiella*, *C. ombrodelta* and *Gatesclarkeana* spp. Higher intraspecific genetic variation was observed in *Conopomorpha* species complex as compared to *Cryptophlebia* species complex.

Gopurenko *et al*. (2021) reported the use of DNA barcoding for investigating the population and species genetic diversity of cocoa pod borer infecting cocoa plantations in Papua New Guinea. They revealed, DNA barcodes from 94.4% (169 moths) of 179 moths matched to reported *C. cramerella* sequence accessions. Remaining 10 moths were genetically unrelated to *C. cramerella,* differing by more than 10%. Of these, four moths were closely related (92.3%) to *C. litchiella* than to *C. cramerella* (88.9%). Three other specimens were matched (99.5-100% similarity) to tortricid moth, *T. zophophanes*.

There are now 97,705 specimens with barcodes from nearly 6,538 species of Crambidae in the Barcode of Life Data System. Likewise, there are about 36,620 specimens with barcodes from nearly 1,906 species of Gracillariidae in the Barcode of Life Data System. Also, there are about 37,842 specimens with barcodes from nearly 3,828 species of Lycaenidae and 76,274 specimens with barcodes from 5,394 species of Tortricidae in the Barcode of Life Data System (Ratnasingham and Hebert, 2007).

2.2. To study the life cycle of litchi fruit borer(s), *Conopomorpha sinensis* Bradley

Sharma and Agrawal (1988) studied the biology of litchi fruit borer, *C. cramerella* and observed five instars during the larval period. Further, they reported the longevity of adults varies from 3.12 to 6.84 days. Hwang and Hsieh (1989) studied the bionomics of cocoa pod borer, *C. cramerella* from Taiwan. The studies revealed that, mean duration of egg, larval and pupal stages were 3.3, 8.8 and 7.0 days respectively. The mean number of eggs deposited by females were 114.1 eggs/female and the rate of egg hatchability was 97%. The longevity of the adult was approximately 6.0-8.0 days.

Singh (1992) reported that, larval and pupal period of *C. sinensis* ranges from 7-8 days and 6-7 days, respectively. Hung *et al*. (2002) studied rearing techniques, eclosion and mating behavior of litchi fruit borer, *C. sinensis*. The egg, larval and pupal period lasted 2.8, 10.3 and 7.1 days, respectively. The adult male and female longevity lasted 20 and 19.3 days, respectively. The fecundity was reported of 234.8 eggs. Eclosion was found mainly in dark, when 96.7% of females and 87.6% of males eclosed in dark.

Qinming *et al*. (2005) investigated the life table studies of the litchi fruit borer, *C. sinensis* under laboratory conditions. The results showed that fecundity of *C. sinensis* on litchi fruits were 160.3 eggs/female compared to 99.6 eggs/female on young shoots. Also, the survival rate of *C. sinensis* feeding on young shoots were 96.4%, while the survival rate on fruits were 90.7%.

Posada *et al*. (2011) distinguished the characteristics of male and female pupae and adults of the cocoa pod borer, *C. cramerella*. They observed that, female pupae can be easily differentiated from male, by having two pairs of tubercles on the sterna of 9th and 10th segments. The female genital opening is located anterior to the first pair of tubercles and have a light brown longitudinal depression. While, male genital opening is conspicuous and longitudinal slit located between the two pairs of tubercles. Furthermore, the adult female abdomen is white, compressed laterally at the tip and hairy anal papillae was seen while, the adult male abdomen is black and robust.

Li *et al*. (2013) studied the effect of temperature (@ 15^{o}C, 20^{o}C, 25^{o}C, 30^{o}C, and 35^{o}C) and supplementary nutrition (*i.e*., diluted honey with water at 0%, 10%, 20%, 30%, 40%, and 50%) on the development, longevity and oviposition of *C. sinensis*. They reported that, temperature had significant effect on the duration of the pupal period, pupal emergence rate, adult longevity and oviposition. Pupal emergence rates were significantly higher at 20°C, 25°C and 30°C than at 15°C and 35°C. The provision of supplementary nutrition significantly increased adult longevity, but there was no significant difference in longevity among a series of concentrations. In addition, temperature had a significant effect on oviposition, with the most eggs being laid at 25°C. There was no significant difference in the numbers of eggs laid at supplementary nutrition levels of 5%, 10%, 20%, 30%, 40% or 50% honey water, although the number laid was approximately 6.33-7.56-fold greater than in the control.

Li *et al*. (2014) carried out an experiment to find out the effects of temperature (@ 15, 20, 25, 30 and 35 ^{0}C), 80% RH and 14:10 h L:D on emergence dynamics of *C. sinensis*. They found that, temperature significantly affected the emergence duration and emergence rate, but not the sex ratio and emergence pattern. Th emergence rates at 20, 25, and 30 ^{0}C were significantly higher than at 15 and 35 ^{0}C. The sex ratio of *C. sinensis* under the different temperature treatments remained at approximately 1:1, and was unaffected by temperature. Further, they noticed that, most moths emerged primarily from 20:00 (4 h after the onset of darkness) to early in the morning.

Zhi *et al*. (2015) carried out an experiment to determine the number of larval instars and developmental duration of each instar of the litchi fruit borer, *C. sinensis* at different temperatures in the laboratory. They revealed, *C. sinensis* has five larval instars. The average head capsule width of the 1st - 5th instar larvae was 0.105, 0.170, 0.265, 0.435 and 0.652 mm respectively. The duration of the 1st, 2nd, 3rd, 4th, and 5th instar larva, prepupa, pupa, pre-oviposition and one life cycle at 20-32^{o}C was 1.17-4.50, 1.40-2.09, 1.00-2.84, 1.18-3.41, 1.37-3.00, 0.69-2.41, 5.35-12.74, 4.22-4.75, and 19.34-41.16 days, respectively.

Jayanthi Mala *et al*. (2017) reported that, larvae of *C. sinensis* is whitish in colour and spin thin transparent cocoon during pupal stage in the wooden cages. They also found, in the field conditions pupation takes place within the leaves. Further, they reported that adult moths were 5 mm in length with a long filiform antenna and narrow fringed forewings. Meng *et al*. (2018) studied the preference choices of *C. sinensis* for litchi based on its host surface characteristics and volatiles. They revealed that, females favored laying their eggs on the convex surface of fruits that had particular volatile characteristics.

Niogret *et al.* (2019) reported that, the moths rest beneath branches during the day with a strong preference for nearly horizontal branches. Females demonstrated a greater capacity for movement after disturbance compared to males (83.0 ± 89.9 cm in 9.1 ± 9.5 versus 57.7 ± 49.2 cm in 6.7 ± 5.3 s for females and males, respectively).

2.3. To study the seasonal incidence of litchi fruit borer(s) and their natural enemies

Among the various fruit borer(s) of litchi, *C. sinensis* is the most devastating pest in Nagaland. The information pertaining to seasonal incidence and natural enemies of litchi fruit borer, *C. sinensis* is very meager. Hence, the available literature was presented below.

2.3.1 Seasonal incidence of litchi fruit borer, *C. sinensis*

Hameed *et al*. (1992) noticed that maximum fruit damage by litchi fruit borer, *C. sinensis* was during May-July and highest population of pest in the August. They reported that from October to March, pest population was not found in orchards but reappeared later in the month of April. Further, they estimated the loss and fruit infestation from 24-32% and 7-70%, respectively. Similarly, Singh (1992) reported that, the extent of fruit damage by *C. cramerella* was positively correlated with the amount of precipitation.

Schulte *et al*. (2007) carried out an experiment at two different elevations *viz.*, low elevation orchard (800 m ASL) and high elevation orchard

(1400 m ASL) in the Mae Sa Valley, Northern Thailand. They found that, fruit infestation rate and fruit growth studied in the low elevation orchard were sigmoidal and showed a highly significant positive correlation ($P \leq 0.01$). Also, fruit infestation rate in the low elevation orchard decreased in the course of fruiting season from March to May, but increased in the high elevation orchard, where no fruits were present within the same period of time. They also concluded that, females of *C. sinensis* clearly prefers fruits rather shoots for oviposition. Further, they reported, in high elevation orchard females oviposit eggs on shoots when fruits aren't available.

Dalui and Sarkar (2021) studied the seasonal incidence and damage potentiality of litchi fruit borer, *C. sinensis* in relation to major abiotic environmental factors. They revealed that, initially 3.3% infestation was observed at 21 days (26th March, 2018) and the attack by the borer gradually increased and reached its peak (42.66%) after 60 days of fruit set (4th May, 2018). Further they reported that, rainfall has little influence on the activity of the pest species, while temperature has a significant impact on the pest, particularly on their larval activity.

2.3.2. Natural enemies associated with litchi fruit borer, *C. sinensis*

Walker (1987) reported a parasitoid, *Chelonus chailini* sp. nov. (Hymenoptera: Braconidae) parasitizing cocoa pod borer, *C. cramerella* through survey in Malaysia. Likewise, Sharma and Agrawal (1988) recorded four parasitoids namely *Mesochorus* sp., *Chelonus* sp., *Bracon* sp., and *Apanteles* sp. parasitizing *C. cramerella* in Bihar, India.

Hwang and Hsieh (1989) reported four species of parasitoids *viz*., *Phanerotoma* sp. and *Apanteles* sp., from pupae of *C. cramerella* while, *Tetrastichus* sp. and *Elasmus* sp. from *C. cramerella* larvae feeding on shoots or leaves. Similarly, Huang *et al*. (1994) reported five hymenopteran parasitoids parasitizing *C. sinensis*. Of these, only *Phanerotoma* sp. was found causing 22% larval mortality. Menzel (2002) recorded two parasitoids, *Phanerotoma* sp. and *Apanteles* sp. parasitizing the litchi fruit borer, *C. sinensis* from Thailand.

Waite and Hwang (2002) reported 10 species of parasitoids parasitizing the litchi fruit borer, *C. sinensis* from Taiwan. Of these, six species *viz*., *Phanerotoma* sp., *Colastes* sp., *Pholestesor* sp. (Braconidae), *Goryphus* sp. (Ichneumonidae), *Tetrastichus* sp. and *Elasmus* sp. (Eulophidae) were found attacking the larval stage. Whereas, three species *viz*., *Paraphylax* sp. (Ichneumonidae), *Phanerotoma* sp. and *Apanteles* sp. were found attacking the pupal stage.

Anupunt and Sukhvibul (2005) reported five parasitoids *viz*., *Phanerotoma* sp., *Colastes* sp., *Pholestesor* sp., *Goryphus* sp. and *Paraphylax* sp. parasitizing litchi fruit borer, *C. sinensis* from Thailand. Belokobylskij and Maeto (2006) described a new parasitoid, *Parachremylus litchii* sp. nov., parasitizing larvae of *C. sinensis* and *C. litchiella* from Thailand.

Schulte *et al*. (2007) recorded the parasitization rates of two parasitoids *viz*., *C. chailini* (90.8%) and *Phanerotoma* sp. (9.2%) on *C. sinensis* in Northern Thailand. Meng *et al*. (2014) reported a new specific primer pair to amplify *C. sinensis* cytochrome c oxidase subunit I (COI) sequence fragment to detect consumption of *C. sinensis* by its predators. They revealed that, *C. sinensis* DNA was found in important predators like *Cheilomenes sexmaculata* Fabricius (Coccinellidae), *Leucauge magnifica* Yaginuma (Tetragnathidae), *Propylea japonica* Thunberg (Coccinellidae) and *Oxyopes sertatus* Koch (Oxyopidae). The detection rates of *C. sinensis* COI DNA found in these predators were 39.3, 36.4, 27.3 and 27.2%, respectively.

Satyagopal *et al*. (2015) reported the occurrence of natural enemies such as Mirid bug (*Campyloneura* sp.), lady bird beetles (*C. sexmaculata*, *Coccinella septumpunctata* Linnaeus and *Brumoides suturalis* Fabricius), lacewings, big eyed bugs (*Geocoris* sp.) and pentatomid bug (*Eocanthecona furcellata* (Wolff)) preying on litchi fruit borer, *C. sinensis*.

2.4. To study the efficacy of various insecticides and bio-pesticides against the litchi fruit borer(s)

Among the various fruit borers infesting litchi crop, *C. sinensis* is the most economically important pest. Hence, the management aspects of *C. sinensis* was presented below.

Schulte *et al*. (2007) conducted a field trial in 2005 to find out the efficacy of one bio-pesticide (*Bacillus thuringiensis* var. *aizawai* (florbac) and 2 insecticides *viz*., spinosad (spinosyn A, spinosyn B and spinosyn C) and imidacloprid against litchi fruit borer, *C. sinensis*. It was found that, spinosyn B and spinosyn C each at 6.25 g and 12.5 g a.i/L were effective in controlling fruit borer infestation by 7.5% and 8.5% followed by florbac 8500 IU/mg by 15%, spinosyn A by 15.5% and imidacloprid by 16.5%.

Kumar *et al*. (2014a) consecutively carried out field trials for two years at research farm of National Research Centre for Litchi, Mushahari, Muzaffarpur, Bihar during 2010-2011. They founded that, *Trichogramma chilonis* cards @ 50,000 eggs/ha in blend with nimbicidine 0.5% (12.65%) and kamdhenu keet niyantrak 5% (12.30%) were found equally effective in managing the fruit borer infestation, closely followed by kamdhenu keet niyantrak 5% (14.35%). Further, the highest fruit borer reduction over control (70.29%) was recorded in *Trichogramma cards* @ 50,000 eggs/ha blended with nimbicidine 0.5% (69.34%) and kamdhenu keet niyantrak 5.0% (65.00%).

A field trial was conducted successively for two years during 2014 - 2015 at ICAR-National Research Centre for Litchi, Mushahari, Muzaffarpur, Bihar to evaluate the efficacy of Insect Growth Regulators (IGR’s) for managing litchi fruit borer, *C. sinensis*. The studies revealed that, novaluron 10 EC @ 0.015% recorded least infestation by 9.62% and 4.70% during early and mid-stage respectively, closely followed by diflubenzuron 25 WP @ 0.03% by 9.87% and 5.73%. However, at harvest stage, diflubenzuron 25 WP 0.03% recorded the lowest borer infestation (12.39%), followed by novaluron 10 EC 0.015% (13.67%) compared to control (59.35%) (Srivastava *et al*., 2017).

Field experiments were conducted to evaluate some new mixture formulations of insecticides against litchi fruit borer, *C. sinensis* during 2013 and 2014. The experiments were conducted at the Horticultural Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. The studies revealed that, chlorantraniliprole 9.3% + lambda cyhalothrin 4.6% 150 ZC @ 35 g a.i/ha provided the best result both in terms of minimum fruit infestation (12.12%) and maximum yield (95.92 kg/plant), followed by chlorantraniliprole 10% + thiamethoxam 20% 300 SC @ 150 g a.i/ha (13.10% mean fruit infestation). Thiamethoxam 25 WG was found to be the least effective with 22.88% mean fruit infestation (Alam *et al*., 2019).

Ranjan *et al*. (2019) carried out an experiment to find out the efficacy of two bio-pesticides *viz*., spinosad 45 SC and neem seed kernel extract 4% against litchi fruit borer, *C. sinensis*. A field trial was conducted at the Dr. Rajendra Prasad Central Agricultural University, Research Farm, Samastipur, Bihar during 2012 and 2013. The results revealed that, spinosad 45 SC @ 0.045% twice at flush stage and fruit colour break stage was effective in managing fruit borer infestation by 6.30%, followed by neem seed kernel extract 4% (9.50%).

Upadhyay *et al*. (2020) evaluated the efficacy of five insecticides *viz*., azadirachtin 0.15% EC, chlorantraniliprole 18.5% SC, flubendiamide 39.35% SC, lambda cyhalothrin 2.5% EC and dimethoate 30% EC against litchi fruit borer, *C. sinensis*. The study was carried out at Eastern Terai of Nepal, Regional

Agricultural Research Station (RARS), Sunsari consecutively for two years during 2015 and 2016. Among the tested insecticides, Chlorantraniliprole (18.5% SC) and Flubendiamide (39.35% SC) each at 1ml/3lit of water were found to be most effective, followed by lambda cyhalothrin (2.5% EC), dimethoate (30%EC) and azadirachtin (0.15% EC).

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The experimental study entitled "Study on life cycle of litchi fruit borer(s) and their management" has been conducted in two different locations *i.e.*, Experimental Research Block, Department of Horticulture, School of Agricultural Sciences, Medziphema campus, Nagaland University, Nagaland and Farmer's farm, Medziphema, Nagaland during 2022 and 2023. The biology of litchi fruit borer, *C. sinensis* Bradley was studied at Department of Entomology, SAS, Medziphema campus. The details of materials used and methods adopted during the course of investigations are presented here under different headings.

3.1 Geographical situation

The Experimental Research Block, Department of Horticulture, School of Agricultural Sciences, Medziphema campus, Nagaland University was situated at 25^{o} 45' N latitude and 93^{o} 53' E longitudes at an elevation of 450m above sea level. While, the Farmer's farm, Medziphema was situated at 25^{o} 75' N latitude and 93^{o} 89' E longitudes at an elevation of 407m above sea level.

3.2 Climatic condition and weather

The prevailing climatic condition of both locations was humid and falls under sub-tropical region with an average annual rainfall ranging from 2000-2500 mm, with predominantly high humidity of 70-90%. The mean temperature ranged from 21^{o} to 32^{o} C during summer and during winter from 10-15^{o} C, rarely goes below 8^{o} C in winter. The soil was sandy loam, acidic in nature with P^{H} ranged from 4.5-6.5. The meteorological data during the period of study have been collected from ICAR Regional Research Centre, Jharnapani, Nagaland, and shown in the Table 3.1, 3.2.

Table 3.1 Meteorological data recorded during the litchi fruiting period (*i.e.*, April-June) for the year 2022

Month	Standard Week No.	Temperature (^{0}C)		Relative humidity (%)		Cumulative rainfall (mm)	Wind speed (kmph)	Sunshine hours (h/d)
		Max	Min	Max	Min			
26th March to 01st April	13	30.7	19.3	84	57	0.9	1.984	0.8
2nd April to 8th April	14	29.1	19.8	91	69	10.4	1.863	2.3
9th April to 15th April	15	28.8	19.8	95	78	95.3	2.510	2.9
16th April to 22nd April	16	32.7	19.9	88	62	35.5	2.882	5.5
23rd April to 29th April	17	32.6	20.0	87	61	18.3	1.996	7.1
30th April to 6th May	18	29.3	20.1	91	70	42.2	1.583	4.7
7th May to 13th May	19	31.7	22.7	91	70	74.8	1.188	3.7
14th May to 20th May	20	29.1	21.9	93	81	110.6	0.989	2.2
21st May to 27th May	21	30.8	22.3	93	72	10.9	1.116	3.4
28th May to 3rd June	22	33.3	23.3	93	65	22.5	1.214	4.8
4th June to10th June	23	33.0	24.0	94	74	51.1	1.196	2.9
11th June to17th June	24	30.3	23.3	95	74	46.7	0.872	1.3
18th June to 24th June	25	31.2	23.4	95	75	34.8	0.824	1.8
25th June to 1st July	26	33.3	24.9	93	68	9.9	1.313	4.6

Table 3.2 Meteorological data recorded during the litchi fruiting period (*i.e.*, April-June) for the year 2023

Month	Standard Week No.	Temperature (^{0}C)		Relative humidity (%)		Cumulative rainfall (mm)	Wind speed (kmph)	Sunshine hours (h/d)
		Max	Min	Max	Min			
26th March to 01st April	13	29.7	16.4	90	54	18.2	0.932	3.8
2nd April to 8th April	14	29.1	16.2	91	51	20.1	1.228	6.8
9th April to 15th April	15	34.7	16.2	82	37	0.0	1.051	8.0
16th April to 22nd April	16	35.1	19.7	89	50	27.7	1.229	5.5
23rd April to 29th April	17	31.7	18.3	86	59	20.2	0.953	6.1
30th April to 6th May	18	32.2	20.2	85	60	24.9	0.889	5.4
ay to 13th May	19	34.9	19.5	86	48	0.0	0.927	5.8
14th May to 20th May	20	30.4	20.8	92	63	24.5	0.271	3.6
21st May to 27th May	21	33.4	21.8	80	58	35.2	0.976	5.0
28th May to 3rd June	22	35.8	22.8	85	49	2.5	0.392	9.2
4th June to10th June	23	36.9	24.1	84	61	77.1	0.561	5.1
11th June to17th June	24	30.2	23.6	93	80	107.2	0.276	0.8
18th June to 24th June	25	30.2	23.6	92	77	63.2	0.161	2.5
25th June to 1st July	26	34.1	25.1	91	75	25.0	0.434	4.0

3.3 Experimental Details

The experiment was conducted in an already established litchi orchard, using a Randomized Complete Block Design (RCBD) with a tree spacing of 5x5m in a square system. The objectives seasonal incidence and efficacy studies were carried out under field conditions in two different locations in summer during 2022 and 2023 (Plate 3.1). While, the identification of litchi fruit borer(s) and life cycle studies were studied at the Department of Entomology laboratory, SAS, NU.

3.4 Identification of litchi fruit borer(s)

3.4.1 Identification of litchi fruit borer(s) based on morphological and genital characters

3.4.1.1 Field collection and rearing of immature stages

To characterize the genera and species of litchi fruit borer(s), infested and fallen fruits were collected from litchi orchards. The immature stages of litchi fruit borer(s) were reared in wooden cages (45x30cm) at 25 ± 1°C, RH = 60 ± 5%, and 15:9 hr (light: dark phase) at Entomology Laboratory, SAS, Nagaland University for the adult emergence by adopting the methodology proposed by Doerksen and Neunzig (1976), Tashiro (1976), Genc *et al*. (2003), Rosario *et al*. (2007) and Nagaraj (2014). The rearing room was disinfected with 2 per cent formaldehyde at regular interval to maintain the hygiene.

3.4.1.2 Processing of specimens

The adults after emergence were killed by keeping the specimens in refrigerator for 3-4 hours and later pinned through thorax using nickel insect pins (No. 0, 1, and 2). The adult specimens were mounted on a stretching board, the antenna and wings were stretched properly. Each specimen was labelled with the information pertaining to date of collection, locality, latitude, elevation,

A

B

Plate 3.1 Experimental set-up to study the seasonal incidence and efficacy of various insecticides and bio-pesticides against litchi fruit borer(s) and their natural enemies

A. Experimental Research Block, Department of Horticulture, SAS, Nagaland University, Medziphema, Nagaland; B. Farmers farm, Medziphema, Nagaland name of the collector and host on which it was reared. The specimens were dried properly and preserved in insect boxes having unit trays *i.e*., 45x30cm and were deposited at the Department of Entomology Laboratory (Plate 3.2, 3.3).

3.4.1.3 Identification of collected fruit borer(s) species of litchi and describing of new taxa, if any

The specimens collected were identified up to generic and species level based on the literature published by Bradley (1953), Zimmerman (1978), Bradley (1986), Horak and Komai (2016), Sohn *et al*. (2016), Jayanthi Mala *et al*. (2017), Shashank *et al*. (2018), Chaovalit *et al*. (2019) and Pasam *et al*. (2023). Those species which did not agree with descriptions and figures of already known species were considered as putative species. The specimens were sent to Dr. Shankara Murthy, Associate Professor, Lepidopteran Taxonomist, University of Agricultural Sciences, Raichur, Karnataka.

3.4.1.4 Developing taxonomic key for collected fruit borer(s) species of litchi based on the morphological and genital characters of adults

The morphological characters of adults of different species were highly variable, for example, frons, antenna, proboscis, labial and maxillary palps, *etc*. and also, modifications in male genitalia like uncus, valva, saccus, juxta, gnathos, costa, corona, sacculus, vinculum, tegumen, and aedeagus and in female genitalia, ostium bursae, anterior and posterior apophyses, ductus bursae, corpus bursae, and signum were also variable. In the current study, these variations were studied and utilized for preparation of key through dissection of adults.

Genitalia of adults (male and female) were dissected using the technique described by Clark (1941) and Kirti and Gill (2005) with little modification wherever required. The materials and chemicals used for preparation of genitalia are shown in Plate 3.4. The dried and preserved

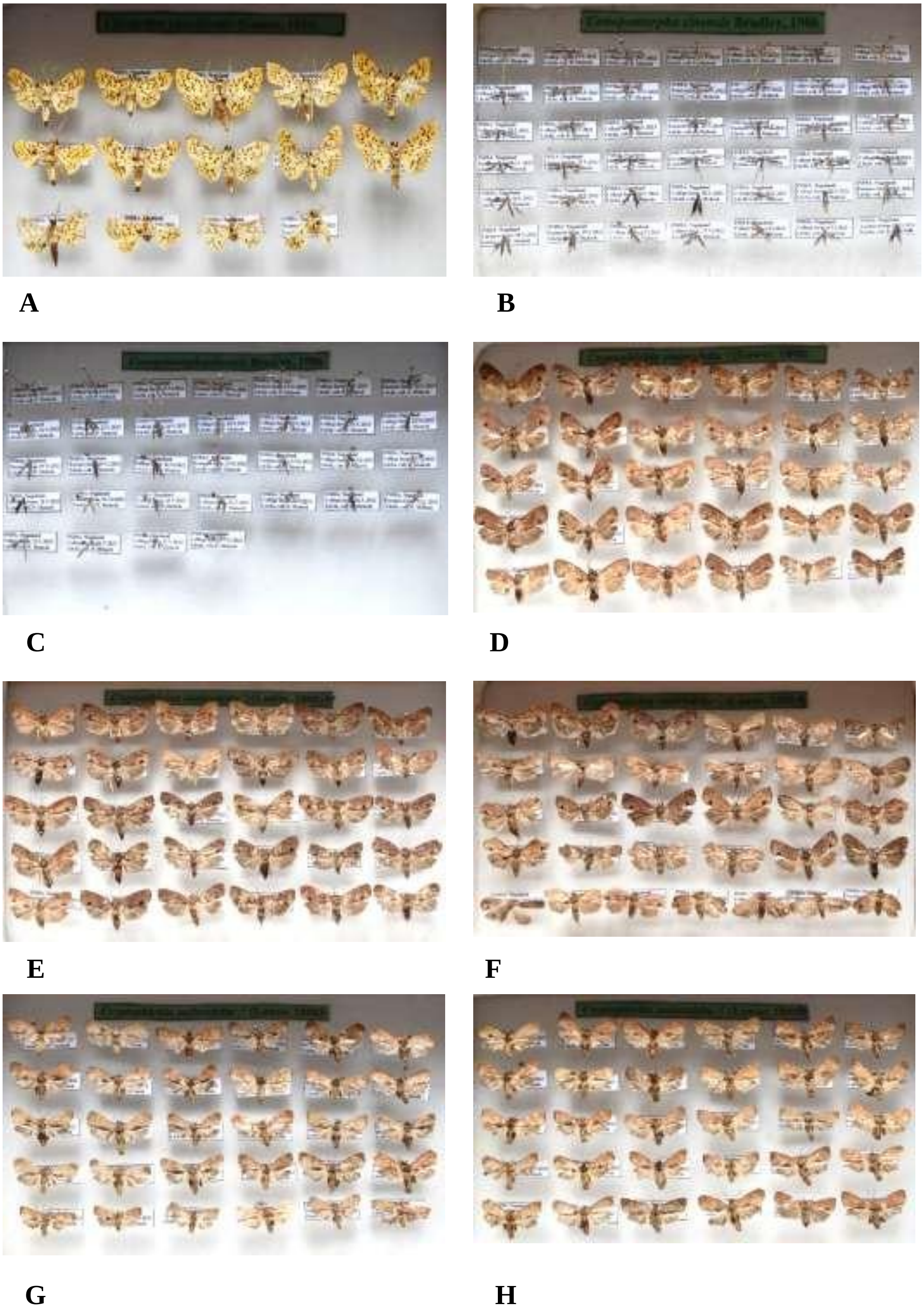


Plate 3.2 Insect specimens used for taxonomic studies

A. *Conogethes punctiferalis*; B and C. *Conopomorpha sinensis*; D, E and F. *Cryptophlebia ombrodelta* ♀; G & H. *Cryptophlebia ombrodelta* ♂

A

B

C

D

Plate 3.3 Insect specimens used for taxonomic studies

A; *Cryptophlebia ombrodelta* ♂**;** B. *Deudorix epijarbus*; C. *Thaumatotibia zophophanes*; D. Dissected specimens

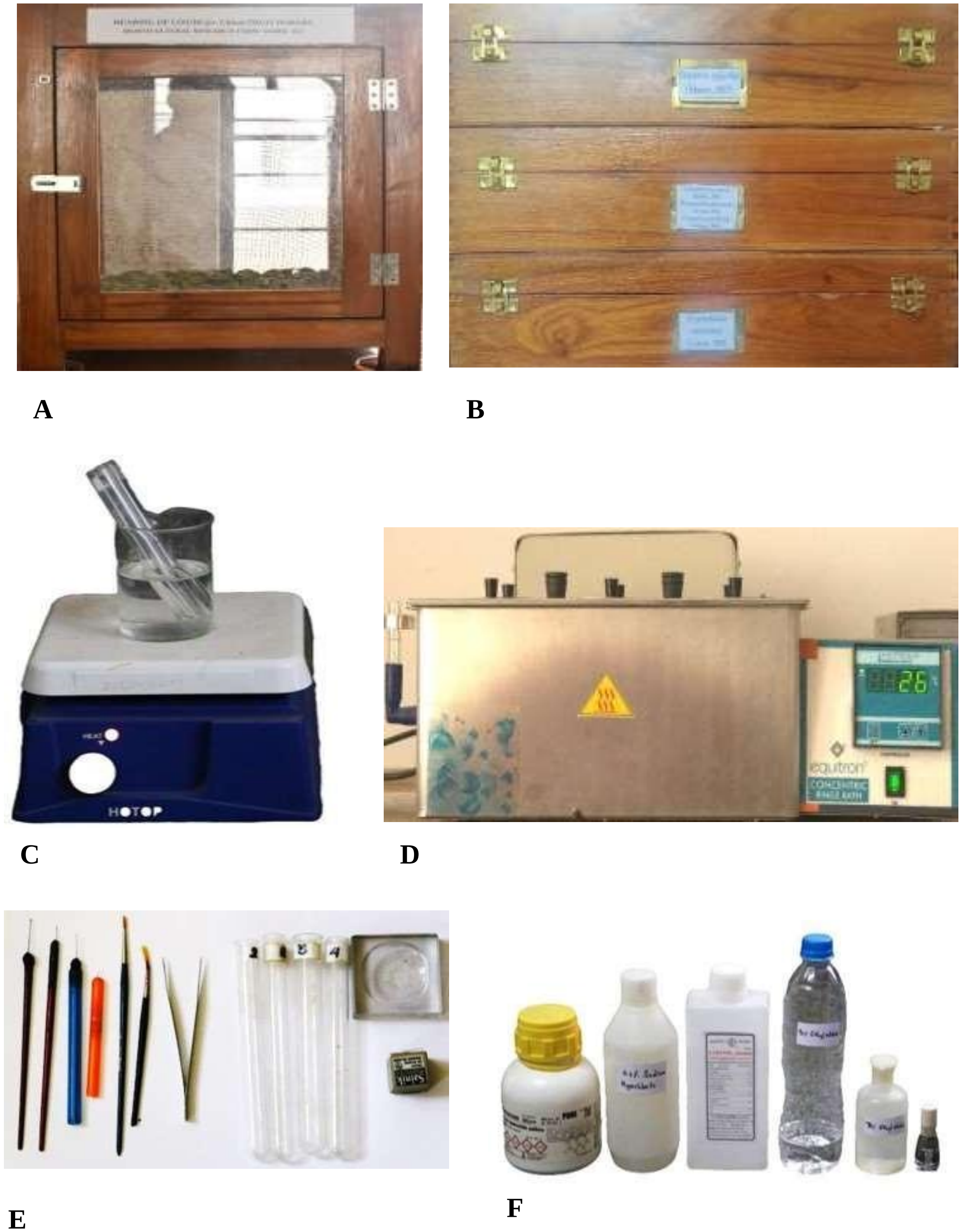


Plate 3.4 Materials and insect specimens used for taxonomic studies

A. Rearing of immature stages of litchi fruit borer(s) using wooden cages; B. Insect specimens used for taxonomic studies; C. Digestion unit; D. Water bath; E. Dissection materials; F. Chemicals specimens were used for studying of genitalia. Before dissection of genitalia, adults were photographed. Then the abdomen was detached from thorax with the help of a fine needle. The abdomen was then transferred to a test tube containing a few milliliters of 10% caustic potash (KOH).

This was heated slowly in a water bath till the convection currents were observed in the solution and then it was kept for cooling. After cooling, the abdomen was transferred to a glass cavity dish containing water and the macerated soft tissues were pressed out with the help of a pair of bent needles mounted on plastic handles. After repeated washings in water, the abdomen was transferred to glycerin in a glass cavity dish for further dissection (separation of genital parts from the genital capsule) and observation was made under a stereoscopic microscope. After the study, the dissected genitalia were preserved in genital vial containing glycerin.

3.4.1.5 Observation and Photographs

The parts of the genitalia were observed and studied using a compound microscope. Before photography, both the male and female genitalia were stained with acid fuschin. The parts of male genitalia were held in the desired position in a cavity slide by means of a small quantity of bee wax. The wax was firmly fix to the bottom of the cavity slide, before placing glycerin to avoid movement while preparing for photography. All the species studied were photographed using Trianocular stereo-zoom microscope (Leica M 205C) with auto montage and images were edited using Adobe photoshop CC 2015 software. Descriptions of genera and species were provided. In case of known species, descriptions of additional characters or variations were observed, if any, were given in addition to earlier description. In the case of putative new species, detailed descriptions are given.

3.4.1.6 Terminology

The definitions / terminologies were drawn from a number of sources, including Mehta (1933), Torre-Bueno (1937), Klots (1970), Bradley (1986), Scoble (1992), Triplehorn *et al*. (2005) and Horak (2006) described the parts of the genitalia of Lepidoptera and their arrangements are also provided.

Aedeagus: tube-like organ of the male genitalia lying between the valves and functioning as a penis, often adorned with spines and useful in determining the species. It houses the vesica.

Ampulla: in male, a process arising from the sacculus, usually thin and tubular on the costal side.

Anal angle: in ventral view, the anterior extremity of the cucullus (in the male).

Anal Papillae: in the female, a paired process at the apex of the ovipositor.

Anellus: in male, the membranous covering of the aedeagus.

Antrum: in the female, a chamber or cavity formed from part of the ostium in some species.

Anterior apophyses: in the female, the pair of elongate processes arising from the eighth sternite.

Basal, basally, basad: closest to the body; towards the body or point of attachment.

Bursa copulatrix: in the female, part of the bag-like structure connected to the ductus bursae, which is used to store sperm. If appendix bursa is also present, these together with the bursa copulatrix constitute the corpus bursae. It is often adorned with spines, which may be distinguishing identification features.

Chaetosema: a group of sensory hairs on the head, near the ocellus.

Cilium (pl. cilia): scale or scales resembling hairs, a row of which usually border the wings, or adorn the antennae or other organs.

Clasper(s): the valves in the male genitalia or parts of the armature. It is also synonymous, in both meanings, with harpe.

Coecum: in the male, a blind sac (part of the aedeagus).

Colliculum: in the female, a small dorsal plate or narrow ring-like sclerite of the ductus bursae.

Coremata: specialized structure on the underside of the male abdomen and the term referred to wide range of scent brushes or hair pencils.

Cornutus (pl. cornuti): in the male, a spine arising from the aedeagus.

Corona: in the male, a row of spines along the outer margin of the cucullus, extending across its inner face.

Corpus bursae: in the female, the bag-like structure connected to the ductus bursae, used to store sperm. Comprises the bursae copulatrix and appendix bursae (which may be absent). It is often adorned with spines, which may be distinguishing features.

Costa, costal: in male genitalia, referring to the uppermost (i.e., posterior) margin of the valva in ventral view. On the wing of a moth, the leading edge.

Cucullus: in male genitalia, the tip of the valva, often necked, rounded and bearing spines.

Dentate: toothed or strongly serrated.

Distal, distally, distad: away from the body or point of attachment.

Diverticulum: a blind side passage, forming a sac or swelling, *e.g.*, in the vesica or bursa copulatrix.

Ductus bursae: in the female, the tube extending from the ostium to the bursa copulatrix.

Ductus ejaculatorius: in the male, the single duct or tube through which the seminal fluid is ejected into the ostium of the female.

Ductus seminalis: in the female, the tube connecting the bursa copulatrix with the oviductus.

Fasciculate: clustered or tufted.

Gnathos: in male genitalia, a hardened part of the vinculum near the uncus, which supports the anal tube.

Harpe: in male genitalia, the hardened clasping organ on the inner face of valva.

Juxta: in male genitalia, a hardened plate-like structure between the valvae which supports the aedeagus.

Lamella ante-vaginalis: in the female, a hardened plate partially surrounding the ostium placed anteriorly.

Lamella post-vaginalis: in the female, a hardened plate partially surrounding the ostium placed posteriorly.

Medial, medially, median: middle; the central area (medio-distal = away, more distant from, the middle).

Ostial plate: in the female, a hardened plate surrounding the ostium.

Ostium: in female genitalia, the external opening.

Ostium bursae: a chamber or cavity formed from part of the ostium (see also antrum).

Ovipositor: in the female, the tubular or valved structure used to deposit the eggs, sometimes extendable beyond the apex of the abdomen.

Pollex: in the male, a process on the valva, usually on the cucullus as an extension of the anal angle. Also, sometimes used to describe a process arising from the median section of the valva.

Posterior apophyses: in the female, the pair of elongate processes arising from the ovipositor. Appendix bursae: in the female, a secondary swelling attached to the bursa copulatrix.

Sacculus: in male genitalia, dominant part of the base of the valva, often adorned with spines.

Saccus: in male genitalia, the lowest part of the vinculum.

Signum (pl. signa): in the female, scleortized spines and plates on the bursa copulatrix.

Socius: in the male, a paired extension of the vinculum.

Sub-genital plate: the plate beneath the genitalia (eighth tergite).

Tegumen: the dorsal half of the large central transverse ring-like part of the male genitalia.

Termen: the outer edge of the wing of a moth, adorned with cilia.

Uncus: in the male, the top part of the vinculum, sometimes forming a large hooked or curved structure.

Valva: the large pair of laterally extending clasping organs of the male genitalia articulating with the vinculum.

Vinculum: in the male, the ventral half of the large central transverse ring-like part of the male genitalia.

3.4.1.7 Preparation of key

Morphological and genital characters of adults of different species exhibited variations. Based on these variations, an illustrated key was prepared for the species of litchi fruit borer(s). The keys were prepared from referring different sources like other published keys, descriptions and an examination of specimens of the groups concerned. Some were taken largely from previously published keys based on study of different authors like, Hampson (1896), Diakonoff (1938), Arora (2000), Timm *et al*. (2007), Shankaramurthy *et al*. (2015), Horak and Komai (2016), Chaovalit *et al*. (2019) and Pasam *et al*. (2023) generally with some changes in wording or organization and adding few more morphological and genital characters.

3.4.2 Identification of litchi fruit borer(s) through DNA barcoding

The field collected litchi fruit borer(s) were used to study DNA barcoding for the confirmation of species identity. The present objective was carried out at Eurofins Genomics India Private Limited, Bangalore.

3.4.2.1 DNA Isolation

The genomic DNA was isolated from legs of adults by employing CTAB method (Saghai-Maroof *et al*., 1984) with slight modification wherever required and DNA samples were stored at -20^{0}C for further studies.

1. Place one leg of an adult in a 1.5 ml effendorf tube using sterile forceps and add 100µl of prewarmed (60^{o}C) CTAB buffer (beta mercaptoethanol)

2. Grinded the legs into a fine paste using a sterile plastic pestle. Then, washed the pestle with another 100µl CTAB extraction buffer with beta mercaptoethanol

3. It was gently mixed the above mixture by inverting the effendorf tube and incubate it at 60^{o}C for at least 1 hr. After incubation, add an equal volume of chloroform: isoamyl alcohol (24:1) and mix by inverting the effendorf tube for several times

4. After adding chloroform: isoamyl alcohol, spin the tube at 12,000 rpm for 10 minutes.

5. After spinning, transfer the aqueous phase solution carefully to a clean tube and discard the rest of the solution

6. Add 0.75 volumes (150µl) of ice-cold isopropanol to the aqueous solution. Then incubate at 20^{o}C for at least 30 min. After incubation, spin the solution at 18,000 rpm for 15 minutes at 4^{o}C

7. Remove the supernatant after spinning and wash the DNA with 500µl of 70 per cent ethanol and again spin at 18,000 rpm for 15 minutes at 4^{o}C

8. Remove the supernatant and dry the pellet at 37^{o}C for 5-10 minutes

9. Finally, resuspend the DNA in 20-30µl TE buffer and store at -20^{o}C

3.4.2.2 DNA purity assessments

The purity and yield of the DNA was assessed spectrophotometrically by calculating the A_{260}/A_{280} ratios to determine protein impurities and DNA concentrations. DNA quantification was done using nanodrop method (Genova

Nano). This method has the ability to measure as low as 0.5-2µl sample of DNA with a very high accuracy and reproducibility. This method also offers the convenience to use traditional cuvette for sample measurements. To assess the purity of DNA and RNA, the absorbance ratio recorded at 260nm and 280nm, respectively.

The 260 nm and 280 nm absorbance ratio were used to evaluate the purity of DNA and RNA, respectively. A proportion of ~1.7 for DNA usually accepted as "pure;" a ratio of ~2.0 for RNA was usually recognized as "pure." In either event, if the proportion was significantly smaller, it may show the existence of protein, phenol or other contaminants that easily absorb at or close to 280 nm. The purified and quantified DNA was stored at -20^{0}C in sterile TE buffer.

3.4.2.3 Primers used

Mitochondrial cytochrome oxidase subunit I gene (*mtCOI* gene) was amplified using the forward primer LCO 1490 and reverse primer HCO 2198, developed by Folmer *et al.* (1994) and Hebert *et al.* (2003). The primers were custom synthesized by Eurofins Genomics India Private Limited, Bangalore.

Mitochondrial Cytochrome Oxidase I (mtCOI)

Name of Primer	Sequence (5' to 3')
LCO 1490	5' GGT CAA CAA ATC ATA AAG ATA TTG G 3'
HCO 2198	5' TAA ACT TCA GGG TGA CCA AAA AAT C 3'

The PCR conditions were optimized in terms of concentration of template DNA, Taq DNA polymerase and $MgCl_2$ concentration. Varying concentration of template DNA from 100 ng to 125 ng in a reaction volume of 20 µl, the 125 ng DNA gave specific bands. A titration of different concentration of Taq DNA polymerase and $MgCl_2$ showed that 0.2µl of 3 units/µl Taq DNA polymerase and 1.6µl of 15mM $MgCl_2$ in a final reaction mixture gave optimum, reproducible and well resolved results. A higher or lower concentration resulted in either sub optimal or lack of complete amplifications.

Master mix for PCR

Reagents	Volume/tube
MQ (Sterile distilled water)	11.2 µl
Taq assay buffer (10X)	4.0 µl
$MgCl_2$	1.6 µl
dNTP's (10 mM)	0.2 µl
Forward primer (5pM)	0.4 µl
Reverse primer (5pM)	0.4 µl
Taq DNA Polymerase (3 u/µl)	0.2 µl
Template (DNA 125 ng)	2.0 µl
Total	20 µl

Reaction mixture was vortexed and centrifuged. Amplifications were performed using Ventri® 96-well thermal cycler (Applied Biosystems® Life Technologies) with following temperature transitions

Steps	Temperature (^{0}C)	Time
1. Initial denaturation	94	5 min.
2. Denaturation	94	1 min.
3. Annealing	52	30 sec.
4. Elongation	72	1 min.
5. Extension	72	5 min.

Thermal cycle was programmed for 35 cycles with one cycle of initial denaturation and steps 2-4 were repeated 35 times.

3.4.2.4 DNA quantification on agarose gel

Agarose gel electrophoresis is the most effective way to separate DNA fragments by their size and visualize them. DNA molecules are separated on the basis of charge by applying an electric field to the electrophoretic apparatus. The DNA is negatively charged at neutral P^H and it will move towards the positive pole. In order to confirm the presence of total DNA isolated from litchi fruit borer specimens were resolved on 1.5 % agarose gel.

1. Agarose (1.5 g) was dispended in 150 ml 5X TAE buffer (242mg/g Tris base, 57.1 ml glacial acetic acid 100 ml of 0.5M EDTA pH 8.0) and 147 ml of distilled water and was heated to get a clear solution
2. After cooling, 5µl of fluorescent dye *i.e.*, ethidium bromide (10 mg/ml) was added to the solution before pouring it into the gel holding tray fitted with comb. It was then kept for 20-30 min for solidification. Further, the gel was kept in a tank containing 1X TAE buffer before loading
3. The DNA samples were mixed with appropriate amount of 6X gel holding dye (0.25% Bromophenol blue and 0.25% xylene cyanol) and loaded into the wells
4. Electrophoresis was carried out at a constant voltage of 100V till the dye moved to the other end of the gel ensuring proper separation of the DNA molecules.
5. The gel was observed under UV light trans laminator and DNA will be documented
6. The confirmed DNA samples were further used for molecular analysis

The PCR products were resolved by electrophoresis using 1 per cent agarose gel in 1X TCA buffer for about 45 min at 80V along with 100bp ladders. The gel was stained with ethidium bromide (0.5µg/ml), viewed under UV Tranilluminator and photographed immediately for further interpretation using Gel-Doc system.

3.4.2.5 Cloning and transformation

After amplification, the products used for cloning. The procedure which was used for cloning is as follows:

The products derived from the amplification of the MtCOI gene litchi fruit borer species were eluted and ligated to the pTZ57R / T TA cloning vector (Clone JET™ PCR Cloning Kit - Thermo Fisher Scientific). For the efficient cloning process, the *Escherichia coli* (DH5α) cells were prepared by growing the *E. coli* culture in Luria- Bertani (LB) broth (containing Nalidixic acid) for overnight, incubated in C-media for 1 hour in 37°C incubator. The ligated PCR products were incubated along with competent DH5α cells. Transformed and non-transformed cells were selected by Blue-white screening. This was a rapid and efficient technique for the identification of recombinant bacteria. For a better selection of transformed cells sub-streaking was followed on LB agar with ampicillin and smeared with Xgal and IPTG. Later, white (transformed colonies with CO-I gene insert) colonies from the sub-streaked plates were selected and subjected to colony PCR for reconfirmation of the correct transformants.

3.4.2.6 Plasmid Isolation

The colony PCR confirmed transformants from the sub-streaked plates were inoculated in LB broth (containing ampicillin) for multiplication. Plasmids were isolated from the multiplied bacterial cells and thus isolated plasmids were visualized on 1 per cent agarose gel electrophoresis and compared with control plasmids (without insert) that provided with ligation kit (Thermo Scientific, Fermentas, Lithuania). These purified recombinant plasmids with the litchi fruit borer species COXI gene were resuspended in 30 µl of nuclease free water. 10 µl plasmid samples from each resuspended replications were sent for Sanger's sequencing. Sequencing reactions were performed in both M13 forward and reverse direction. The rest of the plasmids were preserved in -80°C.

3.4.2.7 Sequencing

The PCR products were purified and dissolved in 0.1 TE by gel extraction / PCR cleanup methods. Purified samples were sequenced at the specific commercial facilities *i.e.*, Eurofins Genomics Private Limited, Bangalore for sequencing.

3.4.2.8 DNA Barcoding analysis

DNA sequences were aligned using ClustalW programme implemented in MEGA ver. 11.0. Software. A homology search was done using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov) and all the sequences generated were deposited in NCBI-GenBank. Multiple sequence alignments were performed using the MUSCLE algorithm function in MEGA ver. 11.0. Software. Nucleotide composition and patterns of nucleotide substitution were performed by the MEGA 11 program (Tamura *et al.*, 2021). The number of transitions and transversions were plotted against sequence divergence values to determine substitution saturation using the program DAMBE (Xia, 2013). Pairwise genetic distance between fruit borers of litchi and their related species were obtained based on Kimura's two parameter model and used for estimating intra and inter-specific genetic distances (K_2P) across the species. The phylogenetic analyses of the fruit borers of litchi and other related species were constructed using the MEGA ver. 11.0 software with neighbor-joining (NJ) method (Saitou and Nei, 1987). The NJ analyses were conducted using the Kimura 2-parameter distance estimate, with 1000 bootstrap replications.

3.5 To study the life cycle of litchi fruit borer(s), *Conopomorpha sinensis* Bradley

The litchi fruit borer(s), *C. sinensis* is an internal feeder found feeding inside the fruits. The biology of *C. sinensis* on litchi fruit was studied under laboratory conditions at Department of Entomology, SAS, Medziphema campus, Nagaland University. During the period of investigation, the mean maximum and minimum temperature, relative humidity were recorded and provided in the Table 3.1 and 3.2. The infested and fallen fruits were collected from the field and were reared up-to adult stage by adopting the methodology proposed by Doerksen and Neunzig (1976), Tashiro (1976), Genc *et al.* (2003), Rosario *et al.* (2007) and Nagaraj (2014) in an incubator where the temperature was maintained below 25°C and relative humidity 80% and 14:10 h L:D (Li *et al.*, 2014).

The freshly emerged, a pair of male and female adults were released into the oviposition cage (25x15cm diameter) with litchi fruits wrapped by tissue paper were kept in conical flask for egg laying. Totally five such replications were maintained. The eggs laid were segregated. Fifty larvae were reared separately on litchi fruits until they reach the pupal stage. The fresh litchi fruits were provided to the larva as and when needed. The adults were fed with 10% honey solution soaked in cotton pads as a food (Plate 3.5). The observations were recorded on the following parameters using Debro DSZ-55 stereomicroscoe with a magnification of 0.7x-4.5x.

3.5.1 Pre-oviposition period

The period taken from the emergence of female moth to the commencement of egg laying was recorded for five females. On this basis, average pre-oviposition period was calculated.

3.5.2 Oviposition period

To work out the oviposition period, dates of first and last egg laid by female moth was recorded. The period between those two dates was considered

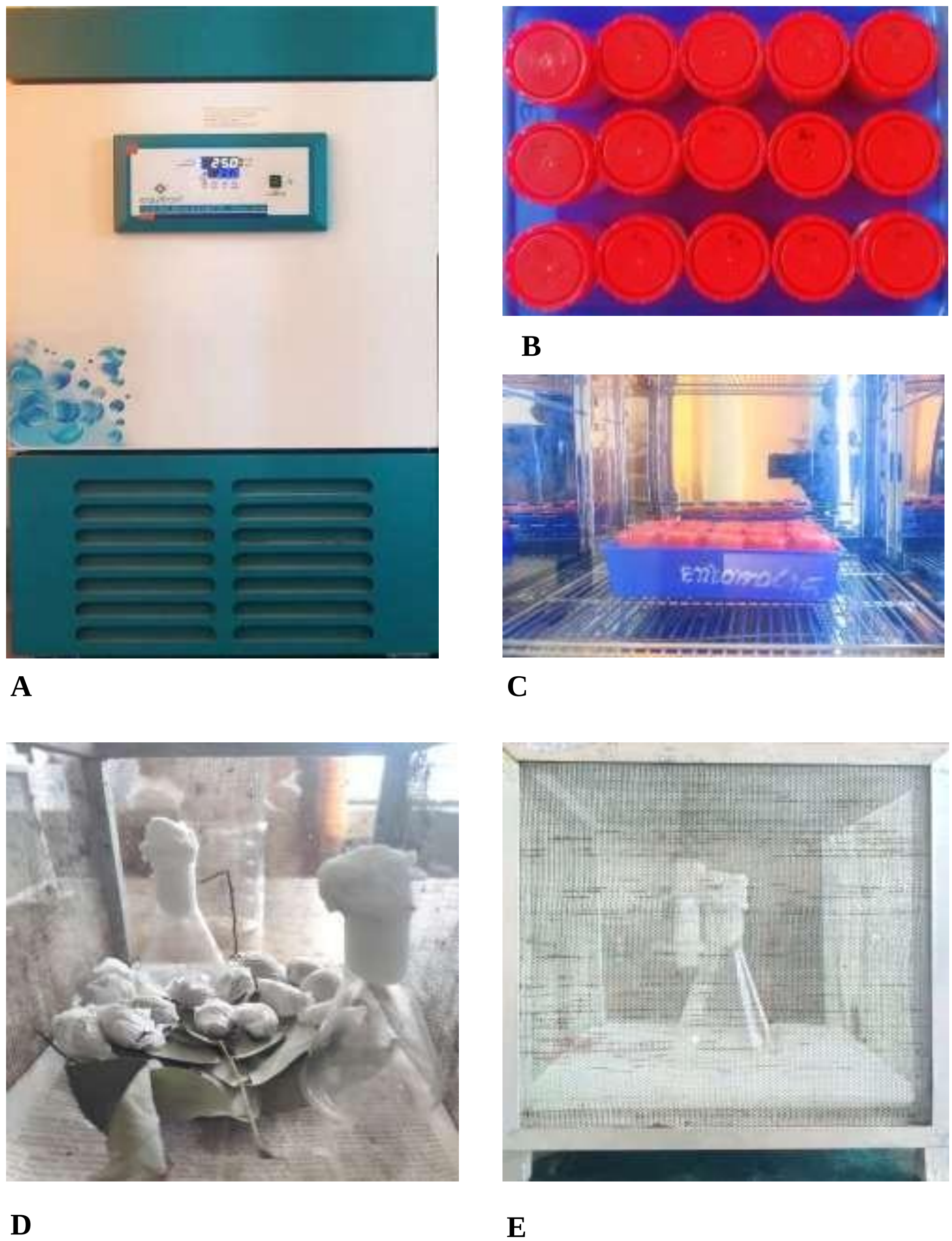


Plate 3.5 Materials utilized to study the biology of *Conopomorpha sinensis* Bradley
A. Incubator; B & C. Biology; D & E. Mating and fecundity of *C. sinenis* (litchi fruits were wrapped by tissue paper for oviposition

as oviposition period. Such oviposition period was recorded for five females and mean oviposition period was worked out.

3.5.3 Fecundity

To record the fecundity, total numbers of eggs laid by each female during oviposition period was counted. Such five females were observed and mean fecundity was worked out.

3.5.4 Incubation period

To study the incubation period, the number of days from egg laid till the hatching of egg was recorded as incubation period. A set of fifty eggs were kept under observation. Observation taken at 12 hr interval.

3.5.5 Egg length and width

The morphometrics of eggs were recorded by using Leica stereo zoom microscope. The average length and breadth were recorded on the basis of ten eggs.

3.5.6 Larval instars and their morphometry

The number of moultings was determined on the basis of casted head capsule. The period between each moulting was recorded as period of corresponding instar. The linear measurements of the larvae were recorded using computerized micrometer scale for ten larvae. Thus, the data obtained were averaged and presented.

3.5.7 Total larval period

The duration of the larval period was recorded as the number of days taken from hatching of egg till last instar larva transformed into pupa.

3.5.8 Pre-pupal period

The period required from full development of larva as indicated by cessation of feeding till complete formation of pupa was recorded and average pre-pupal period was worked out.

3.5.9 Pupal period

To record pupal period, ten freshly formed pupae were kept under observation in plastic covers till emergence of adult and on this basis the average pupal period was worked out. The mean length and breadth of pupa were also recorded by using millimeter scale on the basis of measurements taken for twenty pupae. Observations taken at 12 hr interval.

3.5.10 Adult longevity

Newly emerged adults were separated based on their sexes and released in a separate rearing cage and cotton swab soaked in 10% honey solution was kept suspended in the rearing cage as food for moth. The longevity of five males and females was recorded by observing the duration between emergence and the death of adult.

3.5.11 Total life cycle

The total period for the completion of life cycle was worked out based on the duration of egg, larval, pre-pupal, pupal and adult stage.

3.6 To study the seasonal incidence of litchi fruit borer(s) and their natural enemies

3.6.1 Seasonal incidence of litchi fruit borer(s)

To carry out the above objective, field experiments were conducted during the litchi fruiting season successively from April-June during 2022 and 2023 at two different locations *viz*., Experimental Research Block, Department of Horticulture, SAS, Nagaland University, Nagaland and the Farmers farm

Medziphema, Nagaland. The experiment was laid out in Randomized Complete Block Design with six trees randomly selected from each location and were kept free from insecticide spray during the period of observation. Each tree served as one replication. Good agronomical practices were followed as per recommended package of practices (Kumar *et al*., 2014a).

The number of litchi fruits infested by fruit borers was counted from each replication by visualizing the symptoms of infestation *viz*., a pinhead hole from which little yellowish-brown excreta oozes (Dalui and Sarkar, 2021). Besides, the fruit was assigned to be infested, if a larva is present inside the fruit, or if evidence is found that a larva has developed earlier such as presence of entrance holes or insect excreta (Schulte *et al*., 2007). The observation was taken at weekly interval. The period of observation was 15 days (8th April) to 71 days (30th June) after fruit set. For quantifying the degree of infestation by the fruit borer(s), 100 fruits were randomly selected from each replication. Fruits having the symptom of fruit borer infestation were counted and transformed to percentage value by following formula.

$$\text{Per cent fruit borer(s) infestation} = \frac{\text{Number of fruits infested}}{\text{Total number of fruits}} \times 100$$

3.6.2 Natural enemies associated with litchi fruit borer(s)

To document the natural enemies (*i.e*., predators and parasitoids) of litchi fruit borer(s), the extensive samples of fallen and infested litchi fruits were collected from the litchi orchards during fruiting season and were kept for parasitoid emergence in the laboratory (Sinclair, 1979). The emerged parasitoids were preserved in 90% alcohol for identification and documentation purpose. The coccinellid predators were identified through various publication sources (Poorani and Lalitha, 2018; Poorani, 2023). The parasitoids were identified up to genus level and few are identified up to species level by the taxonomists, Dr. Ankita Gupta, Senior Scientist, Parasitic hymenopteran taxonomist, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India.

3.6.3 Statistical analysis and interpretation of data

Various meteorological parameters like maximum and minimum temperature, maximum and minimum, relative humidity, and rainfall were recorded simultaneously to study the relationship of major abiotic environmental factors with the fruit borer infestation. Data obtained from the study was analyzed using OPSTAT software. Both descriptive and linear multiple regression and analysis of variance were used in showing the relationship between major abiotic environmental factors and fruit borer infestation (Dalui and Sarkar, 2021). Because of their peculiarity in revealing the relation and variability between variables, these statistical techniques were used in the study of both average fruit infestation and mean climatic factors. The critical difference at 5% level of significance was computed. The regression model used in this study was as follows.

$$Y = f(X_1, X_2, X_3, X_4, X_5)\text{.........}(1)$$

$$Y = a + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \varepsilon \text{ ...... } (2)$$

Where, Y=Average infestation (%), X_1=Average temperature, X_2 =Average relative humidity, X_3=Cumulative rainfall, X_4=Wind speed, X_5=Sunshine hours, β=Constant, β_1, β_2, β_3, β_4, and β_5 = coefficient of variation of X_1, X_2, X_3, X_4 and X_5, ε = unexplained variables.

3.7 Efficacy study of various insecticides and bio-pesticides against litchi fruit borer(s)

To carry out this objective, field experiments were carried out in two different locations *viz.*, Experimental Research Block, Department of Horticulture, SAS, Medziphema campus, Nagaland University and Farmers farm, Medziphema, Nagaland during April-June, 2022 and 2023. The experiment was laid out in Randomized Complete Block Design (RCBD) with eight treatments including untreated check. Within each tree, three different directions were recorded as three replications. Good agronomical practices were followed as per recommended package of practices (Kumar *et al.,* 2014a). The various insecticides and bio-pesticides used in the present study against litchi fruit borer(s) were presented in the Table 3.3. The spray fluid was mixed with 0.1% spreader to ensure proper spreading.

3.7.1 Preparation of Neem seed kernel extract 4%

The neem seed kernel extract (NSKE 4%) was prepared freshly by dissolving 400gm of well dried ground powder (8-10% moisture) neem seeds in 10 lit. of water. The seed powder was tied in a cloth immersed in water overnight and stirred well to make a spray suspension (Ranjan *et al*., 2019).

3.7.2 Preparation of Kamdhenu keet niyantrak 5% (Cow urine)

The Kamdhenu keet niyantrak 5% was prepared freshly by dissolving 500 ml of cow urine in 10 lit. of water (Kumar *et al*., 2014a).

Three sprays of all treatments were applied at different intervals during April-June, 2022 and 2023. First spray was given at 13 DAF, second spray at 33 DAF (after fifteen to twenty days of first spray). While, third spray was given at 53 DAF (after fifteen to twenty days of second spray). Spraying was done on outer as well as inner canopy in all the directions of the tree with the help of Knapsack sprayer having hollow cone nozzle (Srivastava *et al*., 2017). Observations were recorded on the basis of damaged fruits *i.e.*, presence of the larvae/excreta. The pre-treatment count was made a day before each spray and post treatment observations were recorded on 7th and 14th day after each spray.

Table 3.3 Insecticides and bio-pesticides evaluated against litchi fruit borer(s)

Sl. No.	Treatments	Trade name	Company	Formulation	Dosage (ml or g/10 lit)
1.	T1: Neem seed kernel extract 4%	-	-	4%	400 ml
2.	T2: *Bacillus thuringiensis* var. *kurstaki*	Dipel	Sumitomo Chemical India Limited	-	50 g
3.	T3: Spinosad 45SC	Tracer	Bayer	45 SC	4.5 ml
4.	T4: Diflubenzuron 25WP	Bi-Larv	Bayer	25 WP	3.0 g
5.	T5: Novaluron 10EC	Rimon	Indofil	10 EC	1.5 ml
6.	T6: Neem oil	-	AgriBegri Trade Link Private Limited	0.2%	20 ml
7.	T7: Kamdhenu keet niyantrak 5%	Cow urine	-	5%	500 ml
8.	T8: Untreated check	-	-	-	-

The formula used for percentage reduction was

$$\text{Percentage reduction} = \frac{\text{Pre}-\text{treatment count} - \text{Post treatment count}}{\text{Pre} - \text{treatment count}} \text{ x } 100$$

3.7.3 Statistical analysis and interpretation of data

The data was analyzed statistically for comparing the treatment means by using OPSTAT software. The transformed values *i.e.*, angular transformation was subjected to one way analysis of variance (ANOVA) by Randomized Complete Block Design. The mean values of different treatments were analyzed with the statistical software along with corresponding standard error mean (S.E.m±). The critical difference at 5% level of significance was computed.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The present investigation on 'Study on life cycle of litchi fruit borer(s) and their management' was carried out during 2022 & 2023 at two locations *i.e.*, Experimental Research Farm, Department of Horticulture, School of Agricultural Sciences, Nagaland University, Nagaland and Farmers orchard, Medziphema, Nagaland. The results on identification of litchi fruit borer(s) based on morphological and genital characters, DNA barcoding of adults, biology of lichi fruit borer(s), seasonal incidence of litchi fruit borer(s) and their natural enemies and efficacy study of certain insecticides and bio-pesticides against litchi fruit borer(s) were presented below.

4.1 Identification of litchi fruit borer(s)

4.1.1 Identification of litchi fruit borer(s) through morphological and genital characters

During the investigation, a total of five identified species were recorded out of 565 specimens collected and reared on its host (Table 4.1 & Plates 4.1 A-F, 4.2 A-F, 4.3 A-F, 4.4 A-B). The studied specimens have shown variations with respect to morphological and genital characters. Based on these characters, it was observed that specimens belonged to four families *viz.*, Crambidae, Gracillariidae, Lycaenidae and Tortricidae. Crambidae, Gracillariidae and Lycaenidae were represented by single species each *viz.*, *Conogethes punctiferalis* (Guenée), *Conopomorpha sinensis* Bradley and *Deudorix epijarbus* (Moore), respectively. Whereas, Tortricidae was represented by two species *viz.*, *Cryptophlebia ombrodelta* (Lower) and *Thaumatotibia zophophanes* (Turner) (Table 4.1 & Plates 4.5 A-F, 4.6 A-D). Among the collected species, *C. sinensis* was found to be predominant with 55.57% followed by *C. ombrodelta* with 22.65%. Other species *i.e.*, *D. epijarbus*, *T. zophophanes* and *C. punctiferalis*

Table 4.1 Fruit borers collected and reared on litchi from Experimental Research Block, SAS, Nagaland University, Medziphema, Nagaland and Farmers farm, Medziphema, Nagaland

Sl. No.	Common name	Scientific name	Family	Subfamily	Damaged parts	Earlier reports cited as a pest across the globe
1.	Castor capsule borer /yellow peach moth	*Conogethes punctiferalis* (Guenée, 1854)	Crambidae	Spilomelinae	Fruits (pulp and seeds)	Singh and Kumar (1992); Kumar *et al*. (2014b); Singh and Kaur (2015); Singh *et al*. (2018); Srivastava *et al*. (2021); Pasam *et al*. (2023).
2.	Litchi shoot and fruit borer/stem end borer	*Conopomorpha sinensis* Bradley, 1986	Gracillariidae	Ornixolinae	Shoots and fruits	Bradley (1986); Waite and Hwang (2002); Nair and Sahoo (2006); Schulte *et al*. (2007); Hung *et al*. (2008); Bai *et al*. (2009); Kumar *et al*. (2014c); Dong *et al*. (2015); Srivastava *et al*. (2015); Yao *et al*. (2015); Fu *et al*. (2016); Jayanthi Mala *et al*. (2017); Srivastava *et al*. (2018); Gupta and Tara (2019); Srivastava *et al*. (2021).
3.	Litchi fruit moth /macadamia nut borer	*Cryptophlebia ombrodelta* (Lower, 1898)	Tortricidae	Olethreutinae	Fruits (pulp and seeds)	Bradley (1953); Zimmerman (1978); Jones (1995); Common (1990); Waite and Hwang (2002); Horak and Komai (2016); Sohn *et al*. (2016); Srivastava *et al*. (2018); Pathania *et al*. (2020); Patel *et al*. (2022)
4.	Anar butterfly/ Fruit borer	*Deudorix epijarbus* (Moore, 1857)	Lycaenidae	Lycaeninae	Leaves, flowers, fruits (pulp and seeds)	Otsuka *et al*. (1991); Waite and Hwang (2002); Srivastava *et al*. (2015); Reddy *et al*. (2016); Gupta and Tara (2019)
5.	Avocado fruit borer/nut borer	*Thaumatotibia zophophanes* (Turner, 1946)	Tortricidae	Olethreutinae	Fruits (pulp and seeds)	Present thesis

A. Formation of webbings within the fruits

B. Presence of granular faecal matter at the entrance hole

C. Mature larva feeding on the pulp as well as nut of the fruit

D. Adult resting on the ventral surface of litchi leaf

E. Larva punctured the peduncle of fruits *i.e.*, developing as well as maturing

F. Larva feeding on the pulp by boring into the fruit

Plate 4.1 A-F. Damage symptoms of fruit borers collected and reared on litchi

A, B, C, & D. *Conogethes punctiferalis* (Guenée, 1854); E & F. *Conopomorpha sinensis* Bradley, 1986

A. Fruits damaged due to boring by larvae near the peduncle

B. Adult resting under the horizontal branches

C. Mature larva on litchi fruit

D. Larval stage holes being plugged by the anal end segment of the larva

E. Fully grown larva fed on the internal content of fruit exclusively on seed

F. Adult resting on the surface of litchi leaf

Plate 4.2 A-F. Damage symptoms of fruit borers collected and reared on litchi

A & B. *Conopomorpha sinensis* Bradley, 1986 ; C, D, E, & F. *Deudorix epijarbus* (Moore, 1857)

A. Newly hatched larva fed on the fruit skin and then tunneled towards the seed

B. Larva came out of the nut through the hole after feeding

C. Larva directly bored into the seed, which is completely eaten

D. Adult (♀) resting on the litchi leaf

E. Larva on the litchi fruit

F. Larva feeding the internal content of the seed

Plate 4.3 A-F. Damage symptoms of fruit borers collected and reared on litchi

A, B, C, & D. *Cryptophlebia ombrodelta* (Lower, 1898); E & F. *Thaumatotibia zophophanes* (Turner, 1946)

A. Larva inside the fruit, feeding on pulp and seed

B. Adult resting on the surface of litchi leaf

Plate 4.4 A-B. Damage symptoms of fruit borers collected and reared on litchi

A & B. *Thaumatotibia zophophanes* (Turner, 1946)

A. *Conogethes punctiferalis* ♂ B. *Conogethes punctiferalis* ♀

C. *Conopomorpha sinensis* ♂ D. *Conopomorpha sinensis* ♀

E. *Cryptophlebia ombrodelta* ♂ F. *Cryptophlebia ombrodelta* ♀

Plate 4.5 A-F. Adult fruit borers collected and reared on litchi

A. *Deudorix epijarbus* ♂

B. *Deudorix epijarbus* ♀

C. Thaumatotibia zophophanes ♂

D. Thaumatotibia zophophanes ♀

Plate 4.6 A-D. Adult fruit borers collected and reared on litchi

Table. 4.2 Species composition and relative abundance of fruit borer species collected and reared on litchi fruits during 2022 & 2023

Sl. No.	Species	No. of individuals	Percentage
1.	*Conogethes punctiferalis* (Guenée)	22	3.90
2.	*Conopomorpha sinensis* Bradley	314	55.60
3.	*Cryptophlebia ombrodelta* (Lower)	128	22. 60
4.	*Deudorix epijarbus* (Moore)	60	10.70
5.	*Thaumatotibia zophophanes* (Turner)	41	7.20
	Total	**565**	**100**

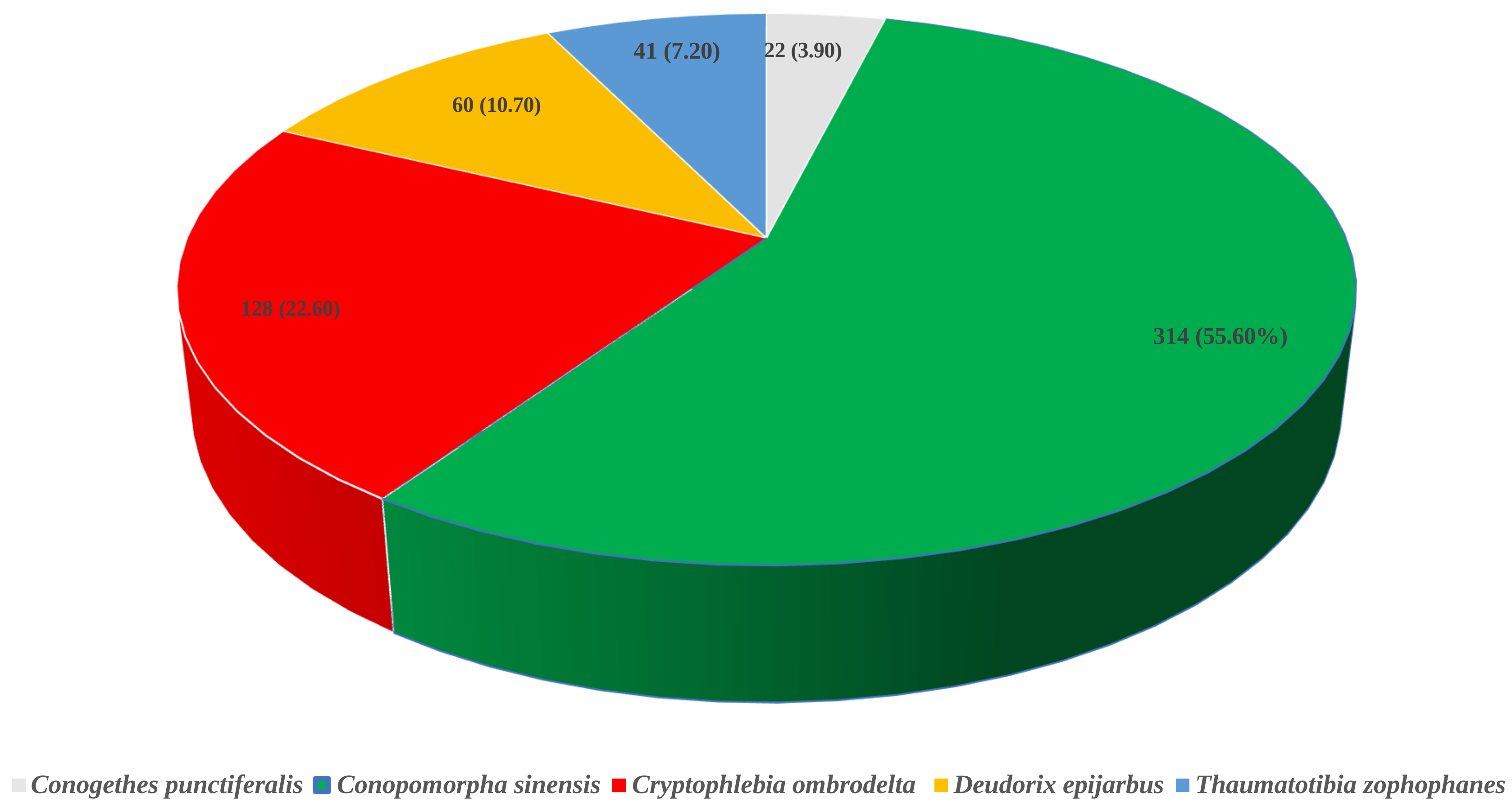


Figure. 4.1 Species composition and relative abundance of fruit borer species collected and reared on litchi fruits during 2022 & 2023

recorded 10.61, 7.25, and 3.89%, respectively (Table 4.2 & Fig 4.1).

Family Crambidae Latreille, 1810 (Plate 4.7)

Diagnosis: Crambids small to medium sized moths, characterized by three segmented labial palps, angled upward or upturned in front of face, often very long; proboscis basally scaled; maxillary palps smaller (sometimes reduced or absent), often with a flattened tuft of scales at the tip; tympanal case open with a wide antero-medial aperture, the conjunctiva and tympanum in a different plane, meets at a distinct angle; praecinctorium present; vein R_5 of the fore wing not normally stalked or fused with R_{3+4}; male genitalia without lateral arms at base of uncus; female genitalia without lobe like ovipositor.

The family Crambidae was represented by a single species, *C. punctiferalis*.

Genus *Conogethes* Meyrick, 1884

= *Conogethes* Meyrick, 1884. *Trans. R. entomol. Soc. Lond*., 1884: 293, 314.

Type species: *Astura punctiferalis* Guenée, 1854 (Type locality: Central India).

Type species: *Botys evaxalis* Walker, 1859; Type locality: India.

Diagnosis: Palpi upturned, conical, and hardly reaching vertex of head; tibia with the outer spurs less than half the length of inner, mid tibiae fringed with spinous hair on outer side.

***Conogethes punctiferalis* (Guenee, 1854) (Plate 4.11)**

= *Astura punctiferalis* Guenée, 1854: 320; *id*., 1854: 347; Walker, 1859: 548; Moore, (1866): 333; Swinhoe, 1885: 872.

Material examined: 1♂: Farmers farm, Dimapur Dist., Nagaland; 09.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: College farm, Dimapur Dist., Nagaland; 10.v.2022, reared on litchi; (coll. P. Mahesh). 1♂: College farm, Dimapur Dist., Nagaland; 04.vi.2022, reared on litchi; (coll. P. Mahesh). 1♂,

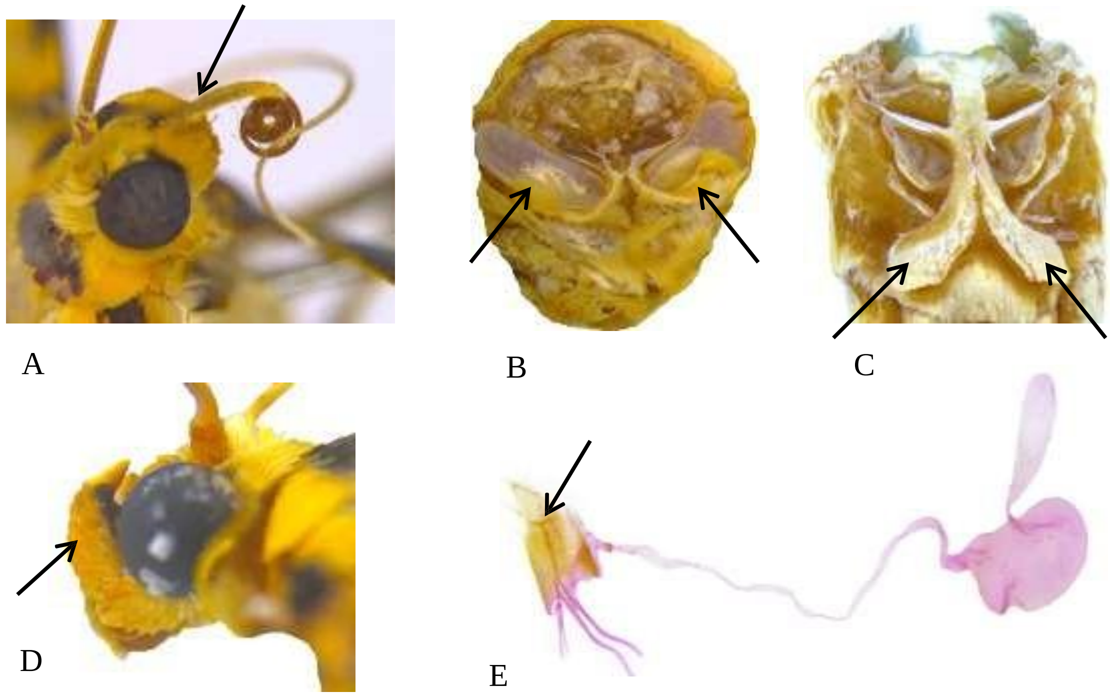


Plate 4.7. Diagnostic characters of the Family, Crambidae (Genus: *Conogethes*)

A. Basally scaled proboscis; B. tympanal case open with a wide antero-medial aperture, the conjunctiva and tympanum in a different plane; C. praecinctorium present; D. labial palpi well developed, porrect or upturned; E. in female genitalia, ovipositor lobes normal

Plate 4.8 Diagnostic characters of the Family, Gracillariidae (Genus: *Conopomorpha*)

A. adult with narrow, long, fringed, slender to lanceolate wings often with metallic markings; B. labial palpi without lateral bristles

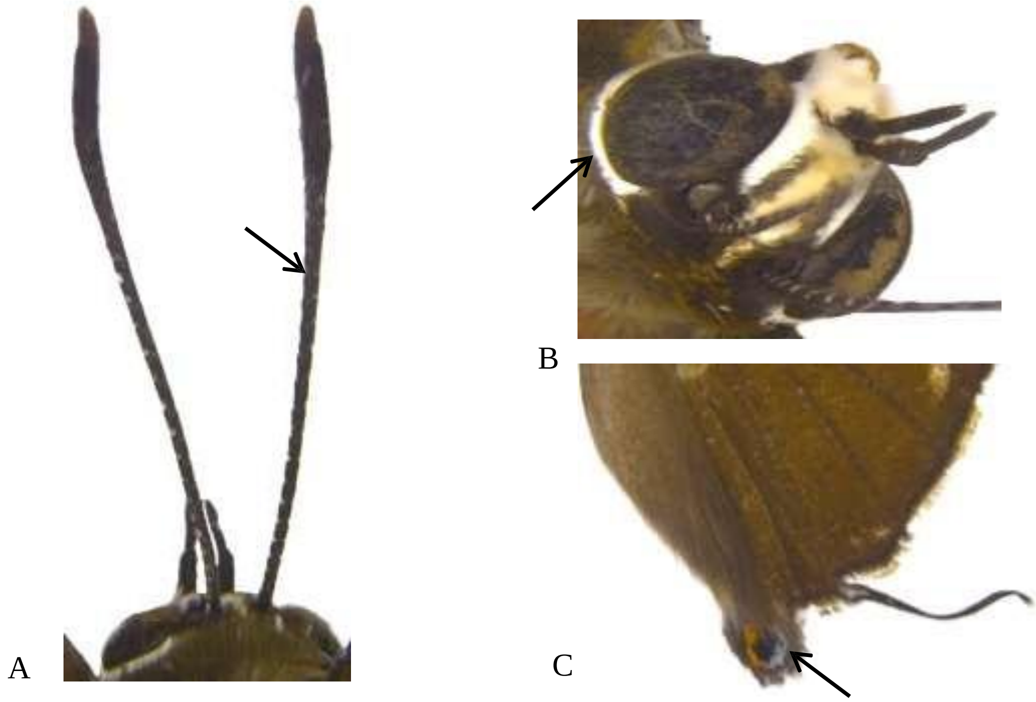


Plate 4.9. Diagnostic characters of the Family, Lycaenidae (Genus: *Deudorix*)

A. Antennae marked with alternating white and black bands; B. compound eyes indented near the base of antennae; C. rear of the hindwing with a thin tail like extending and also have a spot at the base of tail

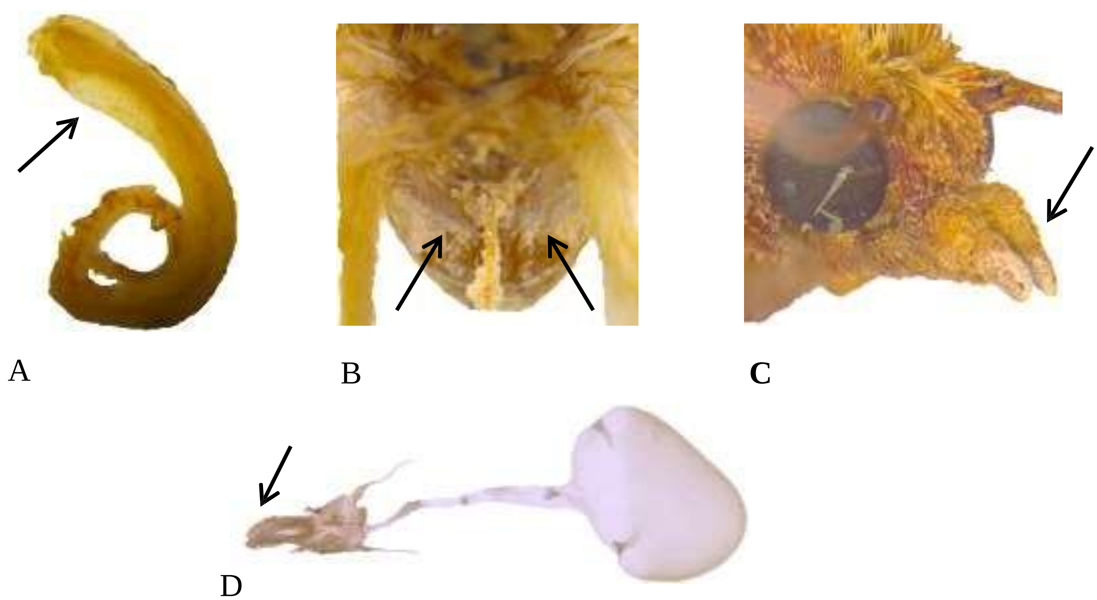


Plate 4.10 Diagnostic characters of the Family, Tortricidae (Genus: *Cryptophlebia*)

A. Naked or unscaled proboscis; B. tympanum and praecinctorium absent; C. maxillary palpi reduced and labial palpi not upturned with apical segment short and blunt; D. in female genitalia, ovipositor lobes flat-like/leaf-like

2♀: College farm, Dimapur Dist., Nagaland; 07.vi.2022, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: Farmers farm, Dimapur Dist., Nagaland; 29.vi.2022, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: Farmers farm, Dimapur Dist., Nagaland; 08.vii.2022, reared on litchi; (coll. P. Mahesh). 1♀: Farmers farm, Dimapur Dist., Nagaland; 14.vii.2022, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: College farm, Dimapur Dist., Nagaland; 05.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: Farmers farm, Dimapur Dist., Nagaland; 08.vi.2023, reared on litchi; (coll. P. Mahesh). 1♀: Farmers farm, Dimapur Dist., Nagaland; 12.vi.2023, reared on litchi; (coll. P. Mahesh).

Description: Adult bright straw-yellow coloured; labial palpi 2nd segment with a narrowly tinted black fuscous; collar and patagia spotted black; dorsal metathorax with three black spots; abdomen with series of black spots on dorsal and lateral sides; male with anal tuft of hairs, more or less black; forewing with black spot at base of costa; three sub-basal black spots and three antemedial; an oblique medial series from lower angle of cell to inner margin; a post medial sinuous series with the spots on veins 5 and 2 displaced inwards; a sub marginal series with the spot-on vein 5 displaced inwards; hindwing with disco cellular spot; a median series highly excurved between veins 2 and 5, and a sinuous sub marginal series.

Male genitalia: Uncus slender, curved ventrally, apical one third swollen and evenly covered with bifurcate setae dorsally; gnathos pointed at tip; lateral arms of tegumen narrow; saccus U-shaped; juxta narrow, elongate, tapering dorsally; valva broad, ovate at the apex and gradually narrowed towards the base; costa broad, tubular; cucullus with tuft of long hairs at distal end; clasper slim, short, sclerotized, oriented anterior ventrad; sacculus rather narrow, sclerotized and tapered with fringed hairs; aedeagus very long, slender, strongly curved, robust near the base, distally with narrow tip, vesica with a thin needle shaped cornutus of almost the length of aedeagus.

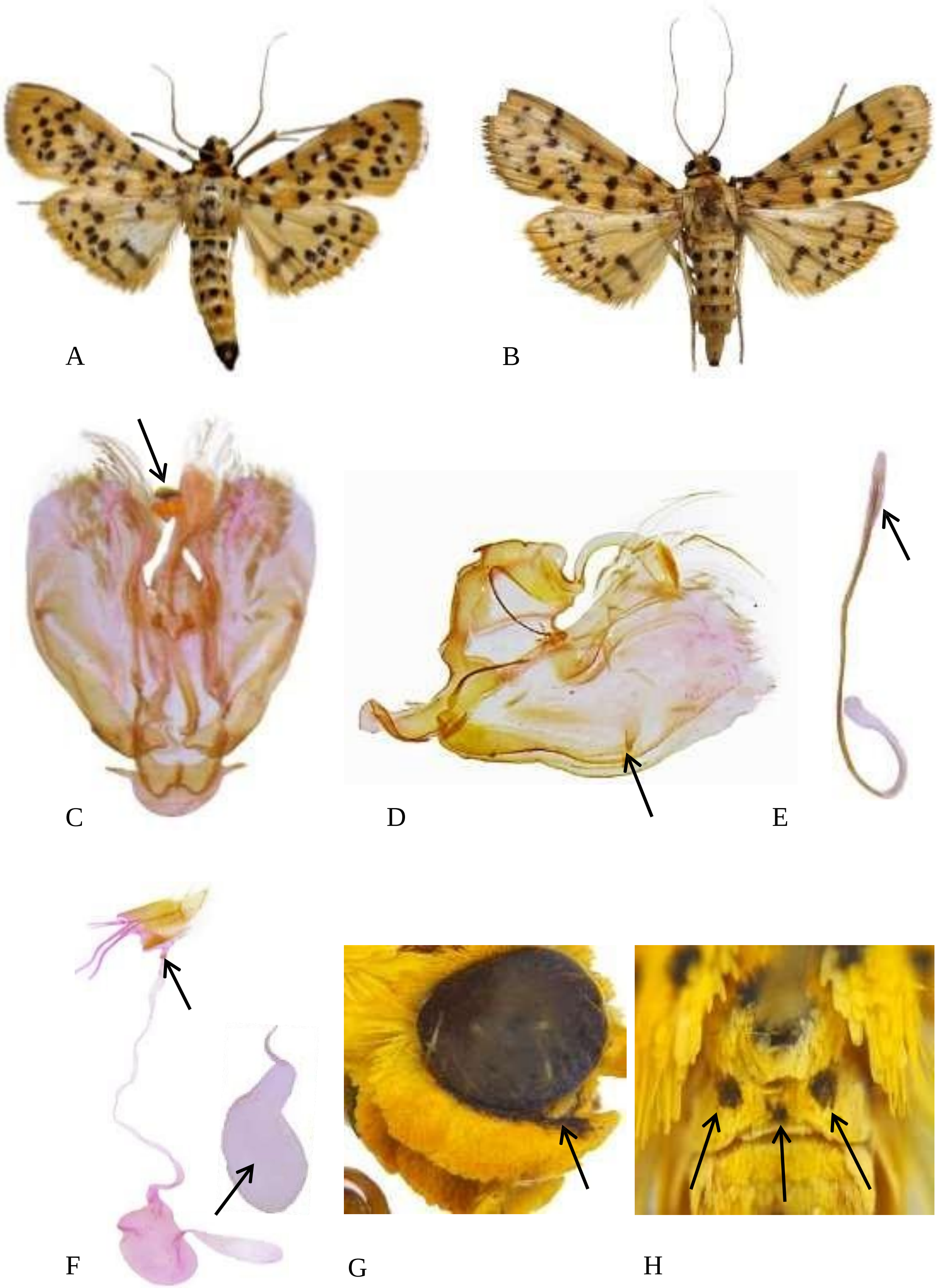


Plate 4.11 Genital and morphological characters of adult *Conogethes punctiferalis* (Guenee)

(A. male; B. female; male genitalia, C. ventral view; D. lateral view; E. aedeagus; F. female genitalia with out signum; G. labial palps black at sides; H. metathorax with three black spots.)

Female genitalia: Ovipositor triangular and covered with mixture of long and short setae; anterior apophyses about as long as posterior apophyses; ostium narrow, membranous, funnel shaped; antrum sclerotized, tubular; ductus bursae narrow, very long, membranous; ductus seminalis originates anterior to antrum; corpus bursae generally ovate, irregular in shape, posterior part granulated slightly, with a membranous appendix bursae attached laterally, signum absent.

Distribution: Throughout India (Andhra Pradesh, Assam, Haryana, Karnataka, Kerala, Maharashtra, Madhya Pradesh, Meghalaya, Punjab, Nagaland, Manipur, Tripura, Tamil Nadu and West Bengal) (Reddy and Shankaramurthy, 2021).

Remarks: The genus *Conogethes* contains several species with a huge impact on economically important crops and other plants. Currently, *Conogethes* comprises 14 recognized species (Nuss *et al*., 2003-2017) distributed in the Palearctic and Indo-Australian region (Shaffer *et al*., 1996). Among these, castor capsule borer/yellow peach moth, *C. punctiferalis* is the most notable one being polyphagous pest (Chakravarthy *et al*., 2012) recorded on 30 plants belonging to 23 plant families from India (Shashank *et al*., 2015). It is reported that *C. punctiferalis* infests 11.36% to 33.33% of litchi fruits during the first fortnight of June in Bihar (Singh and Kumar, 1992). Singh and Kaur (2015) reported about 10% infestation of litchi fruits during May to June in Punjab. The species, *C. punctiferalis* can be distinguished from the sympatric *C. sahyadriensis* by second segment of labial palpi always narrowly tinted with black fuscous whereas, broadly tinted in *C. shayadriensis* and metathorax with three black spots of scales dorsally instead of two black spots of scales in *C. sahyadriensis*. (Shashank *et al*., 2018; Pasam *et al*., 2023)

Family Gracillariidae Stainton, 1854 (Plate 4.8)

Diagnosis: Adults small sized moths, slender; ocelli, chaetosemata absent; antennae filiform, nearly as long as, or longer than forewing; vertex smooth, in few species rough scaled; maxillary palpi slender, porrect, four segmented; labial palpi upturned, three segmented; proboscis without scales; wings lanceolate to linear, usually fringed with piliform scales; in forewings, R_5 reaches to costa or apex, few veins lost, 1A+2A without basal fork; in hindwings, venation often reduced.

Genus *Conopomorpha* Meyrick, 1885

=*Conopomorpha* Meyrick, 1885. *N.Z.Jl. Sci*., 2: 592.

Type species: *Cryptophlebia cyanospila* Meyrick.

Diagnosis: Adult wingspan is 8-15.5 mm; head grey-greyish brown; frons greyish white; thorax and tegula dark brown; forewing narrow, costal and dorsal margins nearly parallel.

***Conopomorpha sinensis* Bradley, 1986 (Plate 4.12)**

Conopomorpha sinensis Bradley, 1986, *Bull. Ent. Res*., 76: 47.

Material examined: 6♂, 4♀: College farm, Dimapur Dist., Nagaland; 17.v.2022, reared on litchi; (coll. P. Mahesh). 4♀, 5♀: College farm, Dimapur Dist., Nagaland; 19.v.2022, reared on litchi; (coll. P. Mahesh). 5♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 19.v.2022, reared on litchi; (coll. P. Mahesh). 5♂, 4♀: College farm, Dimapur Dist., Nagaland; 20.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 3♀: Farmers farm, Dimapur Dist., Nagaland; 29.v.2022, reared on litchi; (coll. P. Mahesh). 3♂, 3♀: College farm, Dimapur Dist., Nagaland; 06.vi.2022, reared on litchi; (coll. P. Mahesh). 6♂, 5♀: College farm, Dimapur Dist., Nagaland; 17.vi.2022, reared on litchi; (coll. P. Mahesh). 5♂, 7♀: Farmers farm, Dimapur Dist., Nagaland; 19.vi.2022, reared on litchi; (coll. P. Mahesh). 4♂, 3♀: College farm, Dimapur Dist., Nagaland; 22.vi.2022, reared on litchi; (coll. P. Mahesh). 6♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 02.vii.2022, reared on litchi; (coll. P. Mahesh). 7♂, 4♀: College farm, Dimapur Dist., Nagaland; 19.vii.2022, reared on litchi; (coll. P. Mahesh). 4♂, 3♀: College farm, Dimapur Dist., Nagaland; 21.v.2023, reared on litchi; (coll. P. Mahesh). 8♂, 3♀: Farmers farm, Dimapur Dist., Nagaland; 21.v.2023, reared on litchi; (coll. P. Mahesh). 4♂, 3♀: College farm, Dimapur Dist., Nagaland; 22.v.2023, reared on litchi; (coll. P. Mahesh). 4♂, 6♀: Farmers farm, Dimapur Dist., Nagaland; 22.v.2023, reared on litchi; (coll. P. Mahesh). 6♂, 4♀: College farm, Dimapur Dist., Nagaland; 24.v.2023, reared on litchi; (coll. P. Mahesh). 3♂, 4♀: College farm, Dimapur Dist., Nagaland; 25.v.2023, reared on litchi; (coll. P. Mahesh). 4♀: Farmers farm, Dimapur Dist., Nagaland; 25.v.2023, reared on litchi; (coll. P. Mahesh). 8♂, 4♀: College farm, Dimapur Dist., Nagaland; 26.v.2023, reared on litchi; (coll. P. Mahesh). 6♂, 7♀: Farmers farm, Dimapur Dist., Nagaland; 26.v.2023, reared on litchi; (coll. P. Mahesh). 5♂, 3♀: College farm, Dimapur Dist., Nagaland; 27.v.2023, reared on litchi; (coll. P. Mahesh). 2♂, 6♀: College farm, Dimapur Dist., Nagaland; 28.v.2023, reared on litchi; (coll. P. Mahesh). 4♀: Farmers farm, Dimapur Dist., Nagaland; 29.v.2023, reared on litchi; (coll. P. Mahesh). 6♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 27.v.2023, reared on litchi; (coll. P. Mahesh). 3♂, 5♀: College farm, Dimapur Dist., Nagaland; 30.v.2023, reared on litchi; (coll. P. Mahesh). 8♂, 5♀: Farmers farm, Dimapur Dist., Nagaland; 30.v.2023, reared on litchi; (coll. P. Mahesh. 5♂, 4♀: College farm, Dimapur Dist., Nagaland; 31.v.2023, reared on litchi; (coll. P. Mahesh). 6♂, 8♀: College farm, Dimapur Dist., Nagaland; 1.vi.2023, reared on litchi; (coll. P. Mahesh). 3♂, 6♀: College farm, Dimapur Dist., Nagaland; 3.vi.2023, reared on litchi; (coll. P. Mahesh). 7♂: Farmers farm, Dimapur Dist., Nagaland; 4.vi.2023, reared on litchi; (coll. P. Mahesh). 4♂, 7♀: College farm, Dimapur Dist., Nagaland; 18.vi.2023, reared on litchi; (coll. P. Mahesh). 6♂, 5♀: Farmers farm, Dimapur Dist., Nagaland; 25.vi.2023, reared on litchi; (coll. P. Mahesh). 3♂: Farmers farm, Dimapur Dist., Nagaland; 19.vi.2023, reared on litchi; (coll. P. Mahesh). 4♂, 4♀: College farm, Dimapur Dist., Nagaland; 26.vi.2023, reared on litchi; (coll. P. Mahesh). 5♂, 6♀: College farm, Dimapur Dist., Nagaland; 27.vi.2023, reared on litchi; (coll. P. Mahesh).

Description: Wingspan 12-15mm in male and female; adults greyish brown with wing apex yellowish brown; head white; thorax greyish fuscous posteriorly; patagium white; labial palpi white, second segment short, scaled ventrally, third segment long, rough scaled; maxillary palpi white; antennae long, one and half-length of forewing, scape greyish fuscous dorsally; forewing with wing markings having the fifth and sixth line descending to dorsum and joining the falcate white line from tornus demarcating brown basal part of wing from pale orange-yellow apical area, 1/3rd apical part of costa with four or five white strigulae and many strigulae scattered randomly along basal part of costa, cilia grey; hindwing silver grey, darker in male, cilia grey, apex suffused white, in male entire underside from near base to apical area strongly irrorate with fine white scales, in female irrorations weaker; legs ochreous-white, fore and mid legs obliquely stripped with fuscous-black, hind leg mixed with black exteriorly; abdomen dark fuscous on dorsal side, white laterally and ventrally, with a wedge like stripes dorsally extending forward from dorsum; in male anal tuft of hairs yellowish white.

Male genitalia: Uncus tubular, membranous; valva broad basally; costa arched; cucullus narrowed distally, distal part scattered with 12-15 stout setae with a dense field of heavy setae in the costal area; tegumen highly reduced; saccus V-shaped; aedeagus long, stout, curved near the base, distal 1/3rd highly sclerotized, with a pair of heavy, sclerotized cornuti, each either trifurcate or bifurcate at the tip.

Female genitalia: Anal papillae with hairs; ostium narrow, smooth; anterior and posterior apophyses short, slightly sclerotized; ductus bursae long, stout, sclerotized at basal 1/3; corpus bursae globular shape with an elongate, dentate patch signum.

Distribution: India (Bihar, Haryana, Karnataka, Punjab and West Bengal) (Dalui and Sarkar, 2021), China (Hainan, Fujian, Guangdong), Nepal, Taiwan,

Plate 4.12 Genital and morphological characters of adult *Conopomorpha sinensis* Bradley
(A. male; B. female; male genitalia, C. dorsal view; D. ventral view; E. aedeagus; F. female genitalia; G. male abdomen; H. female abdomen; I. forewings)

Thailand and Vietnam (Bai *et al*., 2009).

Remarks: Among the fruit borers, *C. sinensis* is an important pest of litchi (Bradley, 1986; Jayanthi Mala *et al*., 2017). Earlier, many literatures (Singh, 1975; Butani, 1977; Lall and Sharma, 1978; Kumar *et al*., 2011) cited, *C. cramerella* as the fruit borer mining litchi fruits in India. However, Bradley (1986) and Jayanthi Mala *et al*. (2017) confirmed this as *C. sinensis* through morphological and genital characters. Chakraborti and Samanta (2005) and Jayanthi Mala *et al*. (2017) reported, litchi fruit borer damage was estimated at 48-74% and 48.4% in West Bengal and Karnataka, respectively. This species can be easily distinguished from *C. cramerella* and other related species by the distinctive purplish fuscous-black hindwings and also by the presence of scattered white scales on the underside. In *C. sinensis*, male genitalia with distal sacculus studded with 12-15 stout setae whereas, absent in other related species *viz*., *C. cramerella*, *C. oceanica* and *C. litchiella* (Bradley, 1986).

Family Lycaenidae Leach, 1815 (Plate 4.9)

Diagnosis: Lycaenids are small, delicate butterflies often distinctively marked with iridescent blue, red, or orange; head is relatively narrow; compound eyes may or may not be hairy, often wrap slightly around the base of antennae; antennae conspicuously marked with alternating black and white bands; maxillary palpi absent; labial palpi usually protrude forward or are slightly ascending; in males, forelegs are reduced; wing colour iridescent blue, copper, or bronze; hair streaks usually have wispy tail filaments at the back of their hindwings; sexual dimorphism is usually well defined; androconial scales usually present on upper side of male forewing.

Genus *Deudorix* Hewitson, 1863; *Illustrations of diurnal lepidoptera. Lycaenidae 1*: (i-viii), (1-228); 2: 1-95; Supplement: 1-48. London

= *Virachola* Moore, 1881 In: Moore, 1880. *The lepidoptera of Ceylon*, 1: 104

(190 pp.). London.

Type-species: *Deudorix perse* Hewitson,

Diagnosis: Adult medium to larger sized; males larger than female, palpi protruding beyond the frons, second segment long, laterally compressed, brushed with scales, third segment short, slender, acuminate; exhibits sexual dimorphism; antennae clubbed; hind wing oval, apex rounded, outer margin showing a slight salient at end of vein 3, vein 2 prolonged to form a short filiform tail, a small rounded lobe between the end of vein 1b and the anal angle, abdominal margin slightly excised between the lobe and the end of vein 1a.

***Deudorix epijarbus* (Moore, 1857) (Plate 4.13)**

= *Thecla epijarbus* Moore, 1858

= *Deudorix coriolanus* Fruhstorfer, 1912

= *Deudorix dido* Waterhouse, 1934

Material examined: 2♂, 2♀: College farm, Dimapur Dist., Nagaland; 05.v.2022, reared on litchi; (coll. P. Mahesh). 2♂: Farmers farm, Dimapur Dist., Nagaland; 05.v.2022, reared on litchi; (coll. P. Mahesh). 3♂, 1♀: College farm, Dimapur Dist., Nagaland; 06.v.2022, reared on litchi; (coll. P. Mahesh). 3♂: Farmers farm, Dimapur Dist., Nagaland; 06.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 07.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 2♀: College farm, Dimapur Dist., Nagaland; 08.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 2♀: College farm, Dimapur Dist., Nagaland; 09.v.2022, reared on litchi; (coll. P. Mahesh). 3♂, 1♀: College farm, Dimapur Dist., Nagaland; 10.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 3♀: College farm, Dimapur Dist., Nagaland; 13.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: Farmers farm, Dimapur Dist., Nagaland; 04.vi.2022, reared on litchi; (coll. P. Mahesh). 2♀: Farmers farm, Dimapur Dist., Nagaland; 15.vi.2022, reared on litchi; (coll. P. Mahesh). 2♂, 1♀: College farm, Dimapur Dist., Nagaland; 07.vii.2022, reared on litchi; (coll. P. Mahesh). 2♂: College farm, Dimapur Dist., Nagaland; 10.vii.2022, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: Farmers farm, Dimapur Dist., Nagaland; 05.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: Farmers farm, Dimapur Dist., Nagaland; 18.vi.2023, reared on litchi; (coll. P. Mahesh). 3♂, 2♀: College farm, Dimapur Dist., Nagaland; 25.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂: Farmers farm, Dimapur Dist., Nagaland; 26.v.2023, reared on litchi; (coll. P. Mahesh). 1♂: Farmers farm, Dimapur Dist., Nagaland; 27.vi.2023, reared on litchi; (coll. P. Mahesh). 2♂: Farmers farm, Dimapur Dist., Nagaland; 01.vii.2023, reared on litchi; (coll. P. Mahesh). 2♀: College farm, Dimapur Dist., Nagaland; 03.vii.2023, reared on litchi; (coll. P. Mahesh).

Description

Male: Adult is a butterfly, upper surface scarlet-red; with a wing expanse of 38 mm; Head and thorax blackish brown in colour; compound eyes well developed, edges encircled with white colour; antennae black, ringed with white, clubbed with a red tip; labial palpi 3 segmented, 1st segment small, blackish dorsally, whitish ventrally, 2nd segment large, stout, white in colour, 3rd segment small, broad, black in colour; forewing upper surface orange being outlined with a broad black band on costal and outer margins, underside brown colour with stripes, costal band with its inner margin curved; hindwing costa, base and abdominal area covered with lilackish, the abdominal fold brown, outer marginal line finely black, anal lobe black with a small red mark in it; tail black, tipped with white, the veins often more or less finely black, underside greyish-brown, markings indicated by their white edges; most of the males have tail in a vertical manner.

Female: Adult is a butterfly, upper surface fulvous-brown; with a wing expanse of 42 mm; compound eyes well developed, edges encircled with white colour; antennae black, ringed with white, clubbed with a red tip; labial palpi three segmented, 1st segment narrowly tinted with black colour, 2nd segment slightly broader than the 1st segment, 3rd segment little broader than the 2nd segment, brownish colour; forewing with fulvous suffusion below the median vein,; hindwing with the abdominal fold pale, entire wing is tinted with fulvous; tail black, tipped with white, the veins often more or less finely black, most of the females have tail in a horizontal and wavy manner.

Sexual dimorphism/Identification of sexes: Male has the tail in a vertical manner whereas in female, the tail is more of a horizontal and wavy manner. The upper side of the female is completely dark greyish brown while, the male upper surface is scarlet-red.

Male genitalia: Uncus not well developed; valva long, broad basally, pointed apically; tegumen usually developed into lateral lobes by a deep convexity, strongly dipping, with rows of setae laterally known as socii; aedeagus elongate, broad basally, blunt apically, with dense setae subapically, vesica with a group of cornuti ending in a robust apical spine.

Female genitalia: Anterior apophyses half the length of posterior apophyses; ductus bursae short, robust, sclerotized basally; bursa copulatrix round, slightly sclerotized at anterior 1/4th, transparent 3/4th, with a pointed signum laterally.

Distribution: India (Assam, Andaman and Nicobar Islands, Jammu and Kashmir, Nagaland, Sikkim), Australia, Borneo, Cambodia, China, Sumatra, Java, Laos, Borneo, Sri Lanka, China, Taiwan, Thailand, Hong Kong, Myanmar, Malaysia, Vietnam, Sri Lanka, Singapore, Fiji, Philippines, Sulawesi and Australia (Otsuka *et al*. (1991); Waite and Hwang, 2002).

Remarks: *D. epijarbus* is considered as a minor insect pest of litchi fruits in Guangdong, China (Srivastava *et al*. (2019); Waite and Hwang (2002). He (2001)), Gupta and Tara (2019) observed, larvae roll the leaves of new growth, severely damaging and destroying new growth flushes and makes holes into the young fruits and feeds on flesh and pulp.

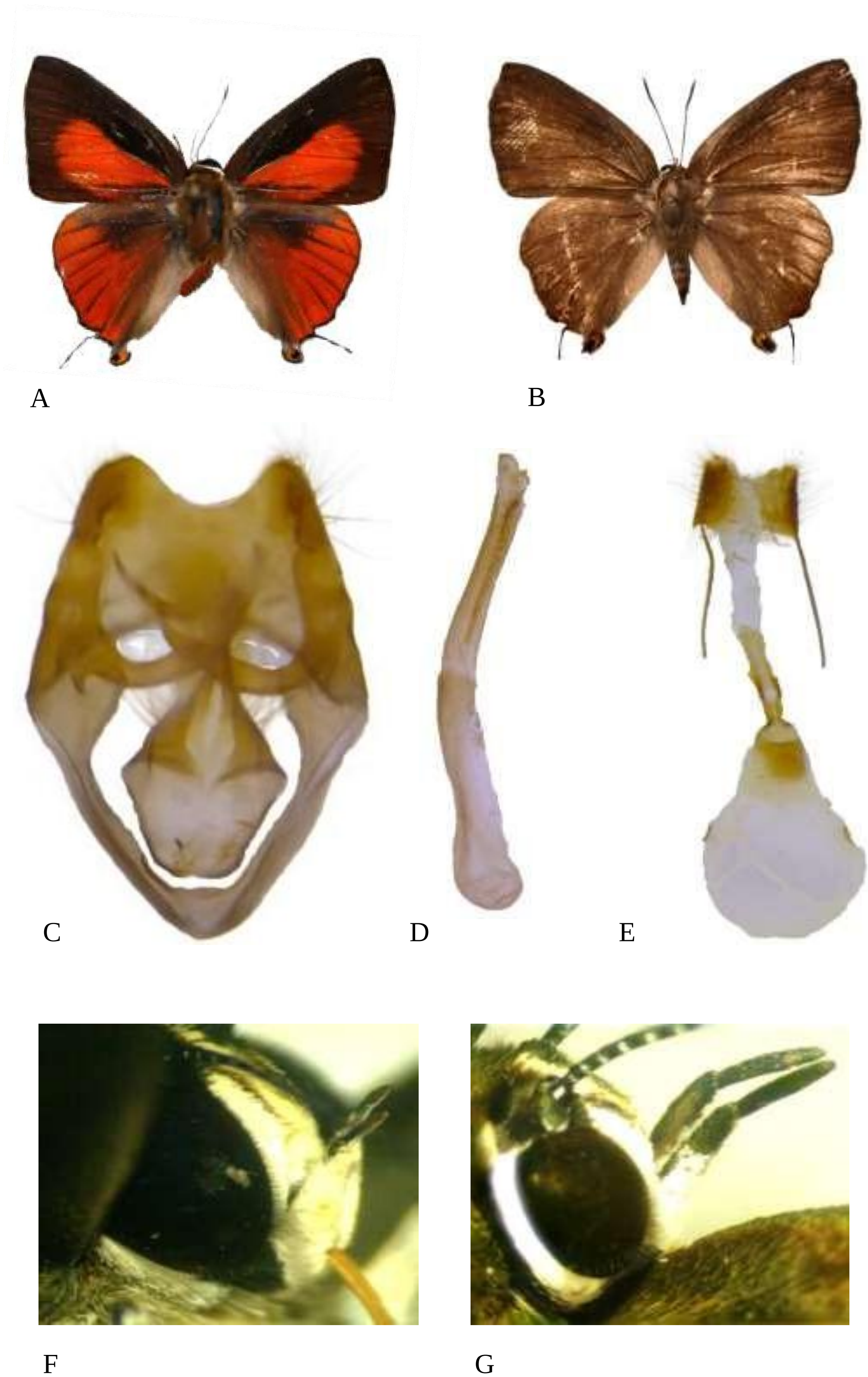


Plate 4.13 Genital and morphological characters of adult *Deudorix epijarbus* (Moore)
(A. male; B. female; male genitalia, C. dorsal view; D. aedeagus; E. female genitalia; F. male labial palpi; G. female labial palpi)

Family Tortricidae Latreille, 1803 (Plate 4.10)

Diagnosis: Adults are small brown or gray moths, characterized by head rough-scaled above; proboscis well developed, unscaled or naked; maxillary palpi reduced; labial palpi three-segmented, generally held horizontally/porrect with apical segment short, blunt; in few species, forewings tend to be curved near the apex, and when folded back, create a bell-shaped outline; hindwings with three anal veins; in female genitalia, ovipositor lobes flat/leaf-like

Genus *Cryptophlebia* Walsingham, 1900
= *Cryptophlebia* Walsingham, 1900: 105. Bradley, 1953: 682; Diakonoff, 1957: 129; Horak *et al*., 1996: 135; Komai, 1999: 56, figs. 40, 45–50, 52, 69–82, 375, 387–389; Komai & Horak, 2006: 435, figs. 108, 110, 915–926.
Type species: *Cryptophlebia perfracta* Diakonoff, 1957.

Diagnosis: Medium to small sized moths; labial palpi medially wide, with short scales; thorax with short scales; forewings broader with a subtriangular pre-tornal spot, having small accessory cell with chorda between R_2 and R_3 to between R_4 and R_5, in rare cases chorda entirely absent; hindwings with M_2 usually close to short stalk of M_3 and CuA_1, rarely distant; males with upcurved anal tuft, dorsal abdomen and hind tibia with hairs; in female genitalia, ductus bursae vary from membranous to sclerotization.

***Cryptophlebia ombrodelta* (Lower, 1898) (Plate 4.14)**
= *Arotrophora ombrodelta* Lower, 1898: 48. Bradley 1953: 682, fig. 1, pl. xxiv fig. 1, pl. xxv fig. 1, 1a (*Cryptophlebia*); Clarke, 1976: 109, fig. 47, pl. 10, figs. c, d; Horak *et al*., 1996: 135; Komai, 1999: 63, figs. 69, 70, 75, 77, 80, 81; Komai & Horak, 2006: 439, figs. 917, 918, 920, 922–925.
= *Cryptophlebia carpophaga* Walsingham, 1899: 106. Bradley 1953: 682.

Material examined: 2♂, 2♀: College farm, Dimapur Dist., Nagaland; 05.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 3♀: Farmers farm, Dimapur

Dist., Nagaland; 13.v.2022, reared on litchi; (coll. P. Mahesh). 4♂, 1♀: College farm, Dimapur Dist., Nagaland; 15.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 15.v.2022, reared on litchi; (coll. P. Mahesh). 4♂, 2♀: College farm, Dimapur Dist., Nagaland; 16.v.2022, reared on litchi; (coll. P. Mahesh). 3♂, 2♀: Farmers farm, Dimapur Dist., Nagaland; 17.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 3♀: Farmers farm, Dimapur Dist., Nagaland; 18.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 19.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 5♀: College farm, Dimapur Dist., Nagaland; 20.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 3♀: College farm, Dimapur Dist., Nagaland; 24.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 4♀: Farmers farm, Dimapur Dist., Nagaland; 25.v.2022, reared on litchi; (coll. P. Mahesh). 3♂: College farm, Dimapur Dist., Nagaland; 28.v.2022, reared on litchi; (coll. P. Mahesh). 3♂, 3♀: College farm, Dimapur Dist., Nagaland; 29.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 3♀: Farmers farm, Dimapur Dist., Nagaland; 30.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 4♀: College farm, Dimapur Dist., Nagaland; 02.vi.2022, reared on litchi; (coll. P. Mahesh). 3♂, 2♀: College farm, Dimapur Dist., Nagaland; 04.vii.2022, reared on litchi; (coll. P. Mahesh). 2♂, 2♀: Farmers farm, Dimapur Dist., Nagaland; 08.vii.2022, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: Farmers farm, Dimapur Dist., Nagaland; 25.v.2023, reared on litchi; (coll. P. Mahesh). 3♂, 1♀: College farm, Dimapur Dist., Nagaland; 27.v.2023, reared on litchi; (coll. P. Mahesh). 3♂, 4♀: College farm, Dimapur Dist., Nagaland; 12.vi.2023, reared on litchi; (coll. P. Mahesh). 3♂: Farmers farm, Dimapur Dist., Nagaland; 12.vi.2023, reared on litchi; (coll. P. Mahesh). 2♂, 1♀: College farm, Dimapur Dist., Nagaland; 18.vi.2023, reared on litchi; (coll. P. Mahesh). 2♀: College farm, Dimapur Dist., Nagaland; 22.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 5♀: College farm, Dimapur Dist., Nagaland; 25.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: College farm, Dimapur Dist., Nagaland; 28.vi.2023, reared on litchi; (coll. P. Mahesh). 3♂, 2♀: College farm, Dimapur Dist., Nagaland; 30.vi.2023, reared on litchi; (coll. P. Mahesh). 2♂, 1♀: College farm, Dimapur Dist., Nagaland; 01.vii.2023, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: Farmers farm, Dimapur Dist., Nagaland; 03.vii.2023, reared on litchi; (coll. P. Mahesh).

Description

Male: Wingspan 15-21mm; head, labial palpi, maxillary palpi, antennae light pinkish brown; thorax darker grayish; abdomen have sex scales dorsally; foreleg and midleg reddish brown, hindleg whitish cream, hind tibia greatly modified, covered with sex scales; forewing moderately wide, pale brown with dark brown streaks in males, dark brown pre-tornal spot faded; hindwing small, barely triangular, whitish near the base, upper surface covered with pocket like sex scales.

Female: Adult wingspan 15-23mm; head, antennae, labial palpi, maxillary palpi, thorax reddish brown in colour; foreleg and midleg reddish brown, hindleg reddish brown to sliver grayish; forewing moderately wide, coloring from pinkish grey to brown with a costal fold, dark brown pre-tornal spot is distinctive; hindwing greyish brown.

Male genitalia: Valva greatly inflated, gradually widened towards apex; costa broad; cucullus dome shaped on outer surface, with three strong marginal spines with distal one closer to ventral one and tuft of hairs on distal end; gnathos small, hook shaped; saccus V-shaped; juxta inverse-trapezoidal; tegumen broad, semi-triangular on upper 1/3, rectangular on lower 2/3; aedeagus long, curved, robust in basal 1/3rd, narrowed gradually in the middle, vesica with a dense, elongate patch of diagonally arranged cornuti.

Female genitalia: Anal papillae broadened dorsally; sterigma narrow, V-shaped, having complete sclerotized ring below ostium; ostium slightly sclerotized laterally; ductus bursae slender at basal 1/3, gradually broadened

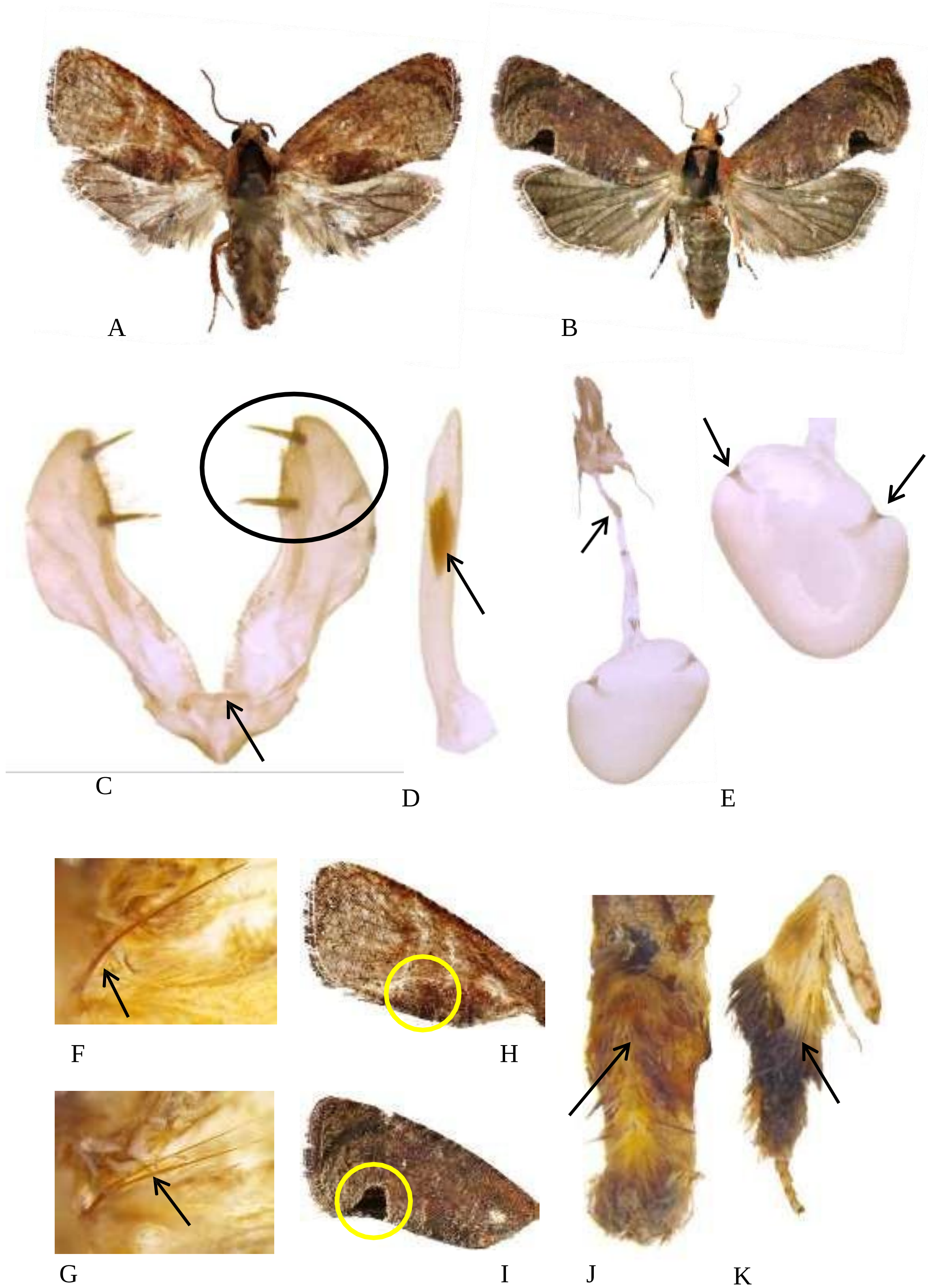


Plate 4.14 Genital and morphological characters of adult *Cryptophlebia ombrodelta* (Lower)
(A. male; B. female; male genitalia, C. dorsal view; D. aedeagus; E. female genitalia with 2 signa in corpus bursae; F. male with single frenulum; G. female with double frenulum; H. in male, dark brown pre-tornal spot is faded; I. in female dark brown pre-tornal spot is distinctive; J. male possess sex scales on abdomen; K. also, male possess sex scales on hind tibia)

towards corpus bursae; corpus bursae globular, anterior 2/3 strongly granulated, with two symmetrical signa, each signum large, sclerotized, curved-like.

Distribution: India (Chhattisgarh, Nagaland, Odisha, West Bengal), Australia, Borneo, Brunei, Caroline Islands, China, Fiji, Gaum, Hawaii, Indonesia, Japan, Korea, Malaysia, Micronesia, Nepal, New Guinea, Philippines, Russia, Sri Lanka, Sumatra Islands, Taiwan, Thailand and Vietnam (Bradley, 1953; Clarke, 1976; Zimmerman, 1978; Jones, 1995; Komai, 1999; Patel, 2016; Singh, 2014; Sohn *et al*., 2016; Pathania *et al*., 2020; Patel *et al*., 2022).

Remarks: The genus *Cryptophlebia*, comprises about 53 species predominantly distributing in Indo-Pacific region (Komai, 2013). Among these, *C. ombrodelta*, commonly known as macadamia nut borer is a serious pest of litchi (Bradley, 1953; Common, 1990). These moths are strongly sexually dimorphic. Adults of *C*. *ombrodelta* are often gets confused with adults of *C. illepida*. These species can be separated only based on genital characters. In *C*. *ombrodelta*, male genitalia valva with three large spines and in female genitalia, sterigma narrow, V-shaped. While in *C*. *illepida*, male genitalia valva with two large spines and in female genitalia, sterigma wide, V-shaped and separate (Bradley, 1953; Horak and Komai, 2016).

Genus *Thaumatotibia* Zacher, 1915

= *Thaumatotibia* Zacher, 1915: 529. Heppner, 1980: 334 (synonymized with *Cryptophlebia*); Horak *et al*. 1996: 135 (as synonym of *Cryptophlebia*); Komai & Horak, 2006: 427, figs. 106, 107, 894–904.

Type species: *Thaumatotibia roerigii* Zacher, 1915 (=*Argyroploce leucotreta* Meyrick, 1913)

Type species: *Eucosma chaomorpha* Meyrick, 1929.

Diagnosis: Small to medium sized, grey brown to blackish brown moths; labial palpi wide with short scales; forewings with a rudimentary M-stem and entirely lacking accessory cell, with a chorda usually coincident with the margin of the discal cell, just before R_3 to R_4, R_3, R_4 and R_5 are approximated at base, R_4 is either equidistant from R_3, R_5 or variable from equidistant to connate to short stalked with R_3; hindwing with M_2 moderately to closely approximated to stalk of M_3 and CuA_1 at base.

Thaumatotibia zophophanes **(Turner, 1946) (Plate 4.15)**

= *Argyroploce zophophanes* Turner, 1946: 217. Horak *et al*., 1996: 135 (*Cryptophlebia*); Komai, 1999: 55, fig. 386 (pupa); Komai & Horak, 2006: 430, figs. 106, 894, 895, 898, 899, 902 (as *Thaumatotibia zophophanes*).

= *Articolla scioessa* Turner, 1946: 218. Horak *et al*. 1996: 135 (*Cryptophlebia*); Komai & Horak 2006: 430 (synonymized with *zophophanes*)

Material examined: 2♂: College farm, Dimapur Dist., Nagaland; 17.v.2022, reared on litchi; (coll. P. Mahesh). 1♂: College farm, Dimapur Dist., Nagaland; 19.v.2022, reared on litchi; (coll. P. Mahesh). 1♂: College farm, Dimapur Dist., Nagaland; 20.v.2022, reared on litchi; (coll. P. Mahesh). 2♂, 1♀: College farm, Dimapur Dist., Nagaland; 22.v.2022, reared on litchi; (coll. P. Mahesh). 1♀: College farm, Dimapur Dist., Nagaland; 23.v.2022, reared on litchi; (coll. P. Mahesh). 1♀: College farm, Dimapur Dist., Nagaland; 25.v.2022, reared on litchi; (coll. P. Mahesh). 1♂: Farmers farm, Dimapur Dist., Nagaland; 26.v.2022, reared on litchi; (coll. P. Mahesh). 2♂: College farm, Dimapur Dist., Nagaland; 27.v.2022, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: College farm, Dimapur Dist., Nagaland; 29.v.2022, reared on litchi; (coll. P. Mahesh). 1♀: Farmers farm, Dimapur Dist., Nagaland; 05.vi.2022, reared on litchi; (coll. P. Mahesh). 2♂: Farmers farm, Dimapur Dist., Nagaland; 11.vi.2022, reared on litchi; (coll. P. Mahesh). 1♂: Farmers farm, Dimapur Dist., Nagaland; 14.vi.2022, reared on litchi; (coll. P. Mahesh). 2♀: Farmers farm, Dimapur Dist., Nagaland; 26.vi.2022, reared on litchi; (coll. P. Mahesh). 2♂, 3♀: College farm, Dimapur Dist., Nagaland; 12.v.2023, reared on litchi; (coll. P. Mahesh). 1♂:

College farm, Dimapur Dist., Nagaland; 12.v.2023, reared on litchi; (coll. P. Mahesh). 1♀: Farmers farm, Dimapur Dist., Nagaland; 24.v.2023, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: Farmers farm, Dimapur Dist., Nagaland; 05.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 1♀: College farm, Dimapur Dist., Nagaland; 18.vi.2023, reared on litchi; (coll. P. Mahesh). 1♀: Farmers farm, Dimapur Dist., Nagaland; 20.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂: Farmers farm, Dimapur Dist., Nagaland; 20.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: College farm, Dimapur Dist., Nagaland; 20.vi.2023, reared on litchi; (coll. P. Mahesh). 2♀: Farmers farm, Dimapur Dist., Nagaland; 22.vi.2023, reared on litchi; (coll. P. Mahesh). 1♂, 2♀: Farmers farm, Dimapur Dist., Nagaland; 20.vi.2023, reared on litchi; (coll. P. Mahesh).

Description: Adult wingspan 13-16mm; male smaller than the female; head and thorax red to blackish brown; thorax with very loose, upcurved paddle shaped grey scales; in male, abdomen greyish brown with anal tuft of hairs; legs blackish brown with tiny white rings on tarsal segments except in hindlegs; abdomen greyish brown; forewing subtriangular, moderately wide, grey-reddish brown with silvery scales, female, forewings have reddish brown studded bands in the costal region; hindwing brownish grey to grey.

Male genitalia: Valva elongate, ovate, wide distally but dilated; uncus sharp, bipartite; gnathos weakly scleortized; tegumen wide, weakly sclerotized; cucullus outer surface slightly rounded, inner surface with a wide band of strong, long spines around distal margin; juxta diamond shaped, basal lobes rounded, small and coarsely spinulose; aedeagus long, sclerotized slightly, evenly sinuate, bulged near the base, narrowly tapered towards tip; vesica membranous with 2–4 small, longitudinal cornuti.

Female genitalia: Anterior and posterior apophyses short; ductus bursae narrow, short, less than the half-length of corpus bursae, with a long ring-shaped sclerite anterior to ostium and a small lateral sclerite at 1/3 from ostium; corpus

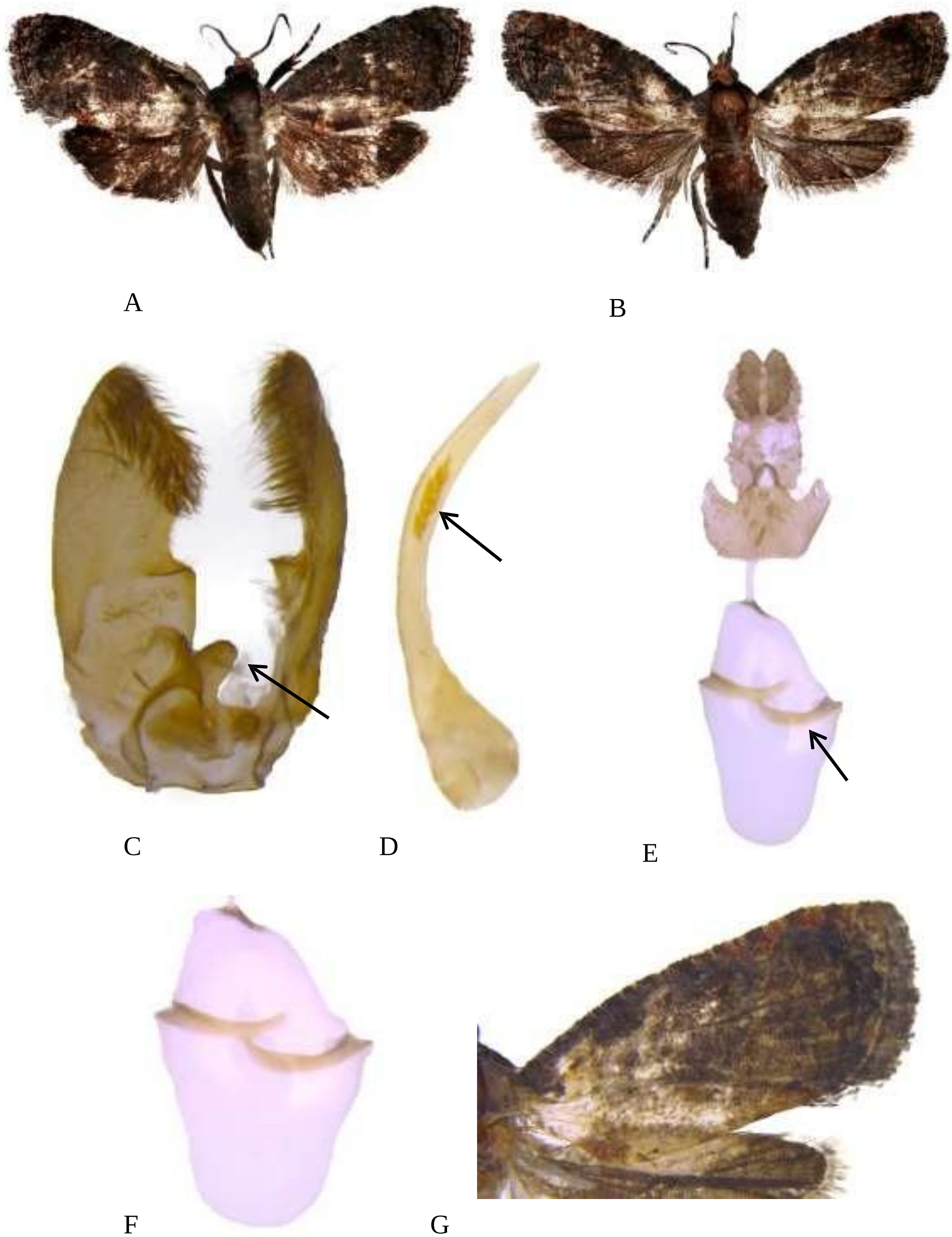


Plate 4.15 Genital and morphological characters of adult *Thaumatotibia zophophanes* (Turner)

(A. male; B. female; male genitalia C. dorsal view; D. aedeagus; E. female genitalia with two signa; F. close-up view of bursa copulatrix; G. female forewings) bursae ovate, with a band of coarse spinules near the entrance, with two long, slender, curved and pointed horn-shaped signa present in the anterior portion.

Distribution: India (Nagaland (present thesis)), Australia (Horak and Komai, 2016); Fiji Islands (Razowski, 2016), Papua New Guinea (Reynolds *et al*., 2019).

Remarks: This is the new record of *T. zophophanes* feeding on litchi fruits from India. Horak and Komai (2016) first reported, *T. zophophanes* on macadamia (Proteaceae) and subsequently, on avocado (Lauraceae). Further, they reared on the fruits of lemon aspen, *Acronychia acidula* F. In the same year, Reynolds *et al*. (2019) reported *T. zophophanes* infesting cocoa fruits in Papua New Guinea. The species, *T. zophophanes* is easily distinguished from *T. aclyta* and *T. maculata* by its forewings being dark without clearly paler distal portion and in the male, hindwings grey and abdomen with an anal tuft. In male genitalia, uncus bipartite whereas, absent in *T. aclyta* and *T. maculata*. While, in female genitalia, *T. zophophanes* can be easily recognized by ductus bursae being narrow, rather than anteriorly widened in *T. aclyta* and *T. maculata* (Horak and Komai, 2016).

4.1.1.1 An illustrated key to the families of adult litchi fruit borer(s) based on morphological and genital characters

1. Adult is a butterfly; eyes well developed with a border of dense white scales, indented near the base of antennae; antennae marked with alternating white and black bands; forelegs not well developed; rear of the hindwing with a thin tail like extending and also have a spot at the base of the tail; in male genitalia, uncus not well defined; tegumen broad, usually developed into lateral lobes by a deep distal convexity, at the base where spine like processes called side lobes or socii present............................ **Lycaenidae Leach, 1815**

-. Adult is a moth; eyes well developed without a border of dense white scales, not indented near the base of antennae; antennae without alternating white and black bands; forelegs well developed; rear of the hindwing without a thin tail like extending and do not have a spot at the base of the tail; in male genitalia, uncus well defined; tegumen broad, usually not developed into lateral lobes **2**

2. Adults with narrow, long, fringed, slender to lanceolate wings often with metallic markings; labial palpi upturned without lateral bristles; fore and hind wings highly reduced; in male genitalia, uncus rudimentary; valva simple; gnathos absent .... **Gracillariidae Stainton, 1854**

-. Adults with wide, short, membranous wings often with gray to reddish brown in colour without metallic markings; labial palpi porrect with lateral bristles; fore and hind wings well developed; in male genitalia, uncus complex; valva flap like, sometimes feather like with curved spines or blade like or rarely divided; gnathos present **3**

3. Proboscis naked or unscaled; tympanum and praecinctorium absent; maxillary palpi look like missing; labial palpi not upturned; in male genitalia, valva flap like or feather like; uncus long and straight; gnathos with central lip curved; aedeagus with cornuti variable shapes; in female genitalia ovipositor lobes leaf like… .... **Tortricidae Latreille, 1803**

-. Basally scaled proboscis; tympanum and praecinctorium present; maxillary palpi well developed; labial palpi porrect or upturned; in male genitalia, valva blade like or divided; uncus short and broad; gnathos with central lip curved or pointed, sometimes turned up dorsally; aedeagus with cornuti variable shapes; in female genitalia ovipositor lobes normal; …... **Crambidae Latreille, 1810**

4.1.1.2 An illustrated key to the species of adult litchi fruit borer(s) based on morphological and genital characters

1. Adult large sized butterfly; eyes with a border of white scales; antenna club-shaped with a long shaft and a bulb at the end, with whitish rings in between the segments; labial palpi protrudes forward; wings without frenulum; in male genitalia, tegumen narrow, usually developed into lateral lobes by a deep convexity, strongly dipping, with rows of setae laterally known as socii; in female genitalia, corpus bursae round. .... ***Deudorix epijarbus***

-. Adult medium to small sized moth; eyes without a border of white scales; antennae filiform without any whitish rings; labial palpi porrect or upturned; wings with a frenulum; in male genitalia, tegumen broad, not usually developed into lateral lobes; in female genitalia, corpus bursae either globular or ovate **2**

2. Adult small sized, greyish brown, with a narrow, long, fringed, slender to lanceolate wings often with metallic markings; labial palpi upturned; in male genitalia, gnathos absent; tegumen highly reduced; in aedeagus, vesica with either bifurcate or trifurcate cornuti; in female genitalia, corpus bursae globular with a dentate patch signum ....***Conopomorpha sinensis***

-. Adult medium sized, with a moderately wide, membranous wing without any metallic markings; labial palpi porrect or straight; in male genitalia, gnathos present; tegumen well developed; in aedeagus, vesica with cornuti of either needle, elongate patch or longitudinal shaped; in female genitalia, corpus bursae ovate with or without signum…..**3**

3. Adult pale straw yellow with numerous small black spots on their body; ocelli absent; proboscis basally scaled; labial palpi second segment narrowly tinted with a black fuscous; dorsal metathorax with three black spots; in male genitalia, uncus well developed; juxta narrow, elongate, tapering dorsally; cucullus with tuft of long hairs at distal end; aedeagus very long, slender, strongly curved near the base; in female genitalia, corpus bursae without

signum ....***Conogethes punctiferalis***

-. Adult reddish brown to light pinkish or blackish brown in colour with a brown pre-tornal spot in forewing; ocelli present; proboscis naked or unscaled; labial palpi medially widened with short scales; dorsal metathorax without any black spots; in male genitalia, uncus reduced or absent; juxta inverse, trapezoidal or diamond shaped; cucullus outer surface doom shaped or rounded; aedeagus long, stout, not curved near the base; in female genitalia, corpus bursae with two signa in anterior portion… **4**

4. Adult reddish brown to light pinkish in colour; presence of a distinct sub-triangular pre-tornal spot in costal forewing; strongly sexually dimorphic; in male genitalia, juxta inverse-trapezoidal; cucullus dome shaped on outer surface with three strong marginal spines with distal one closer to ventral one; in aedeagus, vesica with a dense elongate patch of diagonally arranged cornuti; in female genitalia, corpus bursae with two symmetrical, short, sclerotized, curved like signa… .... ***Cryptophlebia ombrodelta***

-. Adult blackish brown to greyish brown in colour; sub-triangular pre-tornal spot is not distinct in costal forewing; not sexually dimorphic; in male genitalia, juxta diamond shaped; cucullus rounded on outer surface; in aedeagus, vesica with two-four small, longitudinal cornuti; in female genitalia, corpus bursae with two symmetrical, long, slender, sclerotized, pointed, horny like signa ....***Thaumatotibia zophophanes***

In the previous studies also, similar results were obtained by Bradley (1953); Bradley (1986); Jayanthi Mala *et al*. (2017); Shashank *et al*. (2018); and Pasam *et al*. (2023).

4.1.2 DNA barcoding of litchi fruit borer(s)

DNA barcoding is based on a standardized region of mitochondrial genome (648bp of cytochrome *c* oxidase subunit one gene, *COI*). The main purpose of barcoding is the identification of previously described species by comparing a barcode sequence from a specimen to the extensive reference database. It has become a very efficient tool for discovering hidden diversity (*i.e*., cryptic species) and clarifying challenging species complexes. It also enables identification of broken specimens, tissue samples, and various life stages where appropriate morphological characters are lacking and provides an additional tool for examining type specimens.

DNA sequences of mitochondrial genes have been widely used to infer species-level phylogenies, due to the ease of polymerase chain reaction (PCR) amplification and due to maternal inheritance. Several studies have used mtDNA sequences to investigate the phylogenetic relationships of certain groups of butterflies and moths. Of the identified mitochondrial genes, the cytochrome c oxidase subunit I (COI) region has been used commonly, to identify various organisms. In the present study, an attempt was made to compare a barcode sequence from reference database for identified/described species of fruit borers of litchi collected and reared from litchi orchards of Medziphema, Nagaland.

4.1.2.1 Molecular identification

During the study, the genomic DNA was isolated from the legs of five samples of adult litchi fruit borers. Further, the samples were analyzed through nanodrop spectrophotometer, showed that the DNA sample A260/A280 ratios ranged from 1.80 to 1.90 indicating the DNA fraction was pure and could be used for further downstream applications. The yields of genomic DNA ranges from 15.70 to 31.41 ng/µl (Table 4.3).

After quantification, DNA samples were utilized for polymerase chain reaction (PCR) employing LCO-1490 and HCO-2198 mtCOI primers. Among five samples, three samples were amplified and the rest two samples were not amplified. The amplified samples were *Conogethes punctiferalis, Cryptophlebia ombrodelta,* and *Thaumatotibia zophophanes*. Whereas, the non-amplified samples were *Conopomorpha sinensis* and *Deudorix epijarbus*. The fresh specimens of non-amplified samples were again utilized for DNA isolation and PCR amplification by utilizing the similar mtCOI primers. The samples amplified were *Conopomorpha sinensis* and *Deudorix epijarbus*.

4.1.2.2 Sequence comparison and phylogenetic analysis

The raw sequences that were received from Eurofins Genomics India Private Limited, Bengaluru were compared with the BOLD reference database using bioinformatics tools such as NCBI-BLAST. The complete datasets were aligned employing sequence alignment editor, Bio Edit v.7.0.5.3. The compared sequences have shown 97 to 100% similarity, as sequences available in database (Figs 4.2-4.19). Reliability of the clustering pattern in the tree was determined by using the bootstrap test with 1000 replications employing MEGA 11.0.

> LCO1490 forward

5’-GGTCAACAAATCATAAAGATATTGG-3’

Reverse complement 5’-CCAATATCTTTATGATTTGTTGACC-3’

>HCO2198 reverse

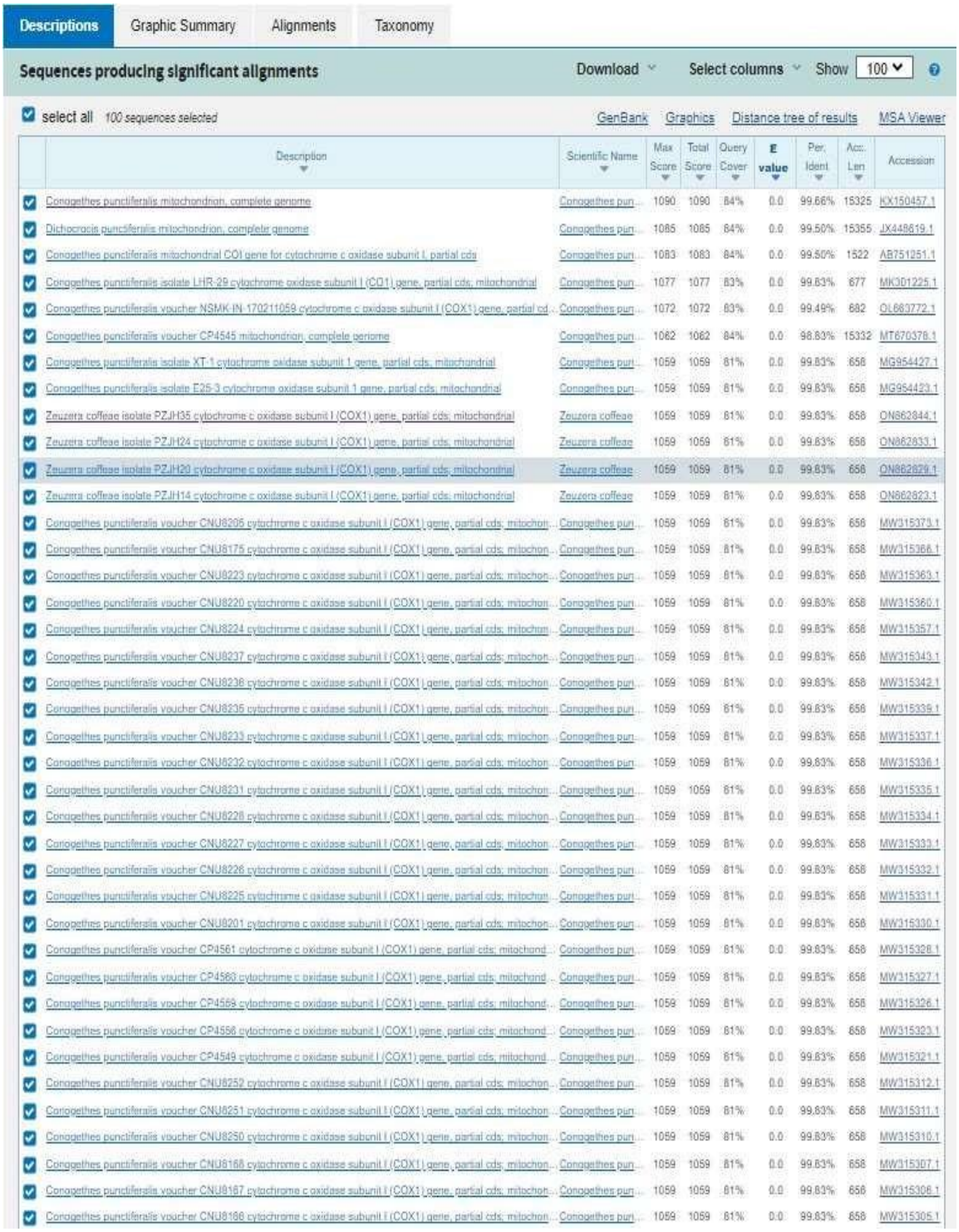
5’-TAAACTTCAGGGTGACCAAAAAATCA-3’

Reverse complement 5’-TGATTTTTTGGTCACCCTGAAGTTTA-3’

***Conogethes punctiferalis* (Guenée)**

GGTCAACAAATCATAAAGATATTGG-Forward primer

TAGCAAAGGGGATGATCCCGTGGAGTGTACGTGGTCACGTAGCAT
ATTATTTTGGTGATTATTTGGTCCTAAATTAGGGAACCCGGGATCA
TAATTGGAGATGATCAAATTTATAATACAATTGTAACAGCTCAGGC
TTTATTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGTTT



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Conogethes punctiferalis mitochondrion, complete genome	Conogethes pun...	1090	1090	84%	0.0	99.66%	15325	KX150457.1
Dichocrocis punctiferalis mitochondrion, complete genome	Conogethes pun...	1085	1085	84%	0.0	99.50%	15355	JX448619.1
Conogethes punctiferalis mitochondrial COI gene for cytochrome c oxidase subunit I, partial cds	Conogethes pun...	1083	1083	84%	0.0	99.50%	1522	AB751251.1
Conogethes punctiferalis isolate LHR-29 cytochrome oxidase subunit I (CO1) gene, partial cds; mitochondrial	Conogethes pun...	1077	1077	83%	0.0	99.83%	677	MK301225.1
Conogethes punctiferalis voucher NSMK-IN-170211059 cytochrome c oxidase subunit I (COX1) gene, partial cd...	Conogethes pun...	1072	1072	83%	0.0	99.49%	682	OL683772.1
Conogethes punctiferalis voucher CP4545 mitochondrion, complete genome	Conogethes pun...	1062	1062	84%	0.0	98.83%	15332	MT670378.1
Conogethes punctiferalis isolate XT-1 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MG954427.1
Conogethes punctiferalis isolate E25-3 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MG954423.1
Zeuzera coffeae isolate PZJH35 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Zeuzera coffeae	1059	1059	81%	0.0	99.83%	658	ON862844.1
Zeuzera coffeae isolate PZJH24 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Zeuzera coffeae	1059	1059	81%	0.0	99.83%	658	ON862833.1
Zeuzera coffeae isolate PZJH20 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Zeuzera coffeae	1059	1059	81%	0.0	99.83%	658	ON862829.1
Zeuzera coffeae isolate PZJH14 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Zeuzera coffeae	1059	1059	81%	0.0	99.83%	658	ON862823.1
Conogethes punctiferalis voucher CNU8205 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315373.1
Conogethes punctiferalis voucher CNU8175 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315366.1
Conogethes punctiferalis voucher CNU8223 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315363.1
Conogethes punctiferalis voucher CNU8220 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315360.1
Conogethes punctiferalis voucher CNU8224 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315357.1
Conogethes punctiferalis voucher CNU8237 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315343.1
Conogethes punctiferalis voucher CNU8236 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315342.1
Conogethes punctiferalis voucher CNU8235 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315339.1
Conogethes punctiferalis voucher CNU8233 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315337.1
Conogethes punctiferalis voucher CNU8232 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315336.1
Conogethes punctiferalis voucher CNU8231 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315335.1
Conogethes punctiferalis voucher CNU8228 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315334.1
Conogethes punctiferalis voucher CNU8227 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315333.1
Conogethes punctiferalis voucher CNU8226 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315332.1
Conogethes punctiferalis voucher CNU8225 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315331.1
Conogethes punctiferalis voucher CNU8201 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315330.1
Conogethes punctiferalis voucher CP4561 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochond...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315328.1
Conogethes punctiferalis voucher CP4560 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochond...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315327.1
Conogethes punctiferalis voucher CP4559 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochond...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315326.1
Conogethes punctiferalis voucher CP4556 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochond...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315323.1
Conogethes punctiferalis voucher CP4549 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochond...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315321.1
Conogethes punctiferalis voucher CNU8252 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315312.1
Conogethes punctiferalis voucher CNU8251 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315311.1
Conogethes punctiferalis voucher CNU8250 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315310.1
Conogethes punctiferalis voucher CNU8168 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315307.1
Conogethes punctiferalis voucher CNU8167 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315306.1
Conogethes punctiferalis voucher CNU8166 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Conogethes pun...	1059	1059	81%	0.0	99.83%	658	MW315305.1

Figure 4.2 NCBI nucleotide BLASTn search results showing submitted sequence has been matched to *Conogethes punctiferalis* (Guenee) with 99.66% similarity COI meta barcoding gene. This identification is solid.

Results Summary Download

Query ID	Best ID	Search DB	Tree	Top %	Graph	Low %
unlabeled sequence	Conogethes punctiferalis	COI FULL DATABASE (includes records without species designation)		98.82		98.17

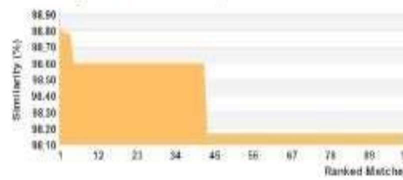
Query: unlabeled_sequence
Top Hit: Arthropoda, Insecta, Lepidoptera, Crambidae, Conogethes, Conogethes punctiferalis (98.82%)

Search Result:

Request Type: COI FULL DATABASE (includes records without species designation)

TREE BASED IDENTIFICATION

Similarity scores of the top 100 matches



Top 20 Matches

Display option: Top 20

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.82	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.79	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.79	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.78	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Crambidae	*Conogethes*	*punctiferalis*		98.6	Published

Sampling Sites For Top Hits (>98% Match)

Figure 4.3 Barcode of life database (BOLD) search results showing submitted sequence has been matched to *Conogethes punctiferalis* (Guenee) with 98.82% similarity COI meta barcoding gene. This identification is solid.

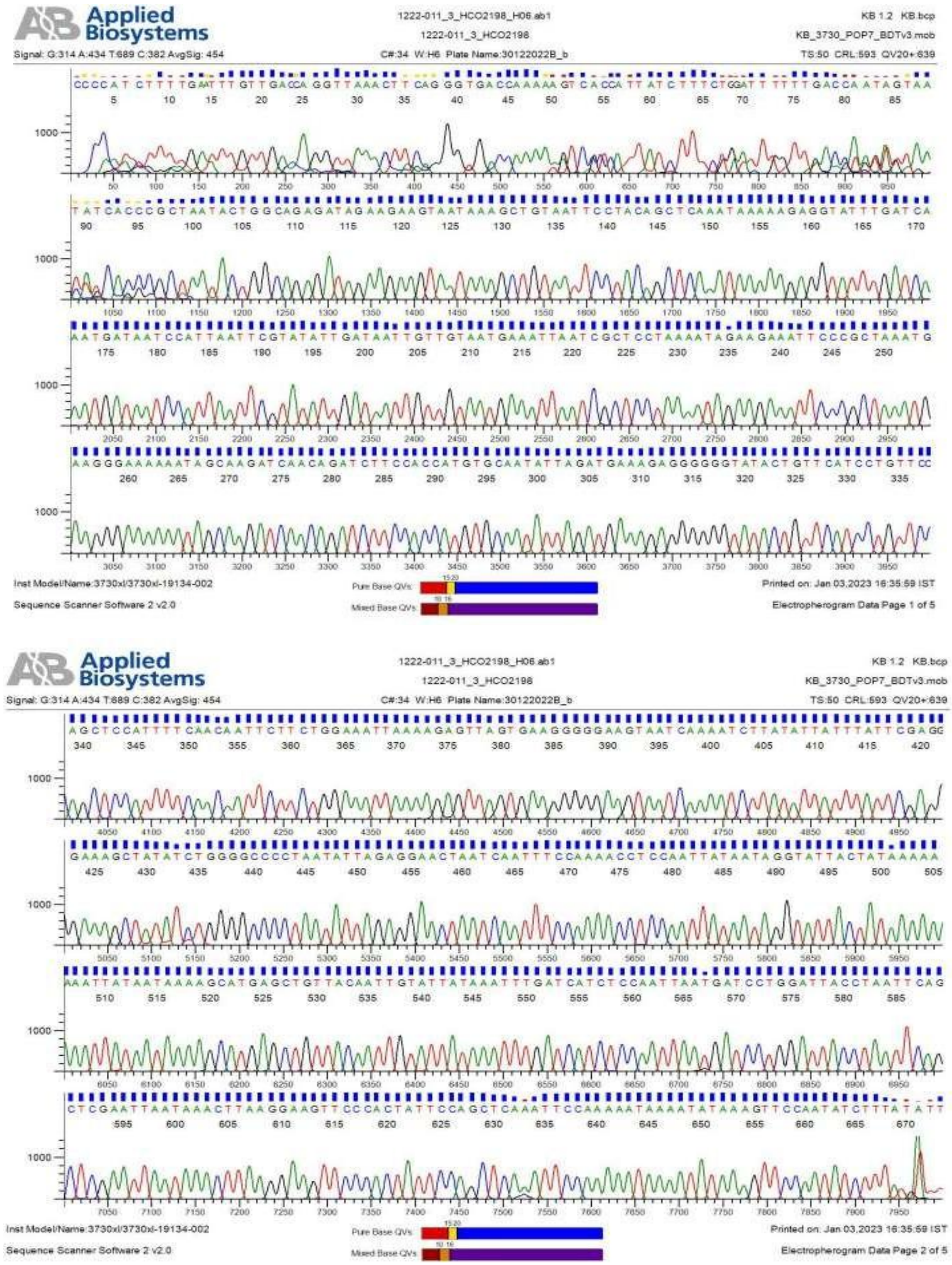


Figure 4.4 DNA sequencing of the cytochrome oxidase subunit I region in *Conogethes punctiferalis* (Guenee) samples. The 3-end of DNA sequencing result is shown with individual nucleotide peaks clearly distinguishable.

BOLD TaxonID Tree

Title	: COI FULL DATABASE includes records without species designati...
Date	: 10-August-2023
Data Type	: Nucleotide
Distance Model	: Kimura 2 Parameter
Marker	: COI-5P
Codon Positions	: 1st, 2nd, 3rd
Labels	: Extra Info, Country & Province, Family
Filters	: Length > 200
Attachment	: Photographs & Spreadsheet

Sequence Count	: 101
Species count	: 3
Genus count	: 3
Family count	: 2
Unidentified	: 5

Figure 4.5 BOLD TaxonID tree of *Conogethes punctiferalis* (Guenee)

GGAAATTGATTAGTTCCTCTAATATTAGGGGCCCCAGATATAGCTT
CCCTAGAATAAATAATATAAGATTTTGATTACTTCCCCCTTCACTAC
TCTTTTAATTTCCAGAAGAATTGTTGAAAATGGAGCTGGAACAGAG
AACAGTATACCCCCCTCTTTCATCAAAATTGCACATGGTGGAAGAC
GGTTGATCTTGCTATTTTTTCCCTTCATTTAGCGGAATTTCTTCTTT
TTAGGAGCGATTAATTTCATTACAACAATTATCAATATACGAATAA
TGGATTATCATTTGATCAATACCTCTTTTTGTTTGAGCTGGGGGAA

TAAACTTCAGGGTGACCAAAAAATCA-Reverse primer

CCCCATCTTTTGAATTTGTTGACCAGGTTAAACTTCAGGGTGACCA
AAGTCACCATTATCTTTCTGGATTTTTTGACCAATAGTAATATCACG
CTAATACTGGCAGAGATAGAAGAAGTAATAAAGCTGTAATTCTAA
GCTCAAATAAAAAGAGGTATTTGATCAAATGATAATCCATTATTCT
ATATTGATAATTGTTGTAATGAAATTAATCGCTCCTAAAAAGAAGA
ATTCCCGCTAAATGAAGGGAAAAAATAGCAAGATCAAAGATCTTC
ACCATGTGCAATATTAGATGAAAGAGGGGGGTATACGTTCATCCTT
TCCAGCTCCATTTTCAACAATTCTTCTGGAAATTAAAGAGTTAGTG
GGGGAAGTAATCAAAATCTTATATTATTTATCGAGGGAAAGCTATT
CTGGGGCCCCTAATATTAGAGGAACTAATATTTCCAAAACCTCCAT
TATAATAGGTATTACTATAAAAAAAATATAATAAAAGCATGAGCG
TTACAATTGTATTATAAATTTGATCATTCCAATTAATGATCCTGGAT
ACCTAATTCAGCTCGAATTAATAAATTAAGGAAGTTCCCACTATTC
AGCTCAAATTCCAAAAATAAAATAAAGTTCCAATATCTTTATATTG
TTTGACCAAAGTAAAGGTTAAACTTGG

***Conopomorpha sinensis* (Bradley)**

GGTCAACAAATCATAAAGATATTGG-Forward primer

TTTCTTTATCACCAATGATTACTTCTATGAACAGAGCGCTCAGTTGA
CTTATGGGTTGATTAGGGCTTTGTTAAAGCTATAATAGCAATTGGA
TATTCATTGTTTGAGCTCACCATATATTTACTGTTGGAATAGATATG
ATACCCCAGCATATTTTACATCTGCAACAATAATTATTGCAATTCCC

ACCGGTATTAAAATTTTTAGTTGATTATCTACCATTCATGGATCTCA
AATTAATTATAGTCCTTCTACTTTATGAAGATTAGGATTTGTATTTT
TTTACTATTGGGGGATTAACTGGAGTAATTTTATCAAATTCATCAAT
GATATTTCCTTACATGATACTTATTATGTTGTAGCACATTTTCATTA
TTTTTATCTATAGGAGCTGTATTTGCTATTATAGGCGGTTTTATTCA
TTATACCCCTTATTTACTGGATTAAATTTAAATCAATATATATTAAA
AATTCAATTTTTTACTATATTTATTGGAGTTAATATAACTTTCTTTCC
TCACACTTTTTAGGGTTAGCTGGTATACCTCGACGATACTCAGATTA
TCCTGATAGTTATACATCATGAAATATCATTTCATCTCTAGGATCTT
ATATTCATTATTAGCTATATTATATATATTAATTATTATTTGAGAAT
CATTCATTATCAACGAGTAGTAATTTTTTCATTAAATTTATCATCTT
CAATTGAATGATATCAATTATTCCCTCCTGCTGAACATTCATATAAT
GAACTACCATTTTAAGAAATTTCTAATATGGCAGATTAGTGCATTG
GAAATA
TAAACTTCAGGGTGACCAAAAAATCA-Reverse primer
TTTTGTGTTTCGGCTACCAGTGGAATGGTTTAGATATCCACACCGTT
TGCTTAAGGGTGATCCAATAGATCAAAGTACTACTCGTTGATAAAT
GAATGATTCTCAAATAATAATTAATATATATAATATACGCTAATAAG
AAATATAAGATCCTAGAGATGAAATGATATTTCATGATGTATAACT
ATCAGGATAATCTGAGTATCGTCGAGGTATACCACCTAACCCTAAA
AGAGTTGAGAAAAGAAAGTTATATTAACTCCAATAAATATAGTAAA
AAATTGAATTTTTAATATATATTGATTTAAATTTAATCCAGTAACTA
AGGGGTATCGTTGAATAAAACCACCTATAATAGCGAATACAGCTCT
ATAGATAAGACATAATGAAAATGTGCTACCACATAATAAGTATCAT
GTAAGGAAGTATCACTTGATGAATTTGCTAAAGTTACTCCAGATATC
CGCCAATAGTAAATAAAAATACAAATCCTAATCTTCATAAAGTAGA
AGGACTATAATTAATTTGAGATCCATGAATTGTAGCTAATCAACTAA
AAATTTTAATACCTGTTGGAATTGCAATAATTATTGTTGCAGGTAAA
ATATGCTCGTGTATCAATATCTATTCCAACAGTAAATATATGTGAGC

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Conopomorpha sinensis mitochondrion, complete genome	Conopomorpha ...	1260	1260	92%	0.0	97.10%	17050	NC_061172.1
Anarta trifolii mitochondrion, complete genome	Anarta trifolii	883	883	89%	0.0	88.45%	15281	NC_046049.1
Spodoptera exempta OKAf01 mitochondrial COI, COII genes for cytochrome oxidase subunit I, cytochrome ...	Spodoptera exe...	870	870	89%	0.0	88.15%	1383	LC582518.1
Spodoptera exempta OKAf02 mitochondrial COI, COII genes for cytochrome oxidase subunit I, cytochrome ...	Spodoptera exe...	865	865	89%	0.0	88.01%	1383	LC582519.1
Tholera decimalis genome assembly, chromosome: 19	Tholera decimalis	861	861	89%	0.0	87.94%	41975962	OW964567.2
Tholera decimalis genome assembly, chromosome: 9	Tholera decimalis	861	861	89%	0.0	87.94%	47040118	OW964557.2
Hyles dahlii mitochondrial coxI (partial), tRNA-Leu and coxII (partial) genes, isolate 23192	Hyles dahlii	859	859	89%	0.0	87.92%	2276	AJ749501.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 7652	Hyles euphorbiae	854	854	89%	0.0	87.79%	2284	LT595335.1
Eudonia lacustrata genome assembly, organelle: mitochondrion	Eudonia lacustrata	854	854	89%	0.0	87.77%	15290	OX387338.1
Xanthodes intersepta mitochondrion, complete genome	Xanthodes inter...	854	854	89%	0.0	87.79%	15366	NC_062099.1
Hyles dahlii mitochondrial coxI gene (partial), tRNA-Leu and coxII gene (partial), isolate 495	Hyles dahlii	854	854	89%	0.0	87.79%	2269	FN386561.1
Hyles dahlii mitochondrial coxI gene (partial), tRNA-Leu and coxII gene (partial), isolate 456	Hyles dahlii	854	854	89%	0.0	87.79%	2269	FN386560.1
Hyles dahlii mitochondrial coxI gene (partial), tRNA-Leu and coxII gene (partial), isolate Nardi	Hyles dahlii	854	854	89%	0.0	87.79%	2264	FN386559.1
Hyles biguttata mitochondrial coxI gene (partial), tRNA-Leu and coxII gene (partial), isolate 510	Hyles biguttata	854	854	89%	0.0	87.77%	2279	FN386547.1
Hyles biguttata mitochondrial coxI gene (partial), tRNA-Leu and coxII gene (partial), isolate 507	Hyles biguttata	854	854	89%	0.0	87.76%	2279	FN386546.1
Hyles biguttata mitochondrial coxI gene (partial), tRNA-Leu and coxII gene (partial), isolate 498	Hyles biguttata	854	854	89%	0.0	87.76%	2269	FN386545.1
Hyles dahlii mitochondrial coxI (partial), tRNA-Leu and coxII (partial) genes, isolate 23195	Hyles dahlii	854	854	89%	0.0	87.79%	2276	AJ749458.1
Hyles dahlii mitochondrial coxI (partial), tRNA-Leu and coxII (partial) genes, isolate 23193	Hyles dahlii	854	854	89%	0.0	87.79%	2265	AJ749457.1
Hyles dahlii mitochondrial coxI (partial), tRNA-Leu and coxII (partial) genes, isolate 16138	Hyles dahlii	854	854	89%	0.0	87.79%	2295	AJ749456.1
Hyles dahlii mitochondrial coxI (partial), tRNA-Leu and coxII (partial) genes, isolate 0030	Hyles dahlii	854	854	89%	0.0	87.79%	2295	AJ749455.1
Oligia fasciuncula genome assembly, organelle: mitochondrion	Oligia fasciuncula	850	850	88%	0.0	87.77%	15350	OY720417.1
Oligia fasciuncula genome assembly, chromosome: 16	Oligia fasciuncula	850	850	88%	0.0	87.77%	21246811	OY720402.1
Tholera decimalis genome assembly, chromosome: 10	Tholera decimalis	850	850	89%	0.0	87.67%	46704437	OW964558.2
Agrotis puta genome assembly, organelle: mitochondrion	Agrotis puta	850	850	89%	0.0	87.67%	15362	OW964197.2
Actinotia intermediata mitochondrion, complete genome	Actinotia interm...	850	850	89%	0.0	87.69%	15352	NC_062107.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 5719	Hyles euphorbiae	848	848	89%	0.0	87.65%	2244	LT595215.1
Rhodafra opheltes mitochondrion genomic DNA containing COI-tRNALeu-COII region, specimen voucher M...	Rhodafra opheltes	848	848	89%	0.0	87.60%	2277	LR700635.1
Rhodafra opheltes mitochondrion genomic DNA containing COI-tRNALeu-COII region, specimen voucher M...	Rhodafra opheltes	848	848	89%	0.0	87.60%	2277	LR700634.1
Hyles euphorbiae euphorbiae mitochondrial coxI gene (partial), tRNA-Leu gene and coxII gene (partial), isol...	Hyles euphorbia...	848	848	89%	0.0	87.65%	2264	FR839520.1
Hyles dahlii mitochondrial coxI (partial), tRNA-Leu and coxII (partial) genes, isolate 695815	Hyles dahlii	848	848	89%	0.0	87.65%	2279	AJ749502.1
Oligia fasciuncula genome assembly, chromosome: 17	Oligia fasciuncula	845	845	89%	0.0	87.55%	21132697	OY720403.1
Oligia fasciuncula genome assembly, chromosome: 6	Oligia fasciuncula	845	845	89%	0.0	87.53%	23411213	OY720392.1
Agrotis clavis genome assembly, organelle: mitochondrion	Agrotis clavis	845	845	89%	0.0	87.52%	15434	OX940948.1
Agrotis clavis genome assembly, chromosome: 17	Agrotis clavis	845	845	89%	0.0	87.50%	25340935	OX940934.1
Tholera decimalis genome assembly, organelle: mitochondrion	Tholera decimalis	845	845	89%	0.0	87.53%	15372	OW964579.2
Actinotia polyodon mitochondrion, complete genome	Actinotia polyodon	845	845	89%	0.0	87.55%	15347	MW697903.1
Catocala electa mitochondrion	Catocala electa	843	843	89%	0.0	87.47%	15575	MN698265.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 1613	Hyles euphorbiae	843	843	89%	0.0	87.52%	2274	LT595481.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 6372	Hyles euphorbiae	843	843	89%	0.0	87.52%	2254	LT595434.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 7568	Hyles euphorbiae	843	843	89%	0.0	87.50%	2252	LT595293.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 7586	Hyles euphorbiae	843	843	89%	0.0	87.50%	2223	LT595289.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 7707	Hyles euphorbiae	843	843	89%	0.0	87.52%	2252	LT595232.1
Hyles euphorbiae mitochondrial genomic DNA containing COI-tRNALeu-COII region, specimen voucher 7706	Hyles euphorbiae	843	843	89%	0.0	87.52%	2252	LT595231.1

Figure 4.6 NCBI nucleotide BLASTn search results showing submitted sequence has been matched to *Conopomorpha sinensis* Bradley with 97.10% similarity COI meta barcoding gene. This identification is solid.

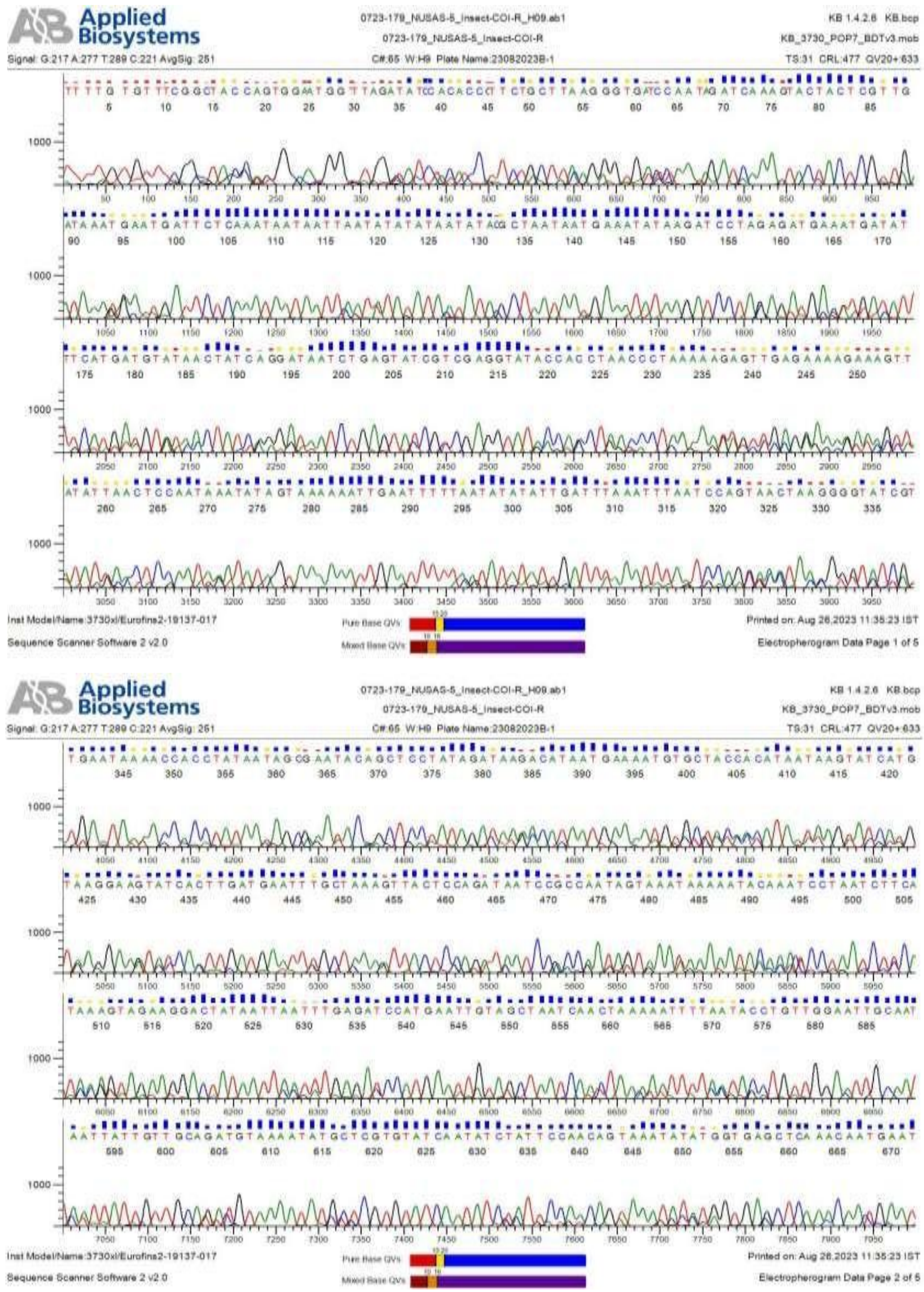


Figure 4.7 DNA sequencing of the cytochrome oxidase subunit I region in *Conopomorpha sinensis* Bradley samples. The 3-end of DNA sequencing result is shown with individual nucleotide peaks clearly distinguishable.

TCAAACAATGAATCCTAATAATCCAATTGCTATTATAGCAAAATTAT
CCCTAAACACCCAAAAGTTTCTTTTTTTCCTCTTTCTTGGAAATAATA
TGAGAAACTAATCCAAATCCTGGTAGAATTAAAATATAACTTCGGG
GTGACGAAAAAATCAAAAT

***Deudorix epijarbus* (Moore)**

GGTCAACAAATCATAAAGATATTGG-Forward primer

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATATTAGGAACAT
TTTAAGAATTTTAATTCGTATGGAATTAGGCACTCCAGGATCTTTAA
TTGGTGATGATCAAATTTATAATACTATTGTAACAGCTCATGCTTTT
ATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGGTTTGG
AAATGATTAGTACCATTAATATTAGGAGCACCTGATATAGCTTTCC
CACGATAAATAATATAAGATTTTGATTATTACCTCCTTCATTAATAT
TATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGAT
GAACAGTTACCCCCCACTTTCATCTAATATTGCTCATAGAGGTTCAT
CAGTTGATCTAGCTATTTTTTCTCTTCATTTAGCAGGTATTTCATCA
ATTTTAGGGCTATCAATTTTATTACAACAATTATTAATATACGAATT
AATAATTTATCCTTTGATCAAATATCTTTATTTATTTGAGCTGTAGG
AATTACTGCATTATTATTATTATTATCTTTACCTGTATTAGCAGGAG
CTATTACAATATTATTAACAGATCGAAATTTAAATACTTCATTTTTT
GACCCAGCAGGGGAGGAGACCCAATTTTATATCAACATTTATTT

TAAACTTCAGGGTGACCAAAAAATCA-Reverse primer

ACAATGTTCAGCAGGGCGGGTAAATTTTGATATCATTCAATAGAAG
TTTATATTTAATGGGAATAAAATAATTCGTTTTTTTACTATAGATTCC
AAATAATAATTAATATTAATATAGTTGCAATTAATGAAATATATGTC
CTAAAGAAGAAATAATATTTCATGATGTGTATGTGTCAGGATAACT
GAATAACGACGAGGTATTCCTGCTAAACCTAAAAAGTGTTGAGAAA
AATGTTAAATTTACTCCGATAAATATTGTAAAAAATTGAATTTTTAA
TAAAAATGGATTTATAGCTAATCCTGTAAATAATGGGTATCAATGA
ATAAATCCTCCTATAATAGCAAATACTGCCCCTATAGATAAACATA

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Select columns Show 50

select all 50 sequences selected GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Deudorix epijarbas voucher NIBGE BUT-00124 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Deudorix epijarbas	1216	1216	100%	0.0	100.00%	658	HQ990434.1
Deudorix staudingeri voucher UMKL-JJW0435 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Deudorix staudin...	1155	1155	100%	0.0	98.33%	658	KF226398.1
Deudorix epijarbas cinnabarus cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Deudorix epijarb...	1151	1151	99%	0.0	98.32%	656	ON436547.1
Deudorix epijarbas voucher 11ANIC-08219 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Deudorix epijarbas	1138	1138	100%	0.0	97.87%	658	JN286135.1
Deudorix epijarbas voucher BBV 307 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Deudorix epijarbas	1133	1133	93%	0.0	99.84%	641	MK348956.1
Deudorix epijarbas voucher USNM:ENT:00705010 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Deudorix epijarbas	1127	1127	100%	0.0	97.57%	658	HQ570669.1
Lepidoptera sp. HP476 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Lepidoptera sp. ...	1122	1122	92%	0.0	99.84%	618	MK044380.1
Deudorix littoralis voucher USNM ENT 00666527 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoch...	Deudorix littoralis	1116	1116	100%	0.0	97.26%	658	GU695463.1
Deudorix diovis voucher USNM ENT 00666518 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Deudorix diovis	1110	1110	100%	0.0	97.11%	658	GU695456.1
Deudorix diovis voucher USNM ENT 00666519 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Deudorix diovis	1105	1105	100%	0.0	96.96%	658	GU695455.1
Deudorix littoralis voucher USNM:ENT:00705057 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoch...	Deudorix littoralis	1048	1048	94%	0.0	97.10%	621	HQ570713.1
Deudorix antalus isolate ME11B047.L001 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Deudorix antalus	1042	1042	100%	0.0	95.14%	999	OP431107.1
Strephonota tephraeus voucher YB-BCI50029 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochond...	Strephonota tep...	1038	1038	100%	0.0	95.14%	658	KP849339.1
Satyrium acaciae voucher RVcoll.07-D848 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1038	1038	100%	0.0	95.14%	658	MN143597.1
Satyrium acaciae voucher RVcoll.14-I431 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1038	1038	100%	0.0	95.14%	658	MN139034.1
Strephonota tephraeus cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Strephonota tep...	1038	1038	100%	0.0	95.14%	658	HM905365.1
Capys alpheus cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Capys alpheus	1035	1035	99%	0.0	95.12%	656	ON436521.1
Satyrium spini voucher RVcoll.11-I540 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1033	1033	100%	0.0	94.98%	658	KP871002.1
Satyrium acaciae voucher RVcoll.08-M701 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1033	1033	100%	0.0	94.98%	658	KP870364.1
Satyrium spini voucher RVcoll.07-C691 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1033	1033	100%	0.0	94.98%	658	KP870275.1
Satyrium acaciae voucher RVcoll.14-F813 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1033	1033	100%	0.0	94.98%	658	MW502943.1
Satyrium acaciae voucher RVcoll.14-G097 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1033	1033	100%	0.0	94.98%	658	MW502740.1
Satyrium acaciae voucher RVcoll16C717 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1033	1033	100%	0.0	94.98%	658	MW501055.1
Lepidoptera sp. BOLD:AAB4662 voucher RVcoll.090806MA6 cytochrome oxidase subunit 1 (COI) gene, partial ...	Satyrium acaciae	1033	1033	100%	0.0	94.98%	658	HM901472.1
Satyrium spini voucher RVcoll.08-J144 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1033	1033	100%	0.0	94.98%	658	GU676563.1
Satyrium spini voucher RVcoll.08-P865 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1033	1033	100%	0.0	94.98%	658	GU675687.1
Satyrium spini voucher Rvcoll.12-M703 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1027	1027	100%	0.0	94.83%	658	KP870853.1
Satyrium spini voucher RVcoll.08-P035 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1027	1027	100%	0.0	94.83%	658	KP870842.1
Satyrium acaciae voucher RVcoll.08-P358 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1027	1027	100%	0.0	94.83%	658	KP870313.1
Satyrium w-album voucher RVcoll.14-B932 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium w-album	1027	1027	100%	0.0	94.83%	658	MW502549.1
Satyrium w-album voucher RVcoll16J617 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium w-album	1027	1027	100%	0.0	94.83%	658	MW501724.1
Satyrium w-album voucher RVcoll.14-F260 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium w-album	1027	1027	100%	0.0	94.83%	658	MW501650.1
Satyrium acaciae voucher RVcoll.14-H308 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1027	1027	100%	0.0	94.83%	658	MW500534.1
Satyrium w-album voucher RVcoll15L326 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium w-album	1027	1027	100%	0.0	94.83%	658	MW499657.1
Satyrium w-album voucher RVcoll15I759 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium w-album	1027	1027	100%	0.0	94.83%	658	MW499629.1
Lepidoptera sp. BOLD:AAB4662 voucher RVcoll.210207TM50 cytochrome oxidase subunit 1 (COI) gene, partia...	Satyrium acaciae	1027	1027	100%	0.0	94.83%	658	HM901486.1
Satyrium spini voucher RVcoll.06-G496 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1027	1027	100%	0.0	94.83%	658	GU676801.1
Satyrium spini voucher RVcoll.06-L922 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1027	1027	100%	0.0	94.83%	658	GU676219.1
Satyrium acaciae voucher RVcoll.08-M088 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1027	1027	100%	0.0	94.83%	658	GU676165.1
Satyrium spini voucher RVcoll.08-M965 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium spini	1027	1027	100%	0.0	94.83%	658	GU675968.1
Satyrium walbum voucher BC ZSM Lep 27091 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Satyrium w-album	1027	1027	100%	0.0	94.83%	658	GU707098.1
Satyrium sp. XQX-2016 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Satyrium sp. XQ...	1022	1022	100%	0.0	94.66%	798	KT236389.1
Satyrium acaciae voucher RVcoll.07-C480 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Satyrium acaciae	1022	1022	100%	0.0	94.66%	658	KP870923.1

Figure 4.8 NCBI nucleotide BLASTn search results showing submitted sequence has been matched to *Deudorix epijarbus* (Moore) with 100% similarity COI meta barcoding gene. This identification is solid.

Results Summary [Download]

Query ID	Best ID	Search DB	Tree	Top %	Graph	Low %
unlabeled_sequence	Deudorix epijarbas	COI SPECIES DATABASE		100.00		95.11

Query: unlabeled_sequence
Top Hit: Arthropoda, Insecta, Lepidoptera, Lycaenidae, Deudorix, Deudorix epijarbas (100%)

Search Result:

The submitted sequence has been matched to *Deudorix epijarbas*. This identification is solid unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases are rare.

A species page is available for this taxon: SPECIES PAGE

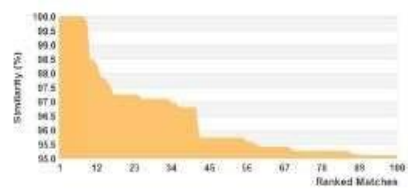
Closest matching BIN (within 3%): BIN PAGE

For a hierarchical placement - a neighbor-joining tree is provided: TREE BASED IDENTIFICATION

Identification Summary

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Arthropoda	100
Class	Insecta	100
Order	Lepidoptera	100
Family	Lycaenidae	100
Genus	*Deudorix*	100
Species	*Deudorix epijarbas*	100

Similarity Scores of Top 100 Matches



Top 20 Matches

Display: Top 20

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Private
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		100	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		99.84	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		98.47	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		98.47	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*staudingeri*		98.32	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		97.86	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		97.86	Published
Arthropoda	Insecta	Lepidoptera	Lycaenidae	*Deudorix*	*epijarbas*		97.71	Published

Figure 4.9 Barcode of life database (BOLD) search results showing submitted sequence has been matched to *Deudorix epijarbus* (Moore) with 100% similarity COI meta barcoding gene. This identification is solid.

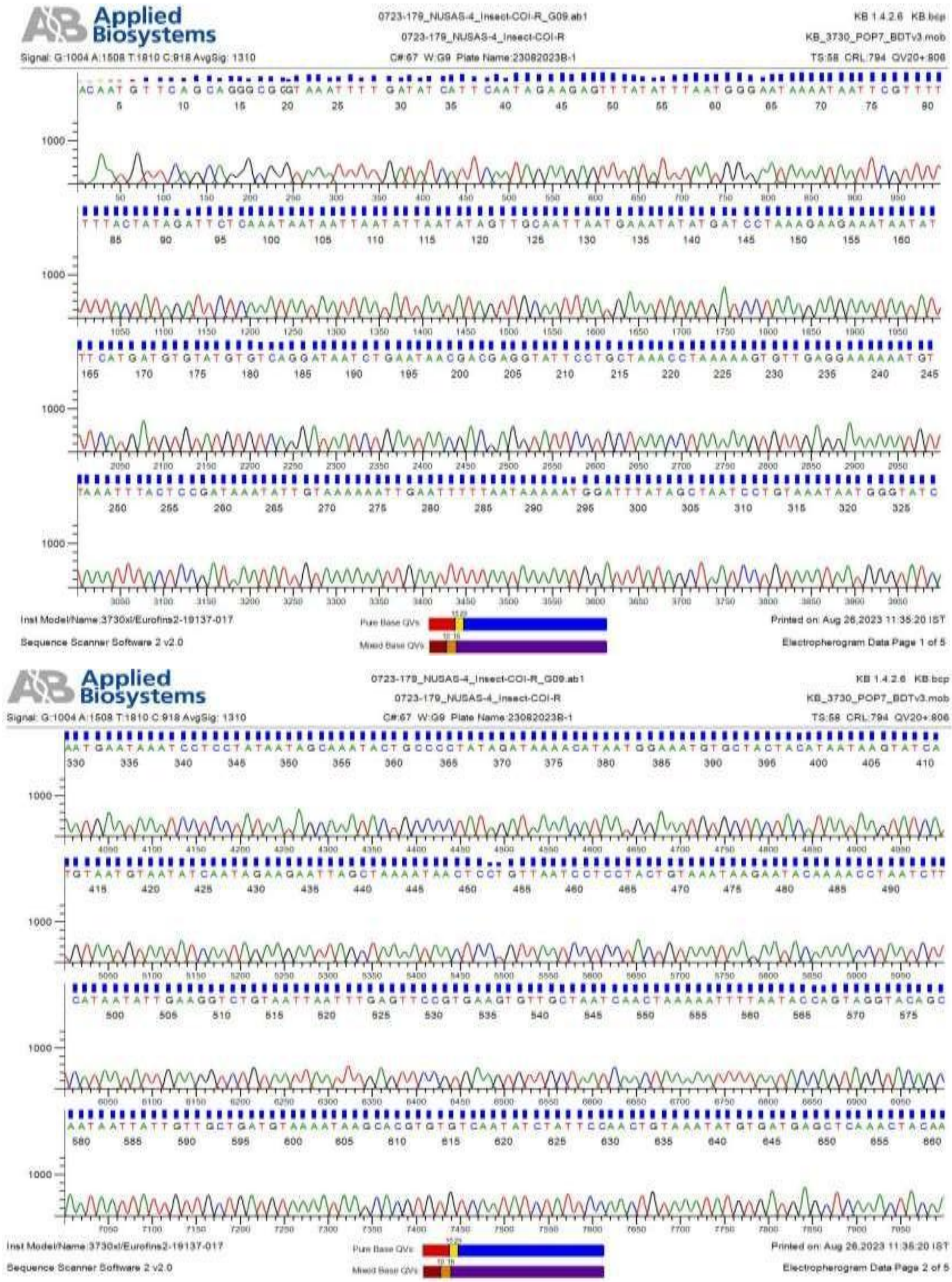


Figure 4.10 DNA sequencing of the cytochrome oxidase subunit I region in *Deudorix epijarbus* (Moore) samples. The 3-end of DNA sequencing result is shown with individual nucleotide peaks clearly distinguishable.

BOLD TaxonID Tree

Title	: COI SPECIES DATABASE Tree
Date	: 14-September-2023
Data Type	: Nucleotide
Distance Model	: Kimura 2 Parameter
Marker	: COI-5P
Codon Positions	: 1st, 2nd, 3rd
Labels	: Extra Info, Country & Province, Family
Filters	: Length > 200
Attachment	: Photographs & Spreadsheet

Sequence Count	: 101
Species count	: 19
Genus count	: 11
Family count	: 1
Unidentified	: 1

Figure 4.11 BOLD TaxonID tree of *Deudorix epijarbus* (Moore)

ATGGAAATGTGCTACTACATAATAAGTATCATGTAATGTAATATCA
ATAGAAGAATTAGCTAAAATAACTCCTGTTAATCCTCCTACTTAAAT
AAGAATACAAAACCTAATCTTCATAATATTGAAGGTCTGTAATTAAT
TTGAGTTCCGTGAAGTGTTGCTAATCAACTAAAAATTTTATACCAGT
AGGTACAGCAATAATTATTGTTGCTGATGTAAAATAAGCACGTGTG
TCAATATCTATTCCAACTGTAAATATGTGATGAGCTCAAACTACAAA
TCCTAATAAACCAATTGCTAATATAGCATAAATTATCCTAAACATCC
AAATGTTTCTCCTCTTTCTTGAGAAATAATATGTGAAATTATTCCAA
ATCCTGGTAAAATTAAAATATATACTTCAGGTGACAAAAAAAATCA
AAATT

***Cryptophlebia ombrodelta* (Lower)**

GGTCAACAAATCATAAAGATATTGG-Forward primer

GCGAGTGCTGATCCCGGCAGTGTAGCGGGTCACTTCGAAAAGAATG
GGTGATTGTTTCCCCCCAGGGGGGGGCCGGGACGGGGCGAGGAGG
ATCGATTCTGCCGTTGTCTAGCGGTGCCGTCATTATAATTTTTTTTAA
GTTAGCGTAGAGAGTGGGGGATTTGGGGATGATTAGGGCCGTGAGA
GGGGGGCCCGGGATATGTCGGTGCCCCGGAAG

TAAACTTCAGGGTGACCAAAAAATCA-Reverse primer

CCCCATTCTTTTGAATTTGTTGACCAGGTTAAACTCCAGGGTGACCA
AAAAATCACCATTATCTTTATGATTTGTTGACCAGGTTAAATTGCAA
GGGCTAAAACTGGTAAAGATAATAATAATAATAAAGCTGTAATACC
AACAGCTCAGACAAATAGTGGTATTTGATCTAATGATATATTATTAG
GTCGTATATTAATAATAGTTGTAATGAAATTTACAGCTCCTAAAATT
GAAGAAATTCCAGCTAAATGAAGAGAAAAAATAGCTAAATCTACTG
AACTTCCTCTATGAGCAATATTAGATGAAAGTGGTGGGTAAACTGTT
CATCCTGTACCTGCTCCGTTTTCTACAATTCTTCTTGAGATTAAAAGT
ATAATAGAGGGGGGTAATAATCAAAATCTTATGTTATTTATTCGAG
GAAAAGCTATATCAGGAGCTCCTAATATTAGGGGAACTAATCAATT
TCCGAATCCTCCAATTATAATAGGTATTACTATAAAGAAAATTATAA

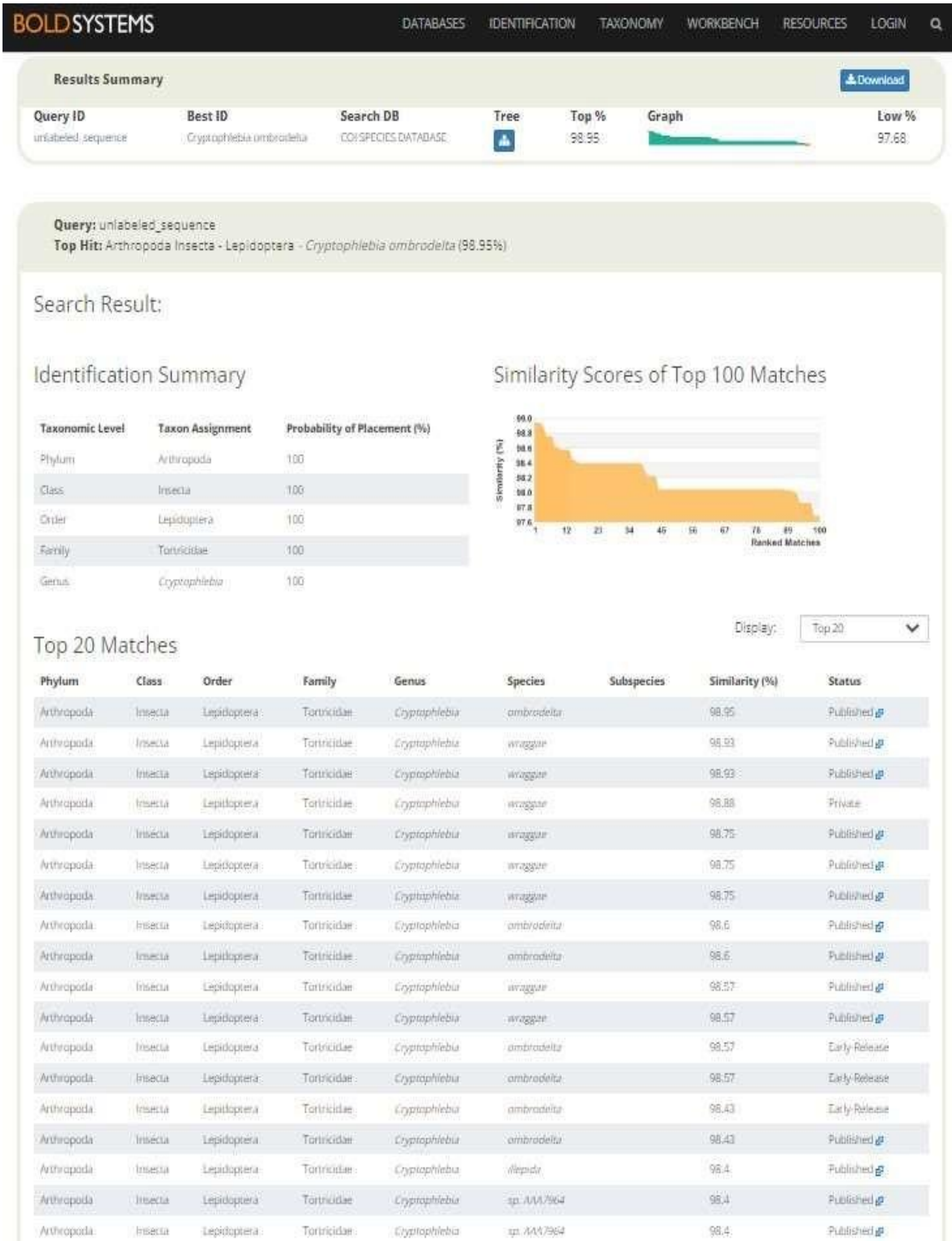
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 50

select all — *0 sequences selected* — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Cryptophlebia ombrodelta isolate KS7B cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Cryptophlebia o...	1031	1031	68%	0.0	98.63%	677	KX150514.1
Cryptophlebia ombrodelta isolate KS8A cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Cryptophlebia o...	1020	1020	68%	0.0	98.28%	677	KX150511.1
Cryptophlebia ombrodelta isolate KS7A cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Cryptophlebia o...	1014	1014	68%	0.0	98.11%	677	KX150513.1
Cryptophlebia wraggae voucher 11ANIC-12851 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia wr...	1011	1011	67%	0.0	98.60%	658	KF401333.1
Cryptophlebia wraggae voucher 11ANIC-12839 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia wr...	1011	1011	67%	0.0	98.60%	658	KF399046.1
Cryptophlebia wraggae voucher 11ANIC-12853 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia wr...	1005	1005	67%	0.0	98.42%	658	KF395486.1
Cryptophlebia sp. AAA7964 voucher USNM:ENT:00721935 cytochrome oxidase subunit 1 (COI) gene, partial c...	Cryptophlebia sp...	994	994	67%	0.0	98.07%	682	KY323086.1
Cryptophlebia illepida voucher JWB-08-0109-1 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Cryptophlebia il...	994	994	67%	0.0	98.07%	658	KF491660.1
Cryptophlebia sp. AAA7964 voucher USNM:ENT:00721136 cytochrome oxidase subunit 1 (COI) gene, partial c...	Cryptophlebia sp...	992	992	67%	0.0	98.07%	658	KY323267.1
Cryptophlebia sp. AAA7964 voucher USNM:ENT:00721139 cytochrome oxidase subunit 1 (COI) gene, partial c...	Cryptophlebia sp...	989	989	67%	0.0	97.96%	658	KY323274.1
Cryptophlebia sp. AAA7964 voucher USNM:ENT:00721043 cytochrome oxidase subunit 1 (COI) gene, partial c...	Cryptophlebia sp...	989	989	67%	0.0	97.90%	658	KY323171.1
Cryptophlebia sp. AAA7964 voucher USNM:ENT:00721663 cytochrome oxidase subunit 1 (COI) gene, partial c...	Cryptophlebia sp...	989	989	67%	0.0	97.90%	658	KY323099.1
Cryptophlebia ombrodelta voucher 11ANIC-12854 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia o...	983	983	67%	0.0	97.72%	581	KF406110.1
Cryptophlebia ombrodelta voucher 11ANIC-12864 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia o...	983	983	67%	0.0	97.72%	658	KF402024.1
Cryptophlebia ombrodelta voucher 11ANIC-12865 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia o...	983	983	67%	0.0	97.72%	658	KF400426.1
Cryptophlebia ombrodelta voucher 11ANIC-12866 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia o...	977	977	67%	0.0	97.55%	658	KF401637.1
Cryptophlebia ombrodelta voucher 11ANIC-12862 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia o...	972	972	67%	0.0	97.37%	658	KF397678.1
Cryptophlebia ombrodelta voucher 11ANIC-12863 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia o...	966	966	67%	0.0	97.20%	658	KF403816.1
Lepidoptera sp. LEPT 58.112 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Lepidoptera sp. ...	913	913	62%	0.0	97.91%	618	MW601827.1
Cryptophlebia semilunaria voucher USNM ENT 00719306 cytochrome oxidase subunit 1 (COI) gene, partial cd...	Cryptophlebia se...	883	883	67%	0.0	94.57%	658	HQ947317.1
Cryptophlebia semilunana voucher USNM:ENT:00676536 cytochrome oxidase subunit 1 (COI) gene, partial cd...	Cryptophlebia se...	883	883	67%	0.0	94.57%	658	KJ592119.1
Cryptophlebia caulicola voucher 11ANIC-12855 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia ca...	872	872	67%	0.0	94.22%	658	KF406793.1
Cryptophlebia sp. BOLD:AAG0340 voucher USNMENT00662052 cytochrome oxidase subunit 1 (COI) gene, p...	Cryptophlebia sp...	872	872	67%	0.0	94.22%	658	HM422451.1
Cryptophlebia stigmata voucher 11ANIC-12844 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia sti...	869	869	67%	0.0	94.05%	658	KF395966.1
Cryptophlebia stigmata voucher 11ANIC-12842 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia sti...	861	861	67%	0.0	93.87%	658	KF400707.1
Cryptophlebia pallifimbria voucher 11ANIC-12849 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoch...	Cryptophlebia pa...	859	859	68%	0.0	94.30%	658	KF402831.1
Cryptophlebia stigmata voucher 11ANIC-12845 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia sti...	857	857	67%	0.0	93.70%	658	KF398556.1
Pseudogalleria inimicella voucher PPBP-0782 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Pseudogalleria i...	854	854	66%	0.0	94.12%	658	KM540976.1
Cryptophlebia peltastica voucher KLM Lep 03097 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoch...	Cryptophlebia pe...	850	850	67%	0.0	93.52%	658	MH415950.1
Cryptophlebia caulicola voucher 11ANIC-12857 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia ca...	850	850	64%	0.0	94.55%	550	KF398478.1
Cryptophlebia stigmata voucher 11ANIC-12840 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Cryptophlebia sti...	850	850	67%	0.0	93.52%	658	KF397258.1
Pseudogalleria inimicella voucher PPBP-0937 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Pseudogalleria i...	848	848	66%	0.0	93.94%	658	KM555010.1
Pseudogalleria inimicella voucher PPBP-0772 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Pseudogalleria i...	848	848	66%	0.0	93.94%	609	KM543770.1
Pseudogalleria inimicella voucher PPBP-0774 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Pseudogalleria i...	848	848	66%	0.0	93.94%	609	KM543247.1
Pseudogalleria inimicella voucher PPBP-0915 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Pseudogalleria i...	848	848	66%	0.0	93.94%	658	KM543233.1
Pseudogalleria inimicella voucher PPBP-1075 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon...	Pseudogalleria i...	848	848	66%	0.0	93.94%	658	KM539588.1
Pseudogalleria inimicella voucher BIOUG<CAN>:10BBLEP-00897 cytochrome oxidase subunit 1 (COI) gene, p...	Pseudogalleria i...	848	848	66%	0.0	93.94%	658	HQ986049.1
Cryptophlebia sp. BOLD:ACM4140 voucher USNM ENT 00795188 cytochrome oxidase subunit 1 (COI) gene, ...	Cryptophlebia sp...	845	845	67%	0.0	93.35%	658	KP850108.1
Cryptophlebia peltastica voucher USNM:ENT:00676849 cytochrome oxidase subunit 1 (COI) gene, partial cds; ...	Cryptophlebia pe...	845	845	67%	0.0	93.35%	658	KJ592112.1
Cryptophlebia sp. JB1 voucher USNM:ENT:00676544 cytochrome oxidase subunit 1 (COI) gene, partial cds; mi...	Cryptophlebia sp...	845	845	67%	0.0	93.35%	658	KJ592071.1
Pseudogalleria inimicella voucher BIOUG<CAN>:09BBLEP-02318 cytochrome oxidase subunit 1 (COI) gene, p...	Pseudogalleria i...	843	843	66%	0.0	93.76%	658	HM427749.1
Pseudogalleria inimicella voucher BIOUG<CAN>:09BBLEP-01373 cytochrome oxidase subunit 1 (COI) gene, p...	Pseudogalleria i...	843	843	66%	0.0	93.76%	658	HM426821.1
Cryptophlebia peltastica voucher USNM:ENT:00676546 cytochrome oxidase subunit 1 (COI) gene, partial cds; ...	Cryptophlebia pe...	841	841	67%	0.0	93.17%	658	KJ592125.1
Cryptophlebia peltastica voucher USNM:ENT:00676549 cytochrome oxidase subunit 1 (COI) gene, partial cds; ...	Cryptophlebia pe...	839	839	67%	0.0	93.17%	658	KJ592414.1

Figure 4.12 NCBI nucleotide BLASTn search results showing submitted sequence has been matched to *Cryptophlebia ombrodelta* (Lower) with 98.63% similarity COI meta barcoding gene. This identification is solid.



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Results Summary Download

Query ID	Best ID	Search DB	Tree	Top %	Graph	Low %
unlabeled_sequence	Cryptophlebia ombrodelta	COI SPECIES DATABASE		98.95		97.68

Query: unlabeled_sequence
Top Hit: Arthropoda Insecta - Lepidoptera - *Cryptophlebia ombrodelta* (98.95%)

Search Result:

Identification Summary

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Arthropoda	100
Class	Insecta	100
Order	Lepidoptera	100
Family	Tortricidae	100
Genus	*Cryptophlebia*	100

Similarity Scores of Top 100 Matches

Display: Top 20

Top 20 Matches

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.95	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.93	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.93	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.88	Private
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.75	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.75	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.75	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.6	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.57	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*wraggae*		98.57	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.57	Early-Release
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.57	Early-Release
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.43	Early-Release
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*ombrodelta*		98.43	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	*illepida*		98.4	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	sp. *AAA7964*		98.4	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Cryptophlebia*	sp. *AAA7964*		98.4	Published

Figure 4.13 Barcode of life database (BOLD) search results showing submitted sequence has been matched to *Cryptophlebia ombrodelta* (Lower) with 98.95% similarity COI meta barcoding gene. This identification is solid

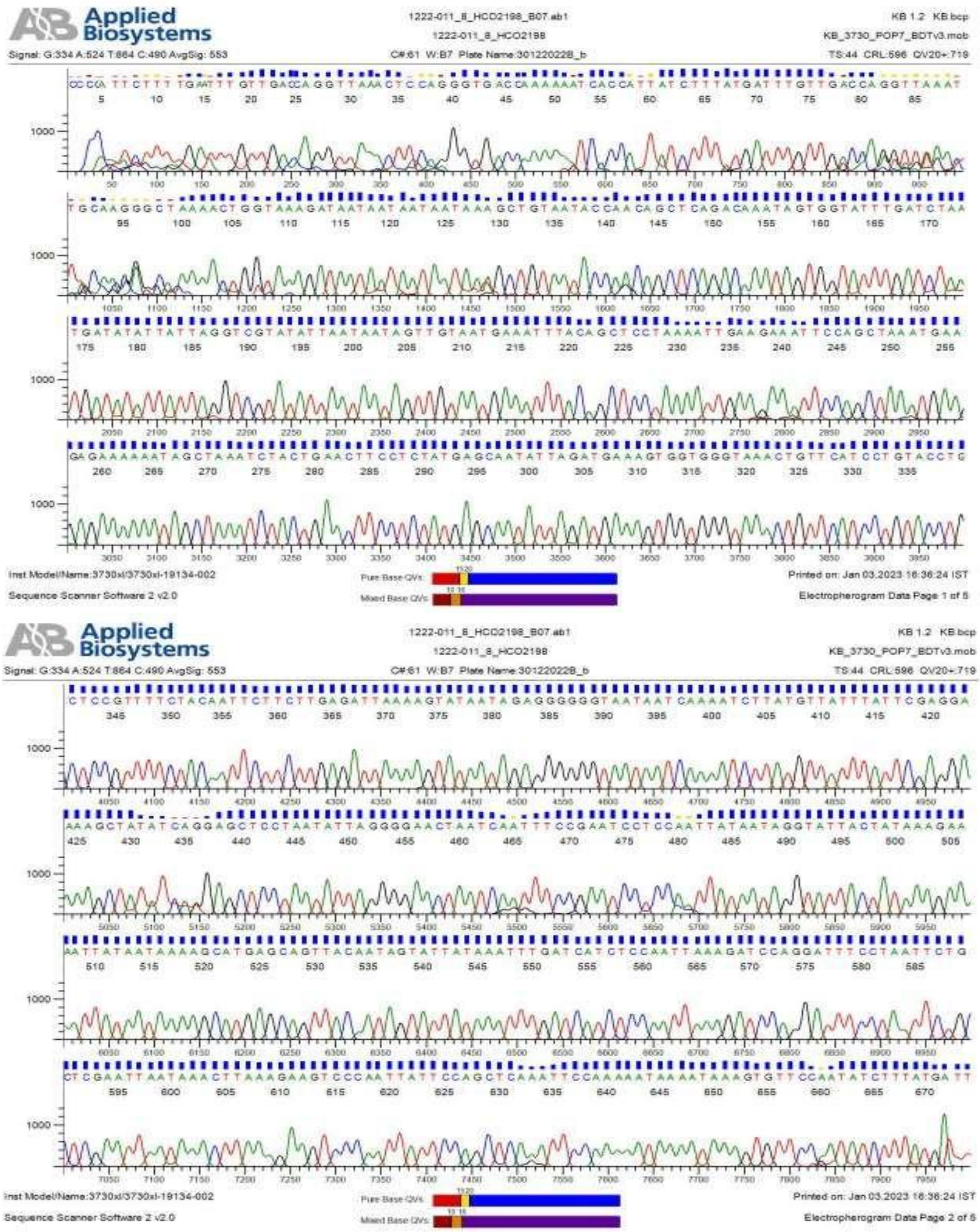


Figure 4.14 DNA sequencing of the cytochrome oxidase subunit I region in *Cryptophlebia ombrodelta* (Lower) samples. The 3-end of DNA sequencing result is shown with individual nucleotide peaks clearly distinguishable.

BOLD TaxonID Tree

Title	: COI SPECIES DATABASE Tree
Date	: 14-August-2023
Data Type	: Nucleotide
Distance Model	: Kimura 2 Parameter
Marker	: COI-5P
Codon Positions	: 1st, 2nd, 3rd
Labels	: Extra Info, Country & Province, Family
Filters	: Length > 200
Attachment	: Photographs & Spreadsheet

Sequence Count	: 101
Species count	: 4
Genus count	: 1
Family count	: 1
Unidentified	: 1

Figure 4.15 BOLD TaxonID tree of *Cryptophlebia ombrodelta* (Lower)

TAAAAGCATGAGCAGTTACAATAGTATTATAAATTTGATCATCTCCA
ATTAAAGATCCAGGATTTCCTAATTCTGCTCGAATTAATAAACTTAA
AGAAGTCCCAATTATTCCAGCTCAAATTCCAAAAATAAAATAAAGT
GTTCCAATATCTTTATGATTTGTTGACCACGTTAAGCGTAACCTGGT
CCGGCAAATCATAAAGATATTGGTGATTTTTTGGTCACCCTGAATTT
TAACCGGGTCAGCTAATCAAAAAGATATTGGTGATTTTTTGGTCACC
CTAAATTTAAACCGGGACAACAAACAAACAGATTGGGTGATTTTTT
GGTCCCA

Thaumatotibia zophophanes **(Turner)**

GGTCAACAAATCATAAAGATATTGG-Forward primer

CTTGTTTTGGTACCTGGAAGTTTAAGCCGGGTCAACAAATCGAAAG
ATATTGGTGATTTTTTGGACCCCTGTAGGATAACCTGGATCATTAAT
GAGATGATCAAATTTATAATACTATTGTAACTGCTCACGCTTTTTTA
TAATTTTTTTCATAGTAATACCTATTATAATTGGAGGATTTGGAATT
GATTAGTACCATTAATATTAGGAGCCCCAGATATAGCTTTCCCCGA
ATAAATAATATAAGATTTTGACTTTTACCCCCCTCAATCATATTATA
ATTTCAAGTAAAATCGTAAAAAATGGAGCTGGAACAGGATGAATTT
ACCCCCCACTTTCATCTAATATTGCCCACAGAGGAAGATCAGTATCT
AGCAATTTTTTCCCTTCATTTAGCTGGAATTTCTTCTATTTTTGGCTG
TAAATTTTATTACAACTATTAGGGTGATACGACCCCCCACTTATCAC
TAGATCAAATACCACTTTTTGTATGAGCTGTAAGTATTACAGCTTTA
CTTTTACTTTTATCTTTACCTGTATTAGCTGGTGCCATCACTTATATT
ATCAAATCGAAATCTTAATACATCATTCTTTGATCCTTCATAGAGGA
GATCCAATTCTTTACCAACATTTATTTTGATTTTTTGGTCCCCTAAGT
TTAACCGCCAGGTTCCGGGTGACAAAAAAATCGGCAATCTTGTGAT
TTGTTGACCAAGTTAGGCTTGGGGGTGGCCAAAAACCGCCAGATTC
TTGGAATTGGTTGACCAGGGAGGACTGG

TAAACTTCAGGGTGACCAAAAAATCA-Reverse primer

CCCTTCGGGTGAATTTGTTGACCAGGTTTAAACTTCAGGGTGACCA

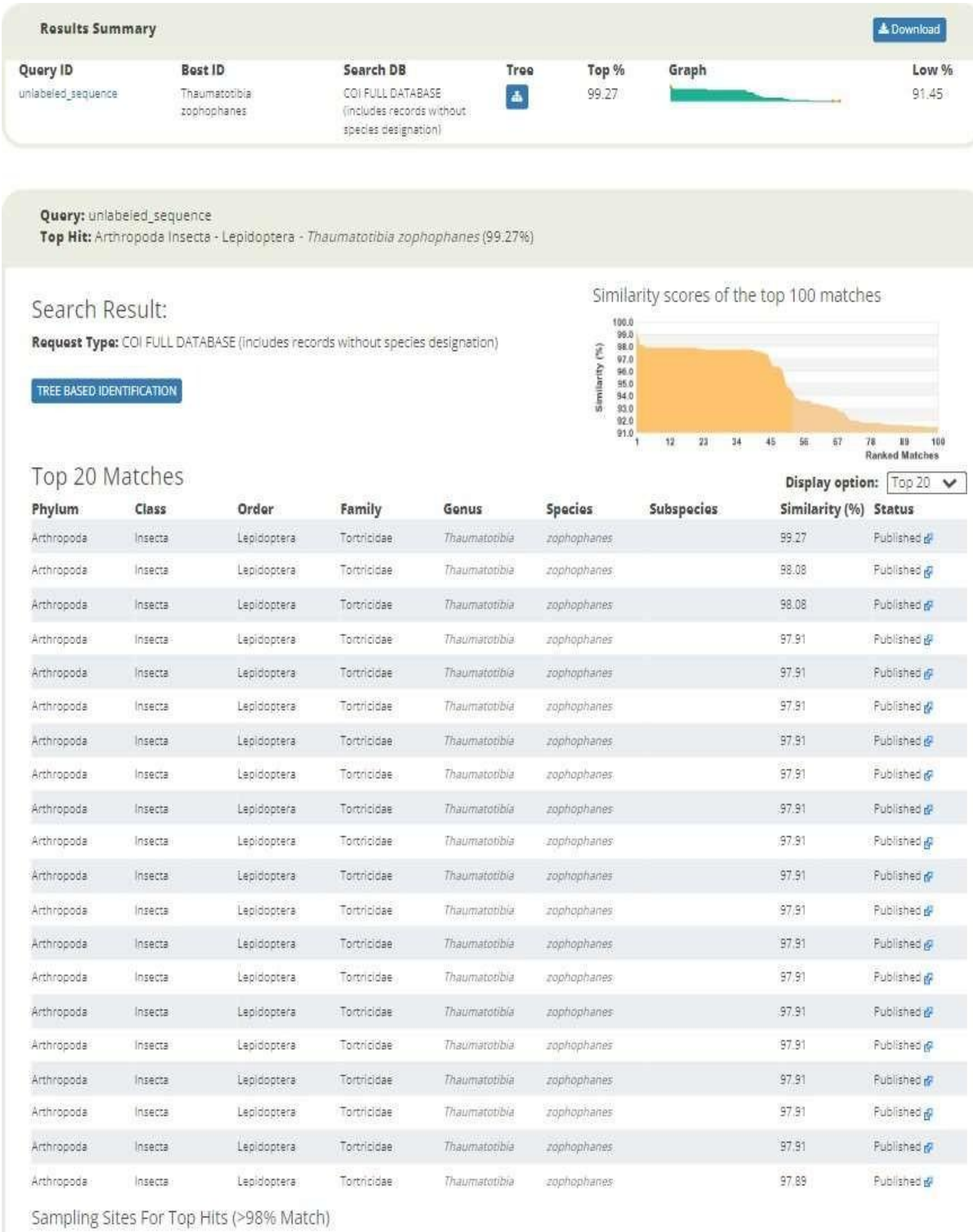
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 50

select all 50 sequences selected — GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Thaumatotibia zophophanes voucher USNM:ENT:00720675 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	1014	1014	67%	0.0	99.29%	604	KY323084.1
Thaumatotibia zophophanes voucher ww27757 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Thaumatotibia z...	1014	1014	67%	0.0	99.29%	895	MW378310.1
Thaumatotibia zophophanes voucher ww26734 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Thaumatotibia z...	1014	1014	67%	0.0	99.29%	895	MW378308.1
Thaumatotibia zophophanes voucher USNM ENT 00868590 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	1014	1014	67%	0.0	99.29%	658	GU695432.1
Thaumatotibia zophophanes voucher USNM:ENT:00721872 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	1009	1009	67%	0.0	99.11%	621	KY323226.1
Thaumatotibia zophophanes voucher USNM:ENT:00724763 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	1009	1009	67%	0.0	99.11%	658	KY323053.1
Thaumatotibia zophophanes voucher 11ANIC-12875 cytochrome oxidase subunit 1 (COI) gene, partial cds; mit...	Thaumatotibia z...	1009	1009	67%	0.0	99.11%	658	KF403386.1
Thaumatotibia zophophanes voucher 11ANIC-12876 cytochrome oxidase subunit 1 (COI) gene, partial cds; mit...	Thaumatotibia z...	1009	1009	67%	0.0	99.11%	657	KF401748.1
Thaumatotibia zophophanes voucher USNM:ENT:00720330 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	1007	1007	67%	0.0	99.28%	610	KY323195.1
Thaumatotibia zophophanes voucher ww27752 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Thaumatotibia z...	1003	1003	67%	0.0	98.93%	895	MW378309.1
Thaumatotibia zophophanes voucher 11ANIC-12874 cytochrome oxidase subunit 1 (COI) gene, partial cds; mit...	Thaumatotibia z...	994	994	66%	0.0	99.27%	550	KF401879.1
Thaumatotibia zophophanes voucher USNM:ENT:00724792 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	986	986	64%	0.0	99.25%	632	KY323135.1
Thaumatotibia zophophanes voucher USNM:ENT:00720347 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	948	948	63%	0.0	99.05%	625	KY323086.1
Thaumatotibia zophophanes voucher USNM:ENT:00724780 cytochrome oxidase subunit 1 (COI) gene, partial ...	Thaumatotibia z...	946	946	63%	0.0	99.24%	621	KY323124.1
Thaumatotibia aclyta voucher 11ANIC-12873 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Thaumatotibia a...	876	876	67%	0.0	94.83%	657	KF396581.1
Thaumatotibia aclyta voucher 11ANIC-12871 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Thaumatotibia a...	870	870	67%	0.0	94.65%	624	KF406064.1
Thaumatotibia maculata voucher YAWCATCR0540 cytochrome oxidase subunit 1 (COI) gene, partial cds; mito...	Thaumatotibia m...	865	865	67%	0.0	94.47%	636	MK019945.1
Thaumatotibia aclyta voucher 11ANIC-12872 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Thaumatotibia a...	861	861	66%	0.0	94.91%	550	KF402553.1
Thaumatotibia maculata voucher 11ANIC-12866 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocho...	Thaumatotibia m...	854	854	67%	0.0	94.12%	658	KF399439.1
Argyrotaenia quercifoliana voucher JDDNA2938 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Argyrotaenia qu...	824	824	70%	0.0	91.98%	1535	JF703043.1
Diedra intermontana voucher JDDNA4548 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Diedra intermont...	822	822	70%	0.0	91.98%	1536	JF703071.1
Lepidoptera sp. BOLD:AAI8988 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Lepidoptera sp. ...	821	821	67%	0.0	93.05%	658	GU654415.1
Acroclita subsequana isolate MrOuali04 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Acroclita subseq...	817	817	70%	0.0	91.81%	687	MW596785.1
Tortricidae gen. tortJanzen01 sp. Janzen01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Tortricidae gen. t...	815	815	67%	0.0	92.87%	658	JQ525205.1
Tortricidae gen. tortJanzen01 sp. Janzen01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Tortricidae gen. t...	815	815	67%	0.0	92.87%	658	JQ531186.1
Lepidoptera sp. BOLD:AAI8988 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Lepidoptera sp. ...	815	815	67%	0.0	92.87%	658	GU654416.1
Phiaris dolosana mitochondrion, complete genome	Phiaris dolosana	813	813	70%	0.0	91.78%	15562	MK962620.1
Capua sp. ANIC9 voucher 11ANIC-09594 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Capua sp. ANIC9	813	813	67%	0.0	92.87%	658	KF399586.1
Archips rosana voucher JDDNA4583 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Archips rosana	813	813	70%	0.0	91.78%	1536	JF703028.1
Choristoneura metasequoiacola mitochondrion, complete genome	Choristoneura m...	811	811	70%	0.0	91.65%	15128	OP747297.1
Choristoneura occidentalis mitochondrion, complete genome	Choristoneura o...	808	808	70%	0.0	91.67%	15536	NC_037393.1
Dracontogena sp. LepUtl 051 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Dracontogena s...	808	808	68%	0.0	92.54%	658	JQ843301.1
Choristoneura murinana voucher Sperling lab #10146 cytochrome c oxidase subunit I (COX1) gene, partial cds...	Choristoneura m...	808	808	70%	0.0	91.67%	1528	MT711510.1
Choristoneura occidentalis voucher GF_FS_OCCNA cytochrome c oxidase subunit I (COX1) gene, partial cds; ...	Choristoneura o...	808	808	70%	0.0	91.67%	1528	MT711305.1
Choristoneura occidentalis voucher GF_FS_bibi10coi cytochrome c oxidase subunit I (COX1) gene, partial cds;...	Choristoneura b...	808	808	70%	0.0	91.67%	1510	MT711304.1
Choristoneura orae voucher FSb218 cytochrome oxidase subunit I (COI) gene, complete cds; tRNA-Leu gene, ...	Choristoneura orae	808	808	70%	0.0	91.67%	2295	DQ792586.1
Choristoneura occidentalis cytochrome oxidase I (COI) gene, complete cds; tRNA-Leu (trnL) gene, complete se...	Choristoneura o...	808	808	70%	0.0	91.67%	2295	L19094.3
Cryptophlebia endophaga voucher 11ANIC-12828 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cryptophlebia en...	804	804	67%	0.0	92.51%	658	KF402830.1
Dracontogena sp. LepUtl 064 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Dracontogena s...	804	804	67%	0.0	92.51%	658	JQ843300.1

Figure 4.16 NCBI nucleotide BLASTn search results showing submitted sequence has been matched to *Thaumatotibia zophophanes* (Turner) with 99.29% similarity COI meta barcoding gene. This identification is solid.



Results Summary

Download

Query ID	Best ID	Search DB	Tree	Top %	Graph	Low %
unlabeled_sequence	Thaumatotibia zophophanes	COI FULL DATABASE (includes records without species designation)		99.27		91.45

Query: unlabeled_sequence
Top Hit: Arthropoda Insecta - Lepidoptera - *Thaumatotibia zophophanes* (99.27%)

Search Result:

Request Type: COI FULL DATABASE (includes records without species designation)

TREE BASED IDENTIFICATION

Top 20 Matches

Display option: Top 20

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		99.27	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		98.08	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		98.08	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.91	Published
Arthropoda	Insecta	Lepidoptera	Tortricidae	*Thaumatotibia*	*zophophanes*		97.89	Published

Sampling Sites For Top Hits (>98% Match)

Figure 4.17 Barcode of life database (BOLD) search results showing submitted sequence has been matched to *Thaumatotibia zophophanes* (Turner) with 99.27% similarity COI meta barcoding gene. This identification is solid

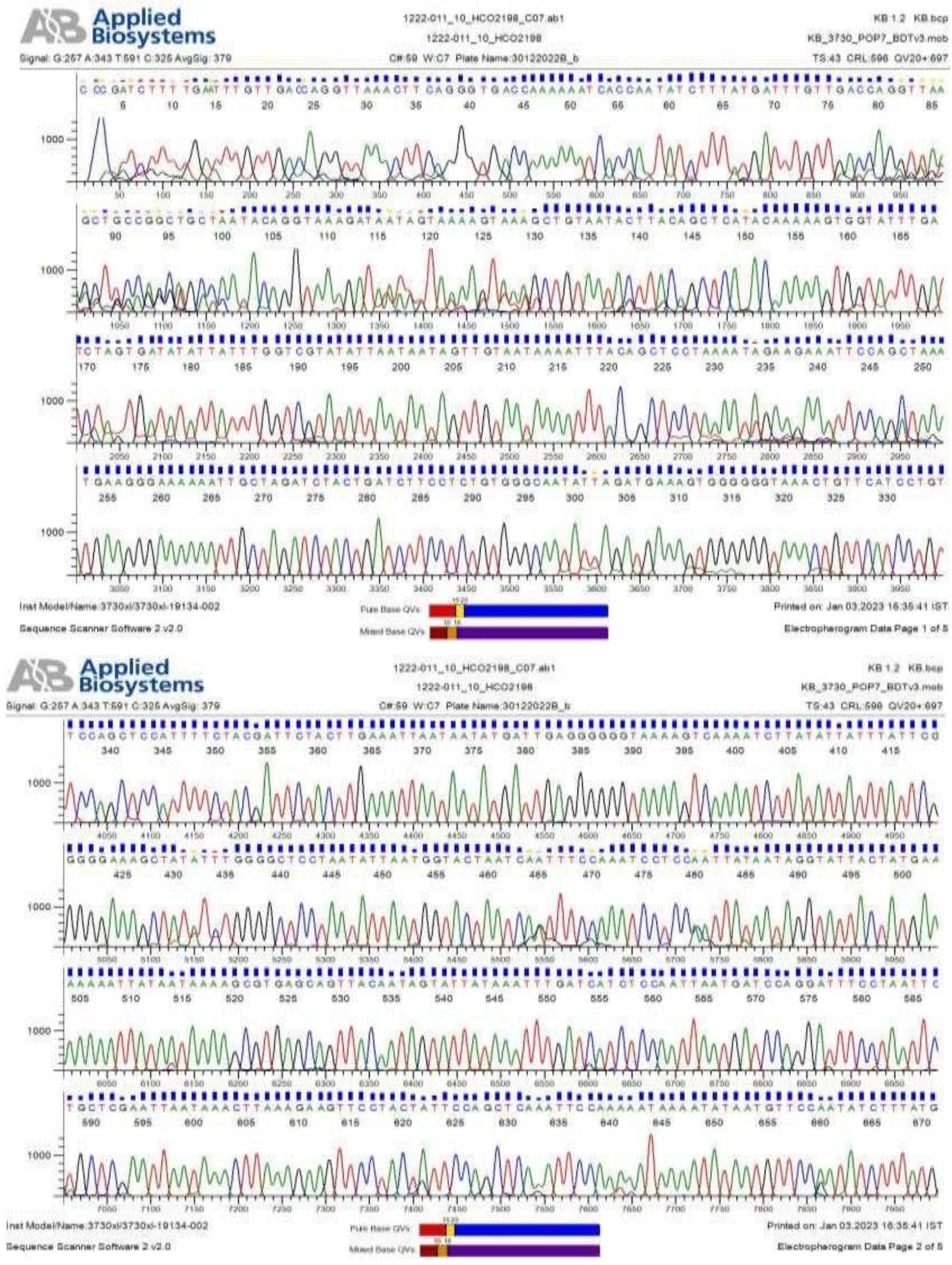


Figure 4.18 DNA sequencing of the cytochrome oxidase subunit I region in *Thaumatotibia zophophanes* (Turner) samples. The 3-end of DNA sequencing result is shown with individual nucleotide peaks clearly distinguishable.

BOLD TaxonID Tree

Title	: COI SPECIES DATABASE Tree
Date	: 14-August-2023
Data Type	: Nucleotide
Distance Model	: Kimura 2 Parameter
Marker	: COI-5P
Codon Positions	: 1st, 2nd, 3rd
Labels	: Extra Info, Country & Province, Family
Filters	: Length > 200
Attachment	: Photographs & Spreadsheet
Sequence Count	: 101
Species count	: 27
Genus count	: 15
Family count	: 2
Unidentified	: 1

Figure 4.19 BOLD TaxonID tree of *Thaumatotibia zophophanes* (Turner)

AAATCACCAATATCTTTATGATTTGTTGACCAGGTTAAATTGCAGGA
GCTAAAACTGGTAAAGATAATAATAATAATAAAGCTGTAATACCAC
AGCTCAGACAAATAATGGTATTTGATCTAATGATATATTATTAGGTC
GTATATTAATAATAGTTGTAATGAAATTTACAGCTCCTAAAATTGA
AGAAATTCCAGCTAAATGAAGAGAAAAAATAGCTAAATCTACTGA
ACTTCCTCTATGAGCAATATTAGATGAAAGTGGTGGGTAAACTGTT
CATCCTGTACCTGCTCCGTTTTCTACAATTCTTCTTGAGATTAGAAG
TATAATAGAGGGAGGTAATAATCAAAATCTTATGTTATTTATTCGA
GGGAAAGCTATATCAGGAGCTCCTAATATTAGGGGAACTAGTCAAT
TTCCAAATCCTCCAATTATAATAGGTATTACTATAAAAAAAATTATA
ATAAAAGCATGAGCAGTTACAATAGTATTATAAATTTGATCATCTC
CAATTAAAGATCCAGGATTTCCTAATTCTGCTCGAATTAATAAACTT
AAAGAAGTTCCAATTATTCCAGCTCAAATTCCAAAAATAAAATAAA
GTGTTCCAATATCTTTATATTTGTTGACCAGAAAAAAGGAAAAACC
GGT

4.1.2.3 Nucleotide substitution pattern

Two types of nucleotide substitution were observed: transition and transversion. As per the rule, transition is more common than transversion. In the current study, the transition between A and G (28.21%) was higher than the transition between T and C (21. 33%). The highest transversion occurred from A to T (5.73 %) and G to T (5.73 %) followed by T to A (4. 57%) and C to A (4. 57%), while the lowest transversion occurred from A to C (2.16%) and G to C (2.14%) followed by T to G (2.14%) and C to G (2.14%) (Table 4.4). The nucleotide frequencies are 31.29% (A), 39.25% (T/U), 14.82% (C), and 14.64% (G). Also, there is a strong AT bias (70. 54%). The transition/transversion rate ratios are k_1 = 6.173 (purines) and k_2 = 3.72 (pyrimidines). The overall transition/transversion bias is R = 2.01, where R = [A*G*k_1 + T*C*k_2]/[(A+TG) *(T+C)]. The above results are in accordance with the previous work carried out

Table. 4.3 Quantification of DNA using nanodrop spectrophotometer

Sl. No.	Litchi fruit borers	Code number	Concentration (ng/µl)	A260/A280 ratio
1.	*Conogethes punctiferalis* (Guenée)	NUSAS01	31.41	1.95
2.	*Conopomorpha sinensis* Bradley	NUSAS02	30.45	1.90
3.	*Deudorix epijarbus* (Moore)	NUSAS03	25.65	1.85
4.	*Cryptophlebia ombrodelta* (Lower)	NUSAS04	11.20	1.92
5.	*Thaumatotibia zophophanes* (Turner)	NUSAS05	15.70	1.88

Table. 4.4. Maximum composite likelihood estimates of the pattern of nucleotide substitution from 61 individuals of 18 species of fruit borers of litchi and their related species

	A	T	C	G
A	-	*5.73*	*2.10*	**13.20**
T	*4.57*	-	**7.74**	*2. 14*
C	*4.57*	**21.33**	-	*2.14*
G	**28.21**	*5.73*	*2.16*	-

Note: Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in *italics*.

on mtCOI gene of genus, Junonia and fruit borers of litchi by Win et al. (2015) and Srivastava et al. (2017) respectively, where they found strong AT bias of approximately 71.1% and overall transition/transversion bias of 2.69.

The program DAMBE was used to plot nucleotide substitutions against sequence divergence values (Fig 4.20). The number of transitions and transversions increased with pairwise divergence. More transitions than transversions occurred at almost every level of divergence. This result showed that substitutions did not become saturated in either transitions or transversions, and so could be used for further phylogenetic analyses. The above results are in accordance with previous work carried out by Win *et al*. (2015), who reported that, number of transitions and transversions increased with pairwise divergence.

4.1.2.4 Intra-specific genetic divergence

The range and mean of intraspecific genetic divergences across all 18 species groups is computed by averaging the K2P distances of all possible combinations of COI sequence variation in a pair-wise manner (Table 4.5). Intraspecific genetic divergences ranged from 0.00% to 0.14% with overall mean of 0.13%. The mean intraspecific genetic distances of *C. punctiferalis* was 0.04 % (range, 0.00-0.10%). In the case of *C. cramerella*, the intraspecific genetic distance was 0.00% (range, 0.00-0.00%). The mean intraspecific genetic distance of *C. litchiella* was 0.00% (range, 0.00-0.00%). Whereas, in *C. sinensis*, the mean intraspecific genetic distance observed was 0.01% (range, 0.00-0.04%). The mean intraspecific genetic distances of *C. ombrodelta* and *D. epijarbus* were 0.04% (range, 0.00-0.14%) and 0.02% (range, 0.00-0.03%), respectively. While, in case of *T. zophophanes*, the mean intraspecific genetic distance is 0.01% (range, 0.01-0.02%).

Minimum intraspecific nucleotide divergence of 0.00% was found in all species except *T. zophophanes* (0.01%), while maximum intraspecific nucleotide divergence of 0.04% found in *C. punctiferalis* and *C. ombrodelta*.

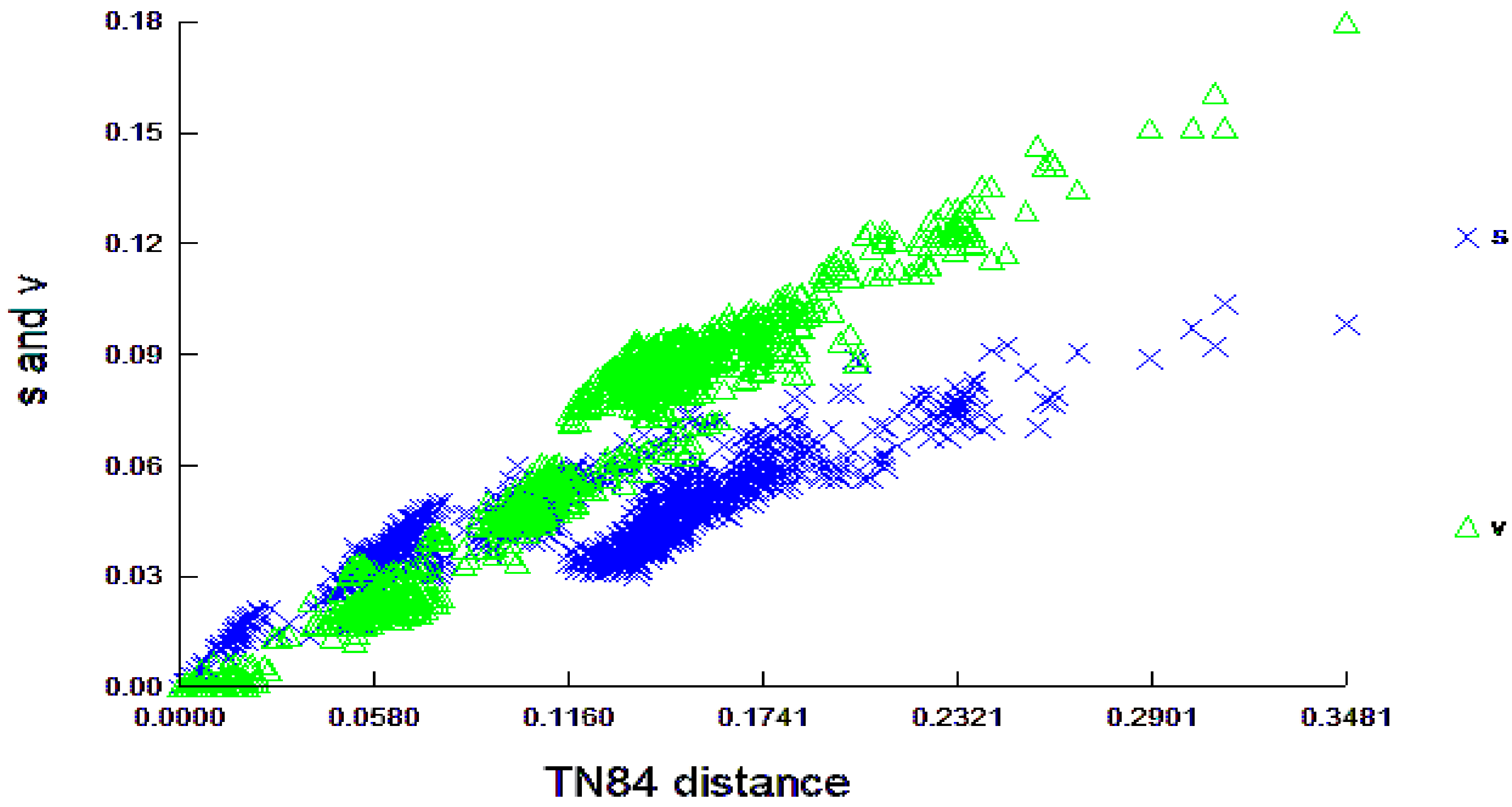


Figure. 4.20 The number of transitions and transversions plotted against the uncorrected pairwise sequence divergence

Table. 4.5 Intraspecific genetic distance (K2P) of fruit borers of litchi based on partial COI sequences that have two or more sequences of fruit borers with minimum, maximum and average values

Species groups	No. of individuals	Intraspecific genetic distance (%)		
		Min.	Max.	Average
Conogethes punctiferalis (Guenée)	5	0.00	0.10	0.04
Conopomorpha cramerella Snellen	4	0.00	0.00	0.00
Conopomorpha litchiella Bradley	3	0.00	0.00	0.00
Conopomorpha sinensis Bradley	6	0.00	0.04	0.01
Cryptophlebia ombrodelta (Lower)	9	0.00	0.14	0.04
Deudorix epijarbus (Moore)	4	0.00	0.03	0.02
Thaumatotibia zophophanes (Turner)	3	0.01	0.02	0.01

Widely distributed species exhibited high intraspecific divergence. The 0.00% sequence divergence showed that DNA sequences overlapped, possibly due to low variation among individuals of the same species from different geographical locations. In contrast, maximum intraspecific nucleotide divergence of 0.04% indicated that there is a higher degree of variation among individuals of same species from different locations. The above results align with the Lukhtanov *et al*. (2009) and Srivastava *et al*. (2017), who concluded that geographical distance is often associated with an increased genetic divergence, but the increase is too small to impede the identification of species.

4.1.2.5 Inter-specific genetic divergence

The overall range and mean of interspecific genetic distance of fruit borer species is presented (Table 4.6) reveal the values for different species as *C. punctiferalis* (0.17-0.19), *C. cramerella* (0.07-0.17), *C. litchiella* (0.06-0.17), *C. sinensis* (0.07-0.24), *C. caulicola* (0.05-0.17), *C. illepida* (0.02-0.13), *C. iridosoma* (0.06-0.18), *C. ombrodelta* (0.03-0.18), *C. pallifimbria* (0.05-0.18), *C. peltastica* (0.06-0.17), *C. rhynchias* (0.05-0.18), *C. semilunana* (0.05-0.18), *C. stigmata* (0.06-0.05), *C. wraggae* (0.02-0.16), *Cryptophlebia* sp. (0.03-0.18), *D. epijarbus* (0.12-0.17), *T. leucotreta* (0.11-0.19) and *T. zophophanes* (0.10-0.24). Sequence divergence at COI of >2% is used for species discrimination in lepidopterans (Hebert *et al*., 2003). Low sequence divergences, ranging from 0% to 1.2% have been found within many species of lepidopteran species (Zakharov *et al*., 2004; Win *et al*., 2015) which may be due to presence of intraspecific hybridization (Hebert *et al*., 2003). Moreover, the gap between maximum intraspecific and minimum interspecific distances has been used for species delinitation in various animal groups (Meyer and Paulay, 2005; Puillandre *et al*., 2012). Variation in the nucleotide sequence is a fundamental property of all living organisms, and may be used for their identification and phylogenetic status.

Table 4.6 Interspecific genetic distance (K2P) of fruit borers of litchi and their related species based on partial COI sequences that have two or more sequences of fruit borers with minimum, maximum and average values

Spp[a].	***C. c***	***C. l***	***C. s***	***C. ca***	***C. i***	***C. ir***	***C. o***	***C. pa***	***C. pe***	***C. r***	***C. se***	***C. st***	***C. w***	***C. sp.***	***D. e***	***T. l***	***T. z***
C. pu[b]	0.15 0.24 (0.17)	0.15 0.22 (0.17)	0.15 0.29 (0.19)	0.15 0.23 (0.17)	0.15 0.23 (0.17)	0.15 0.24 (0.18)	0.14 0.28 (0.18)	0.15 0.23 (0.18)	0.14 0.24 (0.17)	0.15 0.23 (0.18)	0.16 0.24 (0.18)	0.15 0.23 (0.18)	0.14 0.22 (0.16)	0.15 0.23 (0.18)	0.15 0.24 (0.17)	0.16 0.25 (0.19)	0.14 0.22 (0.17)
C. c		0.07 0.08 (0.08)	0.07 0.08 (0.07)	0.15 0.16 (0.15)	0.15	0.15	0.13 0.36 (0.17)	0.13 0.14 (0.13)	0.14 0.15 (0.15)	0.16	0.16	0.14 0.16 (0.15)	0.15	0.14 0.15 (0.15)	0.14 0.15 (0.14)	0.17 0.18 (0.17)	0.16 0.18 (0.17)
C. l			0.05 0.11 (0.06)	0.14 0.15 (0.15)	0.13	0.14 0.15 (0.14)	0.13 0.22 (0.15)	0.12	0.14 0.15 (0.15)	0.14 0.15 (0.15)	0.14	0.14	0.13 0.14 (0.13)	0.13 0.15 (0.13)	0.12 0.13 (0.12)	0.15 0.16 (0.15)	0.15 0.18 (0.16)
C. s				0.14 0.18 (0.15)	0.13 0.17 (0.14)	0.13 0.17 (0.14)	0.13 0.33 (0.16)	0.12 0.15 (0.12)	0.14 0.22 (0.15)	0.14 0.19 (0.15)	0.13 0.17 (0.14)	0.13 0.16 (0.14)	0.13 0.17 (0.14)	0.13 0.19 (0.14)	0.13 0.17 (0.14)	0.15 0.20 (0.16)	0.16 0.18 (0.24)
C. ca					0.05	0.05 0.06 (0.06)	0.05 0.14 (0.06)	0.05	0.06 0.07 (0.06)	0.05 0.06 (0.06)	0.05 0.06 (0.06)	0.06	0.05	0.05 0.06 (0.06)	0.13 0.14 (0.13)	0.11	0.10 0.11 (0.11)
C. i						0.07	0.00 0.09 (0.03)	0.06	0.07	0.07	0.05	0.05 0.06 (0.06)	0.02	0.00 0.05 (0.03)	0.13	0.11	0.10 0.11 (0.10)
C. ir							0.06 0.16 (0.08)	0.06	0.07	0.07 0.08 (0.08)	0.06	0.06	0.07	0.06 0.07 (0.07)	0.14 0.15 (0.14)	0.13	0.11 0.12 (0.11)
C. o								0.06 0.15 (0.07)	0.06 0.28 (0.09)	0.07 0.16 (0.08)	0.05 0.14 (0.07)	0.05 0.15 (0.07)	0.01 0.11 (0.03)	0.01 0.15 (0.05)	0.13 0.21 (0.15)	0.11 0.21 (0.13)	0.09 0.33 (0.12)

C. pa									0.05 0.06 (0.06)	0.05	0.04 0.05 (0.05)	0.06 0.07 (0.06)	0.05	0.05 0.06 (0.06)	0.13 0.14 (0.14)	0.11	0.10 0.11 (0.11)
C. pe										0.06 0.07 (0.07)	0.06	0.06 0.07 (0.06)	0.07 0.08 (0.07)	0.07	0.15	0.13	0.10 0.12 0.11
C. r											0.05 0.07 (0.05)	0.06 0.07 (0.07)	0.07	0.06 0.07 (0.07)	0.15	0.12	0.12
C. se												0.05	0.05 0.06 (0.05)	0.04 0.07 (0.05)	0.14	0.11 0.12 (0.11)	0.11 0.12 (0.11)
C. st													0.06	0.05 0.07 (0.06)	0.13 0.15 (0.14)	0.10 0.11 (0.11)	0.11
C. w														0.02 0.06 (0.04)	0.13 0.14 (0.14)	0.11	0.09 0.10 (0.10)
C. sp.															0.13 0.14 (0.13)	0.11 0.12 (0.11)	0.10 0.12 (0.11)
D. e																0.14 0.15 (0.14)	0.14 0.16 (0.15)
T. l																	0.12

[a]*C. pu, C. punctiferalis*; *C. c, C. cramerella*; *C. l, C. litchiella*; *C. s, C. sinensis*; *C. ca, C. caulicola*; *C. i, C. illepida*; *C. ir, C. iridosoma*; *C. o, C. ombrodelta*; *C. pa, C. pallifimbria*; *C. pe, C. peltastica*; *C. r, C. rhynchias*; *C. se, C. semilunana*; *C. st, C. stigmata*; *C. w, C. wraggae*; *C. sp., Cryptophlebia* sp.; *D. e, D. epijarbus*; *T. l, T. leucotreta*; *T. z, T. zophophanes*

[b]Range and mean of pair-wise genetic distances of fruit borers of litchi and their related species. The mean values are indicated in parenthesis. The actual value is provided wherever only single pair is involved.

4.1.2.6 Phylogenetic tree analysis

Molecular phylogenetic tree was constructed for the COI gene sequences of fruit borers of litchi and other related species using the Neighbor Joining method. The NJ tree showed that all sequences from the eighteen fruit borer species unambiguously clustered into four separate groups with already published sequences in Genbank and identified as *C. punctiferalis, C. sinensis, C. ombrodelta*, *D. epijarbus,* and *T. zophophanes* (Fig 4.21). Clear groups of the species belonging to *Conogethes*, *Conopomorpha*, *Cryptophlebia*, *Deudorix* and *Thaumatotibia* genera are observed. The samples of *C. ombrodelta* of the family Tortricidae were much closely related to the samples prevailing in Bangladesh rather than other individuals. The species, *C. ombrodelta* is found as a sister group to the clade of *Cryptophlebia* sp. and *Cryptophlebia illepida*. In the present study, *T. zophophanes* was found as a sister group to the clade *T. leucotreta*. Earlier, the genera *Cryptophlebia* and *Thaumatotibia* were often treated as congeneric. However, it is proved that, these species are quite distinct based on male and female genital characters. Moreover, the molecular studies confirmed that they are distinct genera, and are not even sister genera. Hence, in the current study, it was formed as a separate clade clearly distinguishing from the genera *Cryptophlebia*.

The phylogenetic tree analysis showed, *C. sinensis* as a sister group to the clade, *C. litchiella*. Besides, the clade (*C. sinensis* + *C. litchiella*) is much closer to the clade, *C. cramerella* under the family Gracillariidae. The species, *D. epijarbus* of the family Lycaenidae is found very close to the individuals of Malaysia. The species, *C. punctiferalis* was found very close to the specimens prevailing in Pakistan. Thus, the five fruit borer species of litchi have the following relationships: (*C. punctiferalis* + (*C. sinensis* + *D. epijarbus*) + (*T. zophophanes* + *C. ombrodelta*).

Thus, different borer species used in this study constitute a single

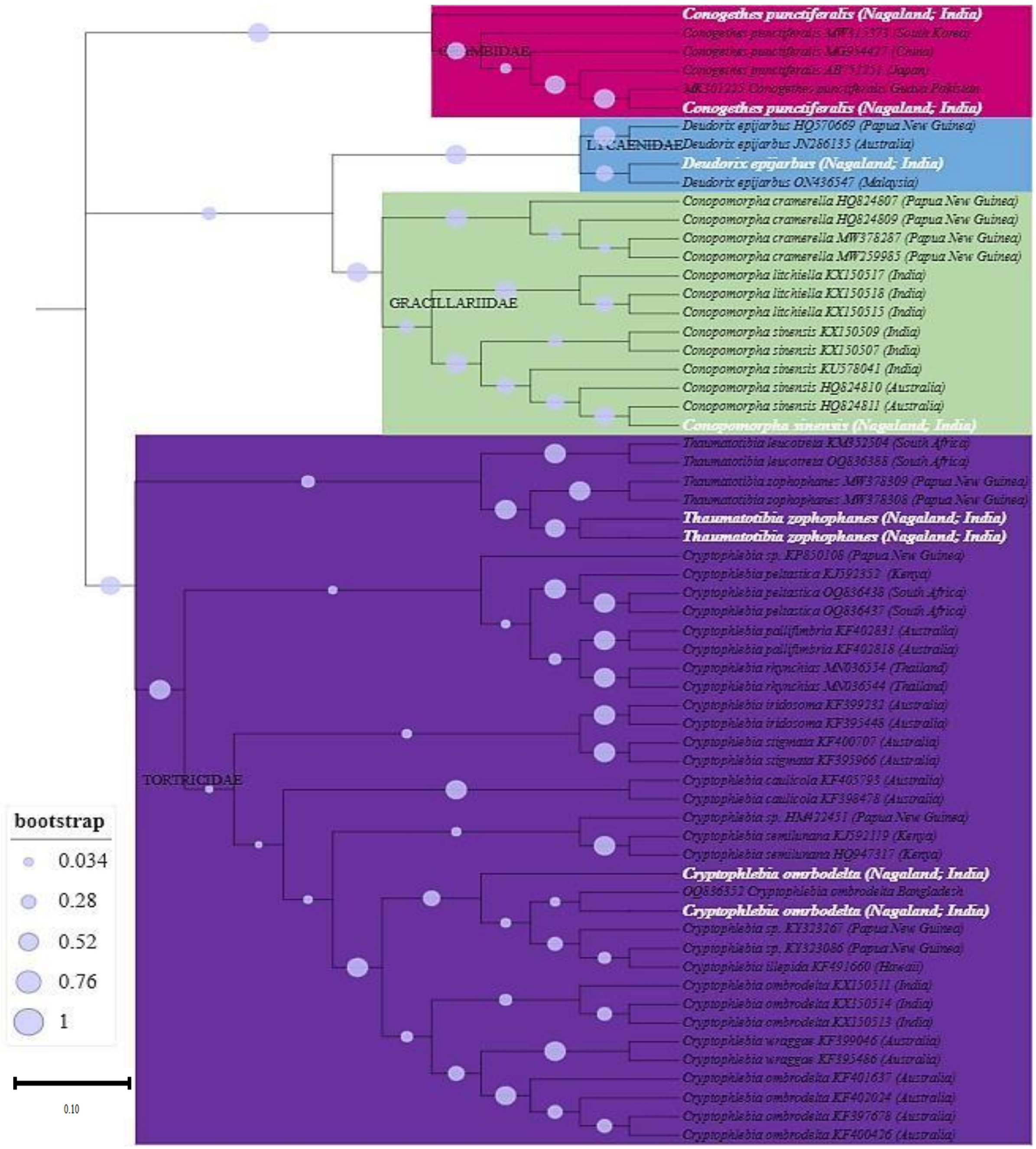


Figure 4.21 Neighbor joining tree showing genetic relationships of litchi fruit borers and their related species collected from litchi and different crops based on COI sequences. Bootstrap values above 50% (1,000 replicates) are show in circles on each clade obtained through kimura 2-parameter model (K2P) distance

lineage of closely related species. In present study, we have not observed adult moths of *C. cramerella*, *C. litchiella* and *Gatesclarkeana* sp. from infested fruits of litchi in Nagaland, which are considered as major fruit borer species of litchi in other states (Bhatia *et al*., 2000; Nair and Sahoo, 2006; Srivastava *et al*., 2017). This was confirmed through genetic variations and phylogenetic analysis among present moths emerged from infested fruit of litchi and NCBI deposited sequences. Therefore, it is concluded that *C. sinensis* as the major species causing infestation in litchi. However, other borer species needs to be confirmed through large sampling from litchi infested fruits.

4.2 To study the biology of litchi fruit borer(s), *Conopomorpha sinensis* Bradley (Lepidoptera: Gracillariidae)

In the present investigation, the biology of *C. sinensis* on litchi was studied at Department of Entomology laboratory, SAS, Medziphema, Nagaland University, Nagaland during April-June, 2022-23. During the period of investigation, the mean maximum and minimum temperatures recorded in the laboratory were 26ºC and 32ºC, respectively with mean relative humidity of 67.6%. The results on the biology of *C. sinensis* on litchi are presented (Tables 4.7, 4.8 & Plates 4.16-4.19) and discussed here.

4.2.1 Egg period

The female moth laid eggs singly on the surface of fruits *i.e*., near the pedicel. The freshly laid eggs were initially light-yellow orange in colour, flattened, and scale like (Plate 4.16). The incubation period ranged from 3.0-5.0 days and a mean of 3.45 days (Table 4.7). The length and width of eggs ranged from 0.42-0.50 mm and 0.20-0.27 mm, respectively. The average length and width of eggs ranged from 0.45 mm and 0.23 mm, respectively (Table 4.8).

4.2.2 Larval instars

During the larval period, the larva moulted four times and thus had five larval instars. The duration taken from first larval instar to last larval instar and their morphometric studies were presented and discussed below.

4.2.2.1 First instar larva

The newly hatched larva is transparent, milky white in colour with well-developed light brownish head capsule. The larval body is covered with dense white hairs on lateral sides (Plate 4.16).

The duration of first instar larvae ranged from 2.0 - 3.0 days with an average of 2.30 days (Table 4.7). The length and width of the larvae ranged from 3.50 - 3.65 mm and 0.28 - 0.32 mm, respectively. The average length and width of the larvae were 3.58 mm and 0.30 mm, respectively. The weight of the larvae ranged from 4.00 - 5.00 mg with an average of 4.50 mg (Table 4.8). The width of head capsules ranged from 0.09 - 0.12 mm. The average width of head capsule was 0.11 mm (Table 4.7 & Plate 4.17).

4.2.2.2 Second instar larva

The second instar larva is stout and creamy white in colour with a distinct brownish head capsule (Plate 4.16).

4.2.2.2 Second instar larva

The second instar larva is stout and creamy white in colour with a distinct brownish head capsule (Plate 4.16).

The duration of second instar larvae ranged from 1.0 - 2.0 days with a mean of 1.60 days (Table 4.7). The length and width of the larvae ranged from 3.62 - 3.85 mm and 0.35 - 0.46 mm, respectively. The average length and width of the larvae were 3.75 mm and 0.42 mm, respectively. The weight of the larvae ranged from 5.00 - 6.00 mg with an average of 5.60 mg (Table 4.8). The width

Table 4.7 Biology of litchi fruit borer, *Conopomorpha sinensis* (Lepidoptera: Gracillariidae)

Stages	Mean ± S.D.	Range
EGG		
Incubation period (days)	3.45 ± 1.13	3.0 - 5.0
LARVA		
I instar	2.30 ± 0.48	2.0 - 3.0
II instar	1.60 ± 0.52	1.0 - 2.0
III instar	1.80 ± 0.42	1.0 - 2.0
IV instar	2.90 ± 0.57	2.0 - 4.0
V instar	2.50 ± 0.53	2.0 - 3.0
Head capsule width*** - I moult	0.11 ± 0.01	0.09 - 0.12
II moult	0.18 ± 0.02	0.13 - 0.21
III moult	0.34 ± 0.01	0.31 - 0.36
IV moult	0.58 ± 0.04	0.53 - 0.66
V moult	0.64 ± 0.06	0.55 - 0.72
Total larval period (days)	11.10 ± 2.52	8.0 - 14.0
PUPA*		
Prepupal period (days)	2.05 ± 0.51	1.0 - 3.0
Pupal period (days)	5.85 ± 0.88	4.0 - 7.0
Total pupal period	7.90 ± 1.39	5.0 - 10.0
ADULT*		
Male adult longevity (days)	5.55 ± 1.00	4.0 - 7.0
Female adult longevity (days)	9.00 ± 1.12	7.0 - 11.0
TOTAL LIFE CYCLE		
Male (days)	30.70 ± 0.52	20.0 - 36.0
Female (days)	32.50 ± 0.43	23.0 - 40.0
Pre-mating period (days)	2.70 ± 0.48	2.0-3.0
Pre-oviposition period (days)	2.30 ± 0.48	2.0-3.0
Oviposition period (days)*	5.70 ± 0.82	5.0-7.0
Fecundity**	33.10 ± 6.84	25-43
Egg hatchability (%)	45.34 ± 7.56	32.0-55.5

Note: n = 10; *mean of 20 pupae/adults; **in numbers; ***values in millimeter; % Percent

Table 4.8 Morphometrics of litchi fruit borer, *Conopomorpha sinensis* Bradley (Lepidoptera: Gracillariidae)

Life stages	Length (mm)		Width (mm)		Weight (mg)	
	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.	Range
EGG	0.45 ± 0.04	0.42-0.50	0.23 ± 0.01	0.20-0.27	-	-
LARVA						
I instar	3.58 ± 0.04	3.50 - 3.65	0.30 ± 0.01	0.28 - 0.32	4.50 ± 0.53	4.00 - 5.00
II instar	3.75 ± 0.08	3.62 - 3.85	0.42 ± 0.04	0.35 - 0.46	5.60 ± 0.52	5.00 - 6.00
III instar	4.41 ± 0.27	3.90 - 4.66	0.56 ± 0.02	0.51 - 0.59	7.70 ± 0.82	7.00 - 9.00
IV instar	7.83 ± 0.41	7.01 - 8.21	0.72 ± 0.05	0.63 - 0.79	13.10 ± 2.28	10.00 - 16.00
V instar	7.86 ± 0.26	7.47 - 8.33	0.92 ± 0.06	0.79 - 1.00	23.90 ± 4.12	17.00 - 29.00
PUPA						
Male/Female*	8.06 ± 0.56	7.35 - 9.21	0.89 ± 0.05	0.81 - 0.96	7.00 ± 1.52	5.00 - 9.00
ADULT						
Male*	6.50 ± 0.08	6.12-6.79	0.50 ± 0.02	0.48-0.52	6.25 ± 0.72	5.00 - 7.00
Female*	6.67 ± 0.05	6.43-6.92	0.52 ± 0.02	0.50-0.54	9.60 ± 1.90	8.00 - 13.00

Note: n=10; *Mean of 20 males/females; S.D. - Standard Deviation of head capsules ranged from 0.13-0.21. The average width of head capsule was 0.18 mm (Table 4.7 & Plate 4.17).

4.2.2.3 Third instar larva

The third instar larva is thick, creamy white in colour with a well-developed blackish head. The prolegs on 1st, 2nd and 3rd abdominal segments are prominent. Larva feed actively on the seed neck (Plate 4.16).

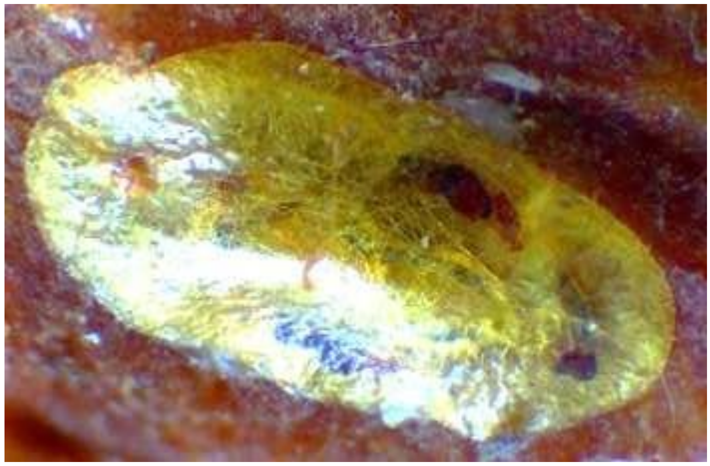
The duration of third instar larvae ranged from 1.0 - 2.0 days with the mean of 1.80 days (Table 4.7). The length and width of the larvae ranged from 3.90 - 4.66 mm and 0.51 - 0.59 mm, respectively. The average length and width of the larvae were 4.41 mm and 0.56 mm, respectively. The weight of the third instar larvae ranged from 7.00 -9.00 mg with an average of 7.70 mg (Table 4.8). The width of third instar head capsules ranged from 0.31 - 0.36 mm. The average width of head capsule was 0.34 mm (Table 4.7 & Plate 4.17).

4.2.2.4 Fourth instar larva

The larva is yellowish cream in colour. Head is brown in colour. The prolegs on 1st, 2nd and 3rd abdominal segments are not well developed. Larva feeding actively on the pulp of fruits *i.e.*, near the pedicel. (Plate 4.16).

The duration of fourth instar larvae ranged from 2.0 - 4.0 days with the mean of 2.90 days (Table 4.7). The length and width of the larvae ranged from 7.01 - 8.21 mm and 0.63 - 0.79 mm, respectively. The average length and width of the larvae were 7.83 mm and 0.72 mm, respectively. The weight of the larvae ranged from 10.00 - 16.00 mg with an average of 13.10 mg (Table 4.8). The width of head capsules ranged from 0.53 - 0.66 mm. The average width of head capsule was 0.58 mm (Table 4.7 & Plate 4.17).

4.2.2.5 Fifth instar larva



Freshly laid egg

1st instar 2nd instar 3rd instar 4th instar 5th instar

Plate 4.16 Egg and larval instars of *Conopomorpha sinensis* Bradley (Dorsal view)

The final instar larva is light green in colour with brownish head. The prolegs on 1st, 2nd and 3rd abdominal segments are well developed. The larva feeds within the seed neck (Plate 4.16).

The duration of fifth instar larvae ranged from 2.0-3.0 days with the mean of 2.50 days (Table 4.7). The length and width of the larvae ranged from 7.47 - 8.33 mm and 0.79 - 1.00 mm, respectively. The average length and width of the larvae were 7.86 mm and 0.92 mm, respectively. The weight of the fifth instar larvae ranged from 17.00 -29.00 mg with an average of 23.90 mg (Table 4.8). The width of the head capsules ranged from 0.55 - 0.72 mm. The average width of head capsule was 0.64 m (Table 4.7 & Plate 4.17).

4.2.2.6 Total larval period (days)

The total larval period of *C. sinensis* ranged from 8.0 - 14.0 days with an average of 11.10 days (Table 4.7).

4.2.3 Pre-pupa

At the end of the fifth instar, larva becomes inactive, cessation of feeding and contracted their body with reduced size. The larva undergoes pupation in a white transparent cocoon either on the upper or lower surface of leaves mostly towards the leaf margin or apex. The cocoon is oval in shape, smooth and brown yellowish in colour. On the surface of cocoon, there are 5-6 small, white bubbles like spheres (Plate 4.17).

The duration of pre-pupa ranged from 1.0-3.0 days with an average of 2.05 days (Table 4.7).

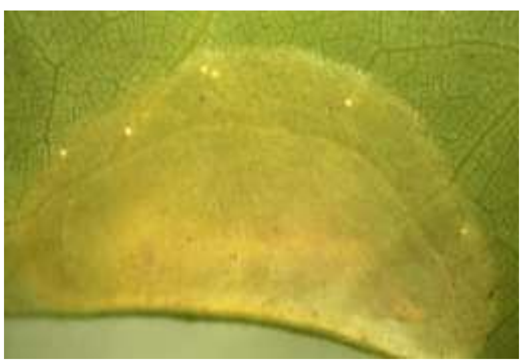
4.2.4 Pupa

The pupa is yellowish in colour, slender and have black prominent eyes in the final stage of development. The appendices like maxillary palpi, labial

1st instar 2nd instar 3rd instar 4th instar 5th instar

Fully grown larva pupate on litchi surface under spinning cocoon

Pupa inside a cocoon



Cocoon with scattered white bubbles

Close-up of white bubble

Exuviae hold on the open cocoon after the emergence of adult

Plate 4.17 Head capsules of different instars and cocoon of *Conopomorpha sinensis* Bradley

palpi, eyes, antennae, legs and wings are clearly visible. The antenna is longer than the caudal apex of the abdomen and is almost 1/3 times longer than the entire pupa. The head possesses a prominent frontal process arises from the front. After the completion of pupal stage, this frontal process is used to cut open the cocoon allowing the adult eclose from the pupal case, which remains half inside the cocoon (Plate 4.17).

The duration of pupa ranged from 4.0 - 7.0 days with an average of 5.85 days (Table 4.7). The length of the pupae, including the body and antenna ranged from 7.35 - 9.21 mm. While, the width of pupae ranged from 0.81 - 0.96 mm. The average length and width of the pupae were 8.06 mm and 0.89 mm, respectively. The weight of the pupa ranged from 5.00- 9.00 mg with an average of 7.00 mg (Table 4.8 & Plate 4.18).

4.2.4.1 Sexual dimorphism in pupa

Male and female pupa are easily distinguished when the pupae are viewed from the ventral side *i.e.*, from 7^{th} to 10^{th} abdominal segments.

4.2.4.1.1 Male pupa

In male, genital opening is present in the middle of the 9^{th} segment and found in between the two marginal tubercles present on the anterior part of 9^{th} segment, and the two tubercles present on 10^{th} segment are located together on the ventrum with prominent spines (Plate 4.18).

4.2.4.1.2 Female pupa

In female, genital opening or slit is located in between the 8^{th} and 9^{th} segment and above the two marginal tubercles located on 9^{th} segment. In the 8^{th} segment, a plateau can be seen and on the seventh segment, a longitudinal ridge is present on anterior segments (Plate 4.18).

Pupa yellowish with well developed eyes, maxillary palpi, labial palpi, proboscis, legs, wings and antenna in the initial stage of development

Pupa turns black in the final stage of development

Pupal head showing a prominent frontal process (ventral and lateral view) arising from the front

Male genital opening located in the middle of 9th segment

Female genital opening or slit located between the 8th and 9th segment

Plate 4.18 Pupal stage of *Conopomorpha sinensis* Bradley

4.2.5 Adult

Adults (male and female) are micro lepidopterans, smaller in size, greyish brown with a yellowish-brown wing apex. Hindwings are silver grey. They are easily recognized by its long silverish antennae, folds back above the wings at rest. In the field, adults can be seen resting below the horizontal branches during day-time (Plate 4.19).

4.2.5.1 Sexual dimorphism in adult

Male and female are differentiated by examining the caudal abdominal segments. In male, the caudal abdominal segment is black in colour and broader. Also, the valva of male genitalia can be seen clearly. Whereas, in female, the caudal abdominal segment is compressed laterally and sterna being white. Also, the ovipositor with hairy anal papillae can be observed (Plate 4.19).

4.2.5.2 Male adult longevity

The longevity of male adult ranged from 4.0 - 7.0 days with an average of 5.55 days (Table 4.7). The length and width of male adults ranged from 6.12-6.79 mm and 0.48-0.52 mm, respectively. The average length and width of male adults were 6.50 mm and 0.50 mm, respectively. The weight of the male adults ranged from 5.00 - 7.00 mg with an average of 6.25 mg (Table 4.8).

4.2.5.3 Female adult longevity

Longevity of female adults ranged from 7.0 - 11.0 days with an average of 9.00 days (Table 4.7). The length and width of female adults ranged from 6.43-6.92 mm and 0.50-0.54 mm, respectively. The average length and width of male adults were 6.67 mm and 0.52 mm, respectively. The weight of the female adults ranged from 8.00 - 13.00 mg with an average of 9.60 mg (Table 4.8).

4.2.6 Pre-mating, pre-oviposition and oviposition period

Male

Female

In male, the caudal abdominal segment is black in colour and broader (left) and the valva of male genitalia (right) can be seen clearly

Female caudal segment is compressed laterally, with white sterna (left) and hairy anal papillae covering ovipositor can be seen (right)

Plate 4.19 Adult stage of *Conopomorpha sinensis* Bradley

The pre-mating, pre-oviposition and oviposition period ranged from 2.0-3.0, 2.0-3.0 and 5.0-7.0 days with an average of 2.70, 2.30 and 5.70 days, respectively (Table 4.7).

4.2.7 Fecundity

Average number of eggs were 33.10 with a range of 25-43 (Table 4.7).

4.2.8 Egg hatchability

The egg hatchability percentage ranged from 32.00-55.50 with an average of 45.34 (Table 4.7).

4.2.9 Total life cycle

The total life cycle of male *C. sinensis* ranged from 20-36 days with an average of 30.70 days. While the female ranged from 23-40 days with an average of 32.50 days (Table 4.7).

The above results are in accordance with Singh (1992), Hung *et al*. (2002), Schulte *et al*. (2007), Srivastava *et al*. (2019), and Niogret *et al*. (2019), who also reported the duration of egg, larva and pupa were 2.8, 10.3 and 7.1 days, respectively. He also reported the longevity of adult male and female were 20.0 and 19.3 days, respectively. In the present study, the findings of head capsule are almost similar with the findings of Zhi (2015), who recorded the average head capsule of 1st, 2nd, 3rd, 4th and 5th instar was 0.150 mm, 0.170 mm, 0.265 mm, 0.435 mm and 0.652 mm, respectively. Further, he reported the duration of egg, 1st, 2nd, 3rd, 4th, 5th instars, pupa and pre-oviposition were 2.09-7.73, 1.17-4.50, 1.40-2.09, 1.00-2.84, 1.18-3.41 1.37-3.00, 5.35-12.74, 4.22-4.75 and 19.34-41.16 days, respectively. The total life cycle was found to be 19.34-41.16 days.

The above findings of egg laying capacity were corroborated with the findings of Srivastava *et al*. (2019), who reported that female moth lays flattened, scale like eggs up to 50 in nos. In contrast, the study of Hung *et al*. (2002) have shown that female moth lays 234.8 eggs. The longevity of adults was in line with the findings of Sharma and Agrawal (1988), who recorded the adult longevity varies from 3.12 to 6.84 days. The information on biology of *C. sinensis* has been randomly scattered across various literatures by the above authors. However, there is no comprehensive information on this biology such as duration, and morphometrics data of all the stages. Hence, in the current study, duration and morphometrics data with images of various life stages were provided.

4.3 To study the seasonal incidence of litchi fruit borer(s) and their natural enemies

The present objective was carried out at two different locations *viz*., Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland and Farmers farm, Medziphema, Nagaland during April-June, 2022 and 2023. During the study, a total of five fruit borers were recorded infesting litchi fruits. Of these, litchi fruit borer, *C. sinensis* was considered as economically important pest. Hence, seasonal incidence of *C. sinensis* and its natural enemies were studied and presented below.

4.3.1 Seasonal incidence of litchi fruit borer, *C. sinensis* during April - June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

The data on the infestation of fruit borer was collected from the field and presented in Table 4.9, 4.10 & Fig 4.22. The litchi fruits set around 5th April, 2022 were considered for taking observation. The initial phase of infestation (0.33%) was found to appear on 16th April *i.e*., 19 days after fruit set when the fruits were small, tender, young, and having no pulp formation. Thereafter, the

Table 4.9 Period of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Medziphema, Nagaland

Sl. No.	Date	Days after fruit set	Infestation out of 100 fruits						Average % fruit borer infestation
			Replication I	Replication II	Replication III	Replication IV	Replication V	Replication VI	
1	09.04.2022	12	0	0	0	0	0	0	0.00
2	16.04.2022	19	0	0	0	1	1	0	0.33
3	23.04.2022	26	2	5	3	2	3	4	3.17
4	30.04.2022	33	3	3	5	3	4	5	3.83
5	07.05.2022	40	8	8	5	5	4	4	5.67
6	14.05.2022	47	17	12	3	5	8	5	8.33
7	21.05.2022	53	28	32	38	29	38	40	34.17
8	28.05.2022	59	42	51	48	50	47	48	47.67
9	04.06.2022	65	44	42	43	45	47	41	43.67
10	11.06.2022	71	38	36	34	38	33	32	35.17
11	18.06.2022	77	27	24	26	31	28	25	26.83
12	25.06.2022	83	18	14	16	13	14	16	15.17

Table 4.10 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Sl. No.	Date	Days after fruit set	Average fruit borer infestation (%)	Average temperature (^{0}C)	Average Relative Humidity (%)	Cumulative rainfall (mm)
1	09.04.2022	12	0.00	24.60	80.50	95.30
2	16.04.2022	19	0.33	24.80	84.00	35.50
3	23.04.2022	26	3.17	26.00	78.00	18.30
4	30.04.2022	33	3.83	26.50	74.00	42.20
5	07.05.2022	40	5.67	24.70	80.00	74.80
6	14.05.2022	47	8.33	27.00	82.00	110.60
7	21.05.2022	53	34.17	25.50	88.00	10.90
8	28.05.2022	59	47.67	27.00	81.00	22.50
9	04.06.2022	65	43.67	28.30	79.00	51.10
10	11.06.2022	71	35.17	28.40	83.00	46.70
11	18.06.2022	77	26.83	27.30	86.00	34.80
12	25.06.2022	83	15.17	27.80	84.00	9.90

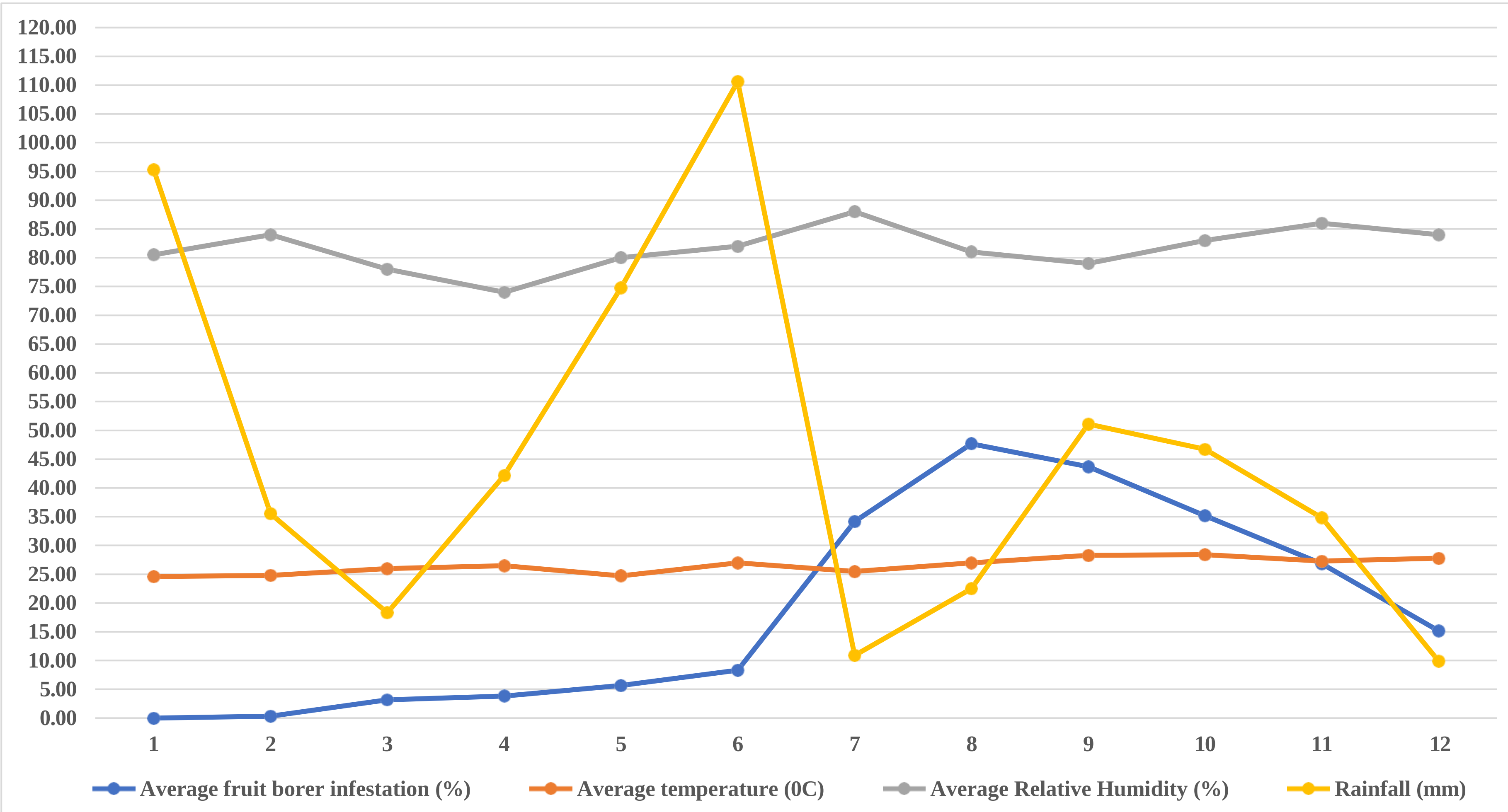


Figure 4.22 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

infestation gradually increased up to 47.67% on 28th May *i.e.*, 59 days after fruit set when the fruits start developing reddish pink coloration leading to the stage of maturation. Then, in an erratic tune the infestation declined to 15.17% on 25th June *i.e.*, 83 days after fruit set.

The regression result for the effect of average temperature, average relative humidity, and average rainfall on the average infestation of fruit by borers was shown in Table 4.11. The results from the analysis showed that the regression coefficient of determination R^2 was 0.518, which means about 51.8% of the variation in infestation was explained by means of temperature, relative humidity, and rainfall. The remaining 48.2% was mostly due to other variables external to the regression model. It is observed from Table 4.11, the average infestation percent of fruit borer is positively correlated with average temperature, average relative humidity, and rainfall. The regression results also reveal that for every unit increase in temperature there is a 77.26% positive effect, for every unit increase in relative humidity there is a 20.06% positive effect, and for every unit increase in rainfall there is a 7.61% positive effect on degree of infestation by fruit borer. It is evident from the present study that the activity of the pest species has a profound influence of average temperature (B=7.726) and is positively correlated with the factors like temperature and relative humidity. Relative humidity was found to have less impact (2.006) than average temperature on fruit infestation. Moreover, from the statistical analysis, it is also evident that rainfall has little influence on the activity of the pest species (B=0.761) because of the fact that fruits were harvested before the rainy season. The regression output shows that all the independent variables are statistically significant ($p<0.05$). This significance indicates that changes in the independent variables correlate with shifts in the dependent variable. Therefore, the regression equation is $Y = 344.767 + 7.727 (X_1) + 2.006 (X_2) + 0.761 (X_3)$.

Table 4.11 Multiple regression result on the effect of mean temperature, relative humidity and rainfall on litchi fruit borer infestation during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Variables	**Coefficients**	**Standard Error**	**t Stat**	**p-value**
Constant	-344.767	134.652	-2.560	0.034
Average temperature (X_1)	7.726	3.258	2.371	0.045
Average relative humidity (X_2)	2.006	1.468	1.366	0.038
Cumulative rainfall (X_3)	0.761	1.190	0.640	0.050
R^2	0.518			
Adjusted R^2	0.337			
F value	2.865			
Regression equation	$Y = -344.767 + 7.726 (X_1) + 2.006 (X_2) + 0.761 (X_3)$			

4.3.2 Seasonal incidence of litchi fruit borer, *C. sinensis* during April - June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

The data on the infestation of fruit borer was collected from the field and presented in Table 4.12, 4.13 & Fig 4.23. The litchi fruits set around 5th April, 2023 were considered for taking observation. The primary phase of infestation (0.17%) was found to appear on 10th April *i.e.*, 13 days after fruit set when the fruits were small, tender, young, and having no pulp formation. Thereafter, the infestation gradually increased up to 48.33% on 29th May *i.e.*, 60 days after fruit set when the fruits start developing reddish pink coloration leading to the stage of maturation. Then, in an erratic tune the infestation declined to 14.83% on 26th June *i.e.*, 84 days after fruit set.

The regression result for the effect of average temperature, average relative humidity, and average rainfall on the average infestation of fruit by borers was shown in Table 4.14. The results from the analysis showed that the regression coefficient of determination R^2 was 0.589, which means about 58.9% of the variation in infestation was explained by means of temperature, relative humidity, and rainfall. The remaining 41.1% was mostly due to other variables external to the regression model. It is observed from Table 4.14, the average temperature, average relative humidity, and rainfall. The regression results also reveal that for every unit increase in temperature there is a 52.72% positive effect, for every unit increase in relative humidity there is a 5.51% positive effect, and for every unit increase in rainfall there is a 2.74% positive effect on degree of infestation by fruit borer. It is evident from the present study that the activity of the pest species has a profound influence of average temperature (B=5.272) and is positively correlated with the factors like temperature and relative humidity. Relative humidity was found to have less impact (0.551) than average temperature on fruit infestation. Also, from the statistical analysis, it is evident

Table 4.12 Period of litchi fruit borer, *C. sinensis* infestation during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Medziphema, Nagaland

Sl. No.	Date	Days after fruit set	Infestation out of 100 fruits						Average % fruit borer infestation
			Replication I	Replication II	Replication III	Replication IV	Replication V	Replication VI	
1	10.04.2023	13	0	0	0	1	0	0	0.17
2	17.04.2023	20	0	2	0	0	1	0	0.50
3	24.04.2023	27	1	0	2	2	0	0	0.83
4	01.05.2023	34	2	5	3	2	2	3	2.83
5	08.05.2023	41	5	2	3	3	4	5	3.67
6	15.05.2023	48	13	12	11	10	12	10	11.33
7	22.05.2023	54	26	36	42	25	31	32	32.00
8	29.05.2023	60	56	52	48	50	42	45	48.83
9	05.06.2023	66	48	45	42	39	40	40	42.33
10	12.06.2023	72	40	35	32	31	32	36	34.33
11	19.06.2023	78	31	29	25	23	23	25	26.00
12	26.06.2023	84	20	18	16	12	10	13	14.83

Table 4.13 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Sl. No.	Date	Days after fruit set	Average fruit borer infestation (%)	Average temperature (^{0}C)	Average Relative Humidity (%)	Rainfall (mm)
1	10.04.2023	13	0.17	23.45	59.36	0.00
2	17.04.2023	20	0.50	27.41	69.43	27.70
3	24.04.2023	27	0.83	25.01	72.50	20.20
4	01.05.2023	34	2.83	26.19	72.57	24.90
5	08.05.2023	41	3.67	27.16	66.71	0.00
6	15.05.2023	48	11.33	25.61	77.36	24.50
7	22.05.2023	54	32.00	27.60	68.86	35.20
8	29.05.2023	60	48.33	29.29	67.29	2.50
9	05.06.2023	66	42.33	30.51	72.57	77.10
10	12.06.2023	72	34.33	26.86	86.36	107.20
11	19.06.2023	78	26.00	26.91	84.36	63.20
12	26.06.2023	84	14.83	29.62	83.43	25.00

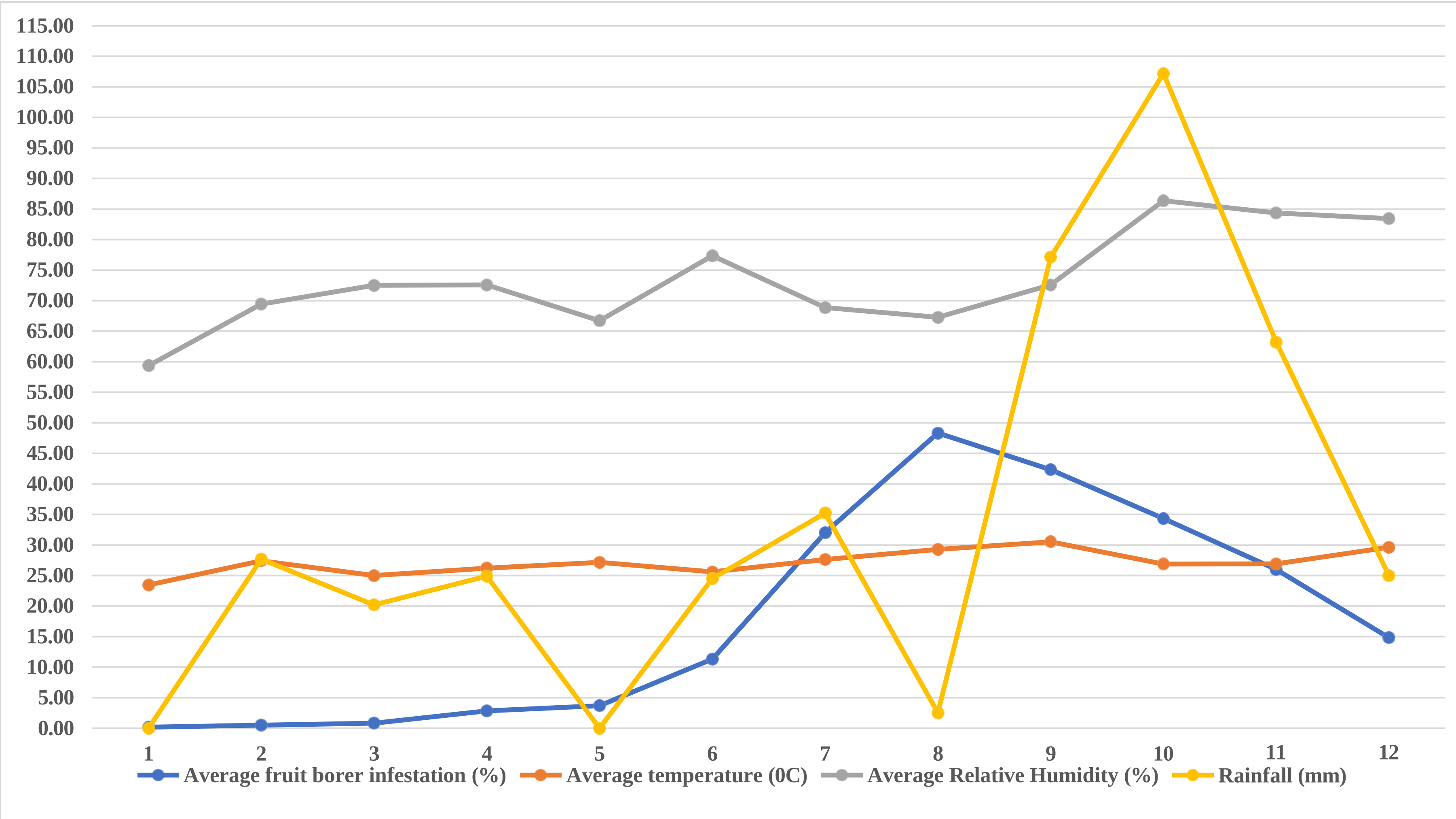


Figure 4.23 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Table 4.14 Multiple regression result on the effect of mean temperature, relative humidity and rainfall on litchi fruit borer infestation during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Variables	**Coefficients**	**Standard Error**	**t Stat**	**p-value**
Constant	-134.824	69.363	-1.352	0.021
Average temperature (X_1)	5.272	2.104	2.505	0.036
Average relative humidity (X_2)	0.551	0.695	0.793	0.045
Rainfall (X_3)	0.274	0.172	1.595	0.014
R^2	0.589			
Adjusted R^2	0.434			
F value	3.821			
Regression equation	$Y = -134.824 + 5.272 (X_1) + 0.551 (X_2) + 0.274 (X_3)$			

that rainfall has little influence on the activity of the pest species (B=0.274). The regression output shows that all the independent variables are statistically significant ($p<0.05$). This significance indicates that changes in the independent variables correlate with shifts in the dependent variable. Therefore, the regression equation is $Y = 93.824 + 5.272 (X_1) + 0.551 (X_2) + 0.274 (X_3)$.

4.3.3 Seasonal incidence of litchi fruit borer, *C. sinensis* during April - June, 2022 at Farmers farm, Medziphema, Nagaland

The data on the infestation of fruit borer was collected from the field and presented in Table 4.15, 4.16 & Fig 4.24. The litchi fruits set around 6th April, 2023 were considered for taking observation. The initial phase of infestation (0.50%) was found to appear on 10th April *i.e.*, 13 days after fruit set when the fruits were small, tender, young, and having no pulp formation. Subsequently, the infestation gradually increased up to 36.50% on 5th June *i.e.*, 66 days after fruit set when the fruits start developing reddish pink coloration leading to the stage of maturation. At that point, in an erratic tune the infestation declined to 11.00% on 26th June *i.e.*, 84 days after fruit set. The regression result for the effect of average temperature, average relative humidity, and average rainfall on the average infestation of fruit by borers was shown in Table 4.17.

The results from the analysis showed that regression coefficient of determination R^2 was 0.580, which means about 58.0% of the variation in infestation was explained by means of temperature, relative humidity, and rainfall. The remaining 42.0% was mostly due to other variables external to the regression model. It is observed from Table 4.17, the average infestation percent of fruit borer is positively correlated with average temperature, average relative humidity, and rainfall. The regression results also reveal that for every unit increase in temperature there is a 55.68% positive effect, for every unit increase in relative humidity there is an 8.08% positive effect, and for every unit increase in rainfall there is a 4.82% positive effect on degree of infestation by fruit borer.

Table 4.15 Period of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 at Farmers Farm, Medziphema, Nagaland

Sl. No.	Date	Days after fruit set	Infestation out of 100 fruits						Average % fruit borer infestation
			Replication I	Replication II	Replication III	Replication IV	Replication V	Replication VI	
1	10.04.2022	13	0	0	0	2	1	0	0.50
2	17.04.2022	20	0	1	0	0	0	1	0.33
3	24.04.2022	27	2	1	2	1	1	2	1.50
4	01.05.2022	34	1	2	1	3	2	3	2.00
5	08.05.2022	41	2	4	5	3	6	3	3.83
6	15.05.2022	48	6	12	15	8	11	6	9.67
7	22.05.2022	54	13	23	14	29	8	6	15.50
8	29.05.2022	60	30	25	34	35	25	25	29.00
9	05.06.2022	66	35	37	40	38	32	37	36.50
10	12.06.2022	72	28	25	32	24	24	23	26.00
11	19.06.2022	78	16	18	25	17	16	13	17.50
12	26.06.2022	84	13	9	15	12	8	9	11.00

Table 4.16 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2022 at Farmers farm, Medziphema, Nagaland

Sl. No.	Date	Days after fruit set	Average fruit borer infestation (%)	Average temperature (^{0}C)	Average Relative Humidity (%)	Cumulative rainfall (mm)
1	10.04.2022	13	0.50	24.60	86.50	95.30
2	17.04.2022	20	0.33	24.80	75.00	35.50
3	24.04.2022	27	1.50	26.00	74.00	18.30
4	01.05.2022	34	2.00	26.50	81.50	42.20
5	08.05.2022	41	3.83	24.70	80.50	74.80
6	15.05.2022	48	9.67	27.00	87.00	110.60
7	22.05.2022	54	15.50	25.50	82.50	10.90
8	29.05.2022	60	29.00	27.00	79.00	22.50
9	05.06.2022	66	36.50	28.30	84.00	51.10
10	12.06.2022	72	26.00	28.40	84.50	46.70
11	19.06.2022	78	17.50	27.30	85.00	34.80
12	26.06.2022	84	11.00	27.80	80.50	9.90

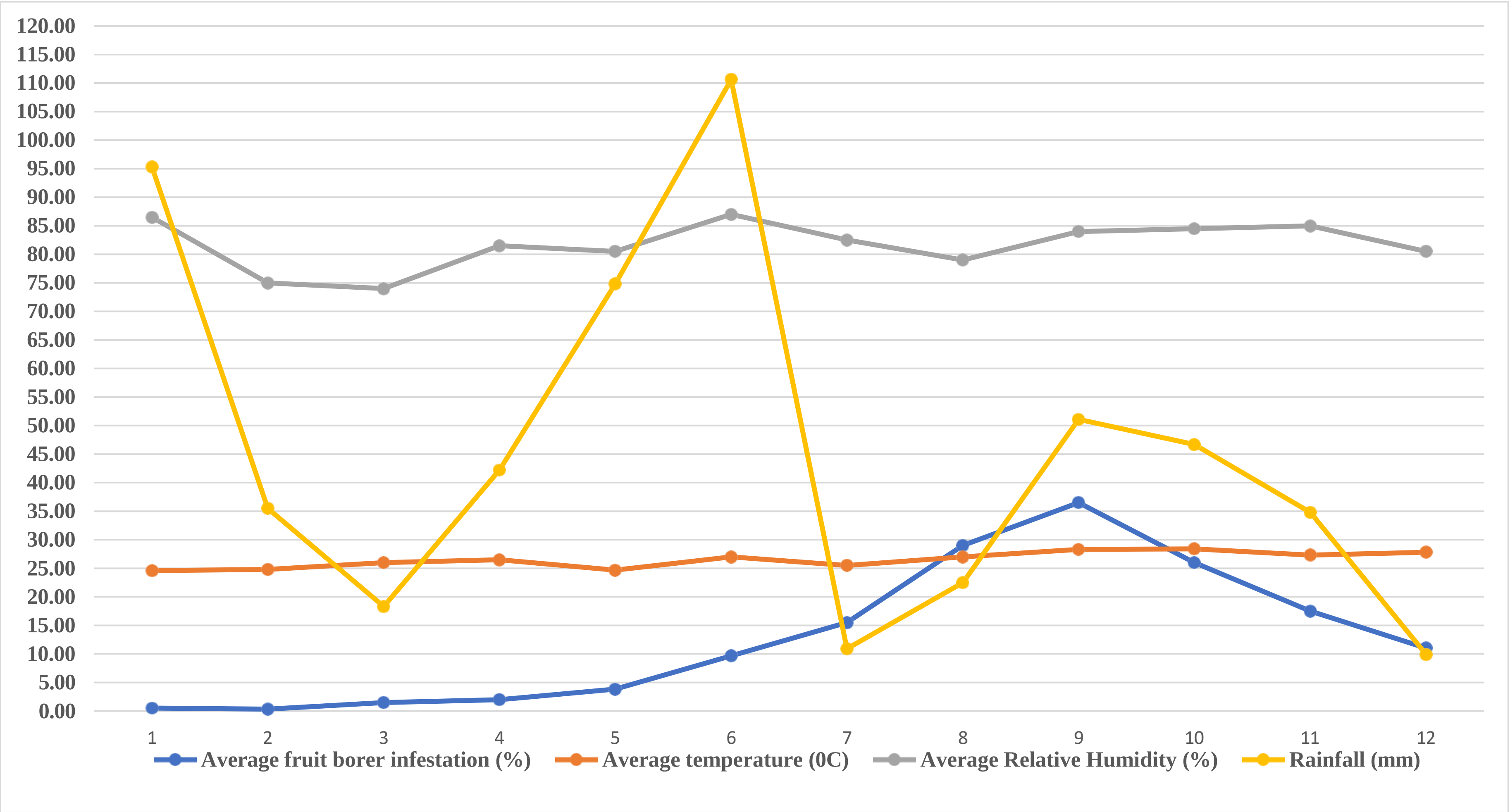


Figure 4.24 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2022 at Farmers farm, Medziphema, Nagaland

Table 4.17 Multiple regression result on the effect of mean temperature, relative humidity and rainfall on litchi fruit borer infestation during April-June, 2022 at Farmers farm, Medziphema, Nagaland

Variables	**Coefficients**	**Standard Error**	**t Stat**	**p-value**
Constant	-196.906	71.729	-2.745	0.025
Average temperature (X_1)	5.568	2.573	2.163	0.042
Average relative humidity (X_2)	0.808	1.032	0.782	0.035
Rainfall (X_3)	0.482	0.128	0.683	0.037
R^2	0.580			
Adjusted R^2	0.423			
F value	3.692			
Regression equation	$Y = -196.906 + 5.568 (X_1) + 0.808 (X_2) + 0.482 (X_3)$			

It is evident that the activity of the pest species has a profound influence of average temperature (B=5.568) and is positively correlated with the factors like temperature and relative humidity. Relative humidity was found to have less impact (B=0.808) than average temperature on fruit infestation. Also, from the statistical analysis, it is also evident that rainfall has little influence on the activity of the pest species (B=0.482). The regression output shows that all the independent variables are statistically significant ($p<0.05$). This significance indicates that changes in the independent variables correlate with shifts in the dependent variable. Therefore, the regression equation is $Y = 196.906 + 5.568 (X_1) + 0.808 (X_2) + 0.482 (X_3)$.

4.3.4 Seasonal incidence of litchi fruit borer, *C. sinensis* during April - June, 2023 at Farmers farm, Medziphema, Nagaland

The data on the infestation of fruit borer was collected from the field and presented in Table 4.18, 4.19 & Fig 4.25. The litchi fruits set around 6th April, 2023 were considered for taking observation. The initial phase of infestation (0.17%) was found to appear on 11th April *i.e.*, 14 days after fruit set when the fruits were small, tender, young, and having no pulp formation. Then, the infestation gradually increased up to 42.00% on 30th May *i.e.*, 61 days after fruit set when the fruits start developing reddish pink coloration leading to the stage of maturation. Later, in an erratic tune the infestation declined to 12.33% on 27th June *i.e.*, 85 days after fruit set.

The regression result for the effect of average temperature, average relative humidity, and average rainfall on the average infestation of fruit by borers was shown in Table 4.20 The results from the analysis showed that the regression coefficient of determination R^2 was 0.620, which means about 62.0% of the variation in infestation was explained by means of temperature, relative humidity, and rainfall. The remaining 38.0% was mostly due to other variables external to the regression model. It is observed from Table 4.20, the average infestation percent of fruit borer is positively correlated with average temperature, average relative humidity, and rainfall. The regression results also reveal that for every unit increase in temperature there is a 45.64% positive effect, for every unit increase in relative humidity there is a 4.81% positive effect, and for every unit increase in rainfall there is a 2.68% positive effect on degree of infestation by fruit borer.

It is evident from the present study that the activity of the pest species has a profound influence of average temperature (B=4.564) and is positively correlated with the factors like temperature and relative humidity. Relative humidity was found to have less impact (B=0.481) than average temperature on fruit infestation. Also, from the statistical analysis, it is also evident that rainfall has little influence on the activity of the pest species (B=0.268). The regression output shows that all the independent variables are statistically significant ($p<0.05$). This significance indicates that changes in the independent variables correlate with shifts in the dependent variable. Therefore, the regression equation is $Y = 80.947 + 4.564 (X_1) + 0.481 (X_2) + 0.268 (X_3)$.

Overall examination of litchi fruit borer, *C. sinensis* population data during April-June, 2022 and 2023 revealed, infestation started after fruit set during early April and continued up to the last week of June with severe infestation during last week of May to early June *i.e*., when the fruits were developing pink colorations. The initial infestation was first observed during 2nd week of April, when the fruits were pea nut size, small, tender, young and having no pulp formation. During this stage, larva after feeding for a while, come out of the fruit and feeds on another fresh fruit. This may be due to unsuitability of the fruit pulp. Also, the infestation was not remarkable in the early stages, due to high acidity and phenol content of fruits. Larva usually feeds on the fruit pulp near the peduncle region and do not enter much deeper into the pulp. Moreover, a single fruit is sufficient to complete the larval growth.

Table 4.18 Period of litchi fruit borer, *C. sinensis* infestation during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Sl. No.	Date	Days after fruit set	Infestation out of 100 fruits						Average % fruit borer infestation
			Replication I	Replication II	Replication III	Replication IV	Replication V	Replication VI	
1	11.04.2023	14	0	0	0	1	0	0	0.17
2	18.04.2023	21	1	2	1	0	0	0	0.67
3	25.04.2023	28	1	0	2	0	2	0	0.83
4	02.05.2023	35	2	2	0	2	3	3	2.00
5	09.05.2023	42	6	9	5	10	7	8	7.50
6	16.05.2023	49	12	14	10	10	13	10	11.50
7	23.05.2023	55	25	22	20	24	30	24	24.17
8	30.05.2023	61	40	40	38	45	41	48	42.00
9	06.06.2023	67	43	39	40	43	37	40	40.33
10	13.06.2023	73	35	33	36	36	33	30	33.83
11	20.06.2023	79	25	27	24	24	25	22	24.50
12	27.06.2023	85	15	14	13	10	12	10	12.33

Table 4.19 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Sl. No.	Date	Days after fruit set	Average fruit borer infestation (%)	Average temperature (^{0}C)	Average Relative Humidity (%)	Cumulative rainfall (mm)
1	11.04.2023	14	0.17	23.45	59.36	0.00
2	18.04.2023	21	0.67	27.41	69.43	27.70
3	25.04.2023	28	0.83	25.01	72.50	20.20
4	02.05.2023	35	2.00	26.19	72.57	24.90
5	09.05.2023	42	7.50	27.16	66.71	0.00
6	16.05.2023	49	11.50	25.61	77.36	24.50
7	23.05.2023	55	24.17	27.60	68.86	35.20
8	30.05.2023	61	42.00	29.29	67.29	2.50
9	06.06.2023	67	40.33	30.51	72.57	77.10
10	13.06.2023	73	33.83	26.86	86.36	107.20
11	20.06.2023	79	24.50	26.91	84.36	63.20
12	27.06.2023	85	12.33	29.62	83.43	25.00

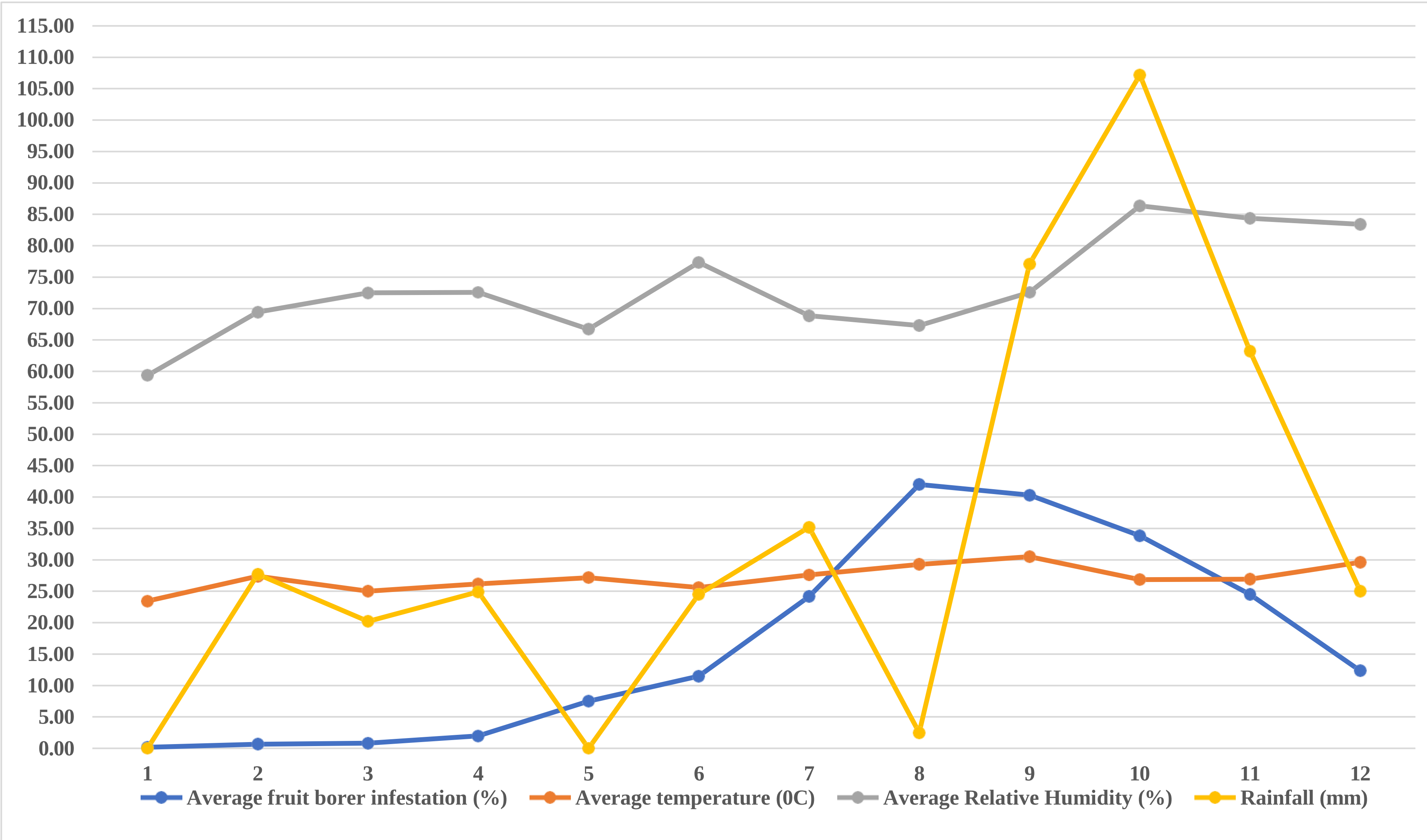


Figure 4.25 Incidence of litchi fruit borer, *C. sinensis* in relation to major environmental abiotic factors during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Table 4.20 Multiple regression result on the effect of mean temperature, relative humidity and rainfall on litchi fruit borer infestation during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Variables	**Coefficients**	**Standard Error**	**t Stat**	**p-value**
Constant	-80.947	59.414	-1.362	0.021
Average temperature (X_1)	4.564	1.802	2.531	0.035
Average relative humidity (X_2)	0.481	0.595	0.809	0.044
Rainfall (X_3)	0.268	0.147	1.818	0.013
R^2	0.620			
Adjusted R^2	0.477			
F value	4.354			
Regression equation	$Y = -80.947 + 4.564 (X_1) + 0.481 (X_2) + 0.268 (X_3)$			

The attack was gradually increased and reached its peak either during last week of May or 1st week of June, when the fruits were developing pink colorations leading to the stage of maturation. Probably, this is due to formation of sugars and low phenol content of the fruit. Later, it was found to decrease gradually. From the present study, it is evident that the activity of the pest species has a profound influence on average temperature and is positively correlated with the factors like temperature, relative humidity and rainfall. However, relative humidity was found to have less impact than average temperature on fruit infestation. Moreover, from the statistical analysis, it is also evident that rainfall has little influence on the activity of the pest's species.

The above studies are in line with Hameed *et al*. (1999), who observed that fruit borer causes maximum infestation during May-June, and its population was insignificant from October to March but reappeared in April. Lall and Sharma (1978) observed the maximum population density in September while, lowest in December. Almost, similar observations were also made by Sharma (1985).

4.3.5 Natural enemies associated with litchi fruit borer, *C. sinensis*

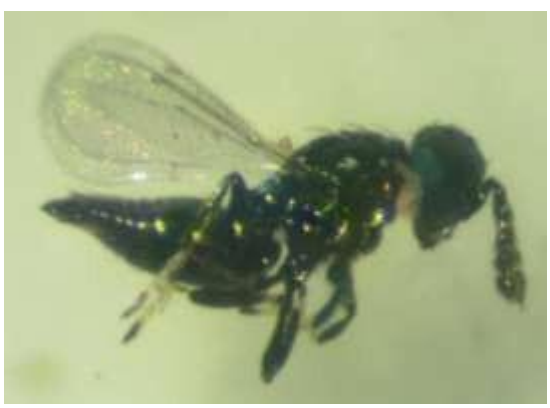
A total of four natural enemies were documented on litchi fruit borer, *C. sinensis*. Of these, one was predator and other three were parasitoids. The only predator documented was six spotted zigzag lady bird beetle, *Cheilomenes sexmaculata* Fabricius. Among the three parasitoids documented, one was chalcid wasp, *Brachymeria euploeae* (Westwood) and the other two were unidentified species belonging to families, Eulophidae and Ichneumonidae (Table 4.21 & Plate 4.20). The present findings are in line with Meng *et al*. (2014) and Satyagopal *et al*. (2015), who also found five species preying on *C. sinensis*.

Table 4.21 Natural enemies associated with litchi fruit borer, *Conopomorpha sinensis* Bradley

Sl. No.	Natural enemy	Scientific name	Family	Order	Predator/Parasitoids
PREDATORS					
1.	Six spotted zigzag lady bird beetle	*Cheilomenes sexmaculata* Fabricius	Coccinellidae	Coleoptera	Insect predator
PARASITOIDS					
2.	Chalcid wasp	*Brachymeria euploeae* (Westwood)	Chalcididae	Hymenoptera	Pupal parasitoid
3.	Eulophid wasp	Unidentified sp.	Eulophidae	Hymenoptera	Larval parasitoid
4.	Ichneumonid wasp	Unidentified sp.	Ichneumonidae	Hymenoptera	Larval parasitoid

A. *Cheilomenes sexmaculata* Fabricius

B. *Brachymeria euploeae* (Westwood)



C. Unidentified sp.

D. Unidentified sp.

Plate 4.20 Natural enemies associated with litchi fruit borer, *Conopomorpha sinensis* Bradley

4.4 Efficacy study of various insecticides and biopesticides against litchi fruit borer(s)

The above objective was carried out during April-June, 2022 and 2023 at two locations *viz*., Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland and Farmers farm, Medziphema, Nagaland to evaluate various insecticides and biopesticides like neem seed kernel extract 4%, *Bacillus thuringiensis* var. *kurstaki*, spinosad 45 SC, diflubenzuron 25 WP, novaluron 10 EC, neem oil 0.2%, kamdhenu keet niyantrak 5%, and untreated check against litchi fruit borer, *C. sinensis* and presented here.

4.4.1 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Pre-treatment count for 13 days after fruit set (First spray)

A day before 13 days after fruit set, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.22 & Fig 4.26).

Seventh day after first spray

The data in Table 4.22 & Fig 4.26 represented that, the lowest number of infested fruits (0.00) were recorded in neem seed kernel extract 4%, diflubenzuron 25 WP, and kamdhenu keet niyantrak 5%. The next effective treatments were *B. thuringiensis* var. *kurstaki*, spinosad 45 SC, and novaluron 10 EC with 0.33 infested fruits. The maximum number of infested fruits (0.66) were recorded in neem oil 0.2% and untreated check.

Fourteenth day after first spray

The perusal of data in Table 4.22 & Fig 4.26 showed that, all the treatments were statistically superior over untreated check (7.33). The least number of infested fruits (0.00) were recorded in spinosad 45 SC and *B. thuringiensis* var. *kurstaki*. Whereas, the treatments like neem seed kernel extract 4%, diflubenzuron 25 WP, and kamdhenu keet niyantrak 5% recorded 0.66 infested fruits. The maximum infestation was noticed in trees with novaluron 10 EC and neem oil 0.2% (1.00).

Per cent reduction over untreated check for first spray

The highest fruit borer reduction was observed in spinosad 45 SC (94.50%), closely followed by *B. thuringiensis* var. *kurstaki* (92.92%). The average fruit borer reduction was recorded in diflubenzuron 25 WP (87. 59%), novaluron 10 EC (84.64%) and neem seed kernel extract 4% (83.50%). The lowest fruit borer reduction was noticed in neem oil 0.2% (79.25%) followed by kamdhenu keet niyantrak 5% (80.12%) (Table 4.22 & Fig 4.26).

Pre-treatment count for 33 days after fruit set (Second spray)

A day before 33 DAF, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.22 & Fig 4.26).

Seventh day after second spray

The data in Table 4.22 & Fig 4.26 represented that, the lowest number of infested fruits were recorded in novaluron EC (3.35), and was on par with spinosad 45 SC (3.64) and *B. thuringiensis* var. *kurstaki* (3.66). The next effective treatment was diflubenzuron 25 WP (4.07), followed by neem seed kernel extract 4% (5.53). The maximum number of infested fruits were observed in neem oil 0.2% (9.82), followed by kamdhenu keet niyantrak 5% (7.54). Yet, all the treatments shown superior results over untreated check (26.17).

Fourteenth day after second spray

The perusal of data in Table 4.22 & Fig 4.26 presented that, all treatments were statistically significant over untreated check (30.86). The least borer infestation was observed in spinosad 45 SC (3.33), followed by *B. thuringiensis* var. *kurstaki* (4.06). The treatments, novaluron 10 EC (5.20) and diflubenzuron 25 WP (5.66) were on par with each other. The next effective treatment was neem seed kernel extract 4% (7.38), followed by kamdhenu keet niyantrak 5% (8.33). The highest fruit borer infestation (8.66) was recorded in neem oil 0.2%.

Per cent reduction over untreated check for second spray

The data in Table 4.22 & Fig 4.26 showed that, maximum fruit borer reduction was observed in spinosad 45 SC (85.17%), closely followed by *B. thuringiensis* var. *kurstaki* (82.33%). The treatment diflubenzuron 25 WP (78.54%) was on par with the novaluron 10 EC (78.40%). The minimum fruit borer reduction was recorded in neem oil 0.2% (57.24%), followed by kamdhenu keet niyantrak 5% (64.46%) and neem seed kernel extract 4% (67. 73%).

Pre-treatment count for 53 days after fruit set (Third spray)

A day before 53 DAF, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.22 & Fig 4.26).

Seventh day after third spray

The data in Table 4.22 & Fig 4.26 represented that, the lowest infestation was recorded in spinosad 45 SC (3.14), followed by *B. thuringiensis* var. *kurstaki* (4.06). The next effective treatment was diflubenzuron 25 WP (4.24), followed by novaluron EC (5.18 infested). The maximum infestation was recorded in neem oil 0.2% (10.25), followed by kamdhenu keet niyantrak 5% (7.50), and neem seed kernel extract 4% (8.03). However, all the treatments were superior over the untreated check (47.42).

Table 4.22. Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)														
		13 DAF (First spray)					33 DAF (Second spray)					53 DAF (Third spray)				
		PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC
Neem seed kernel extract 4%	400	2.00	0.00	0.66	0.33	83.50	20.00	5.53	7.38	6.46	67.73	24.12	8.03	8.61	8.32	65.51
Bacillus thuringiensis var. *kurstaki*	50	2.33	0.33	0.00	0.17	92.92	21.85	3.66	4.06	3.86	82.33	22.85	4.06	5.00	4.53	80.18
Spinosad 45 SC	4.5	3.00	0.33	0.00	0.17	94.50	23.50	3.64	3.33	3.49	85.17	20.50	3.14	4.08	3.61	82.39
Diflubenzuron 25 WP	3.0	2.66	0.00	0.66	0.33	87.59	22.67	4.07	5.66	4.87	78.54	19.67	4.24	5.66	4.95	74.83
Novaluron 10 EC	1.5	4.33	0.33	1.00	0.67	84.64	19.79	3.35	5.20	4.28	78.40	21.00	5.18	6.08	5.63	73.19
Neem oil 0.2%	200	4.00	0.66	1.00	0.83	79.25	21.61	9.82	8.66	9.24	57.24	24.89	10.25	11.27	10.76	56.76
Kamdhenu keet niyantrak 5%	500	1.66	0.00	0.66	0.33	80.12	22.33	7.54	8.33	7.94	64.46	23.12	7.50	9.82	8.66	62.55
Untreated check	-	4.33	6.66	7.33	7.00	-	17.23	26.17	30.86	28.52	-	38.55	47.42	50.36	48.89	-

PTC: Pre-treatment count; DAF: Days after fruit set; DAS: Days after spraying; ROC: Reduction over control

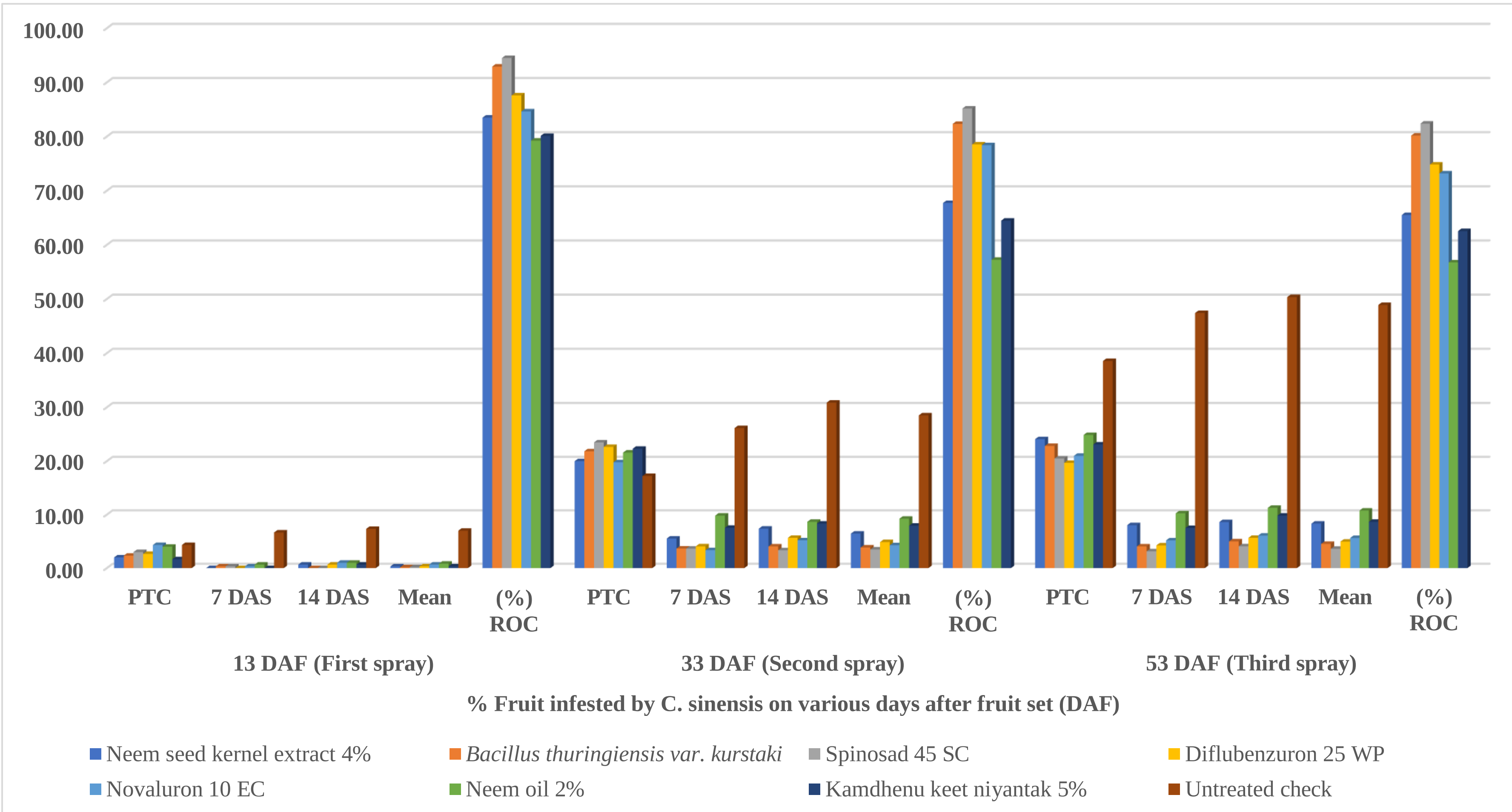


Figure. 4.26 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Fourteenth day after third spray

The least number of infested fruits were recorded in spinosad 45 SC (4.08). The treatment, *B. thuringiensis* var. *kurstaki* (5.06) was on par with the treatment diflubenzuron 25 WP (5.66). The next effective treatment was novaluron 10 EC with 6.08 infested fruits followed by neem seed kernel extract 4% (8. 61 infested fruits). The maximum infested fruits were recorded in neem oil 0.2% (11.27 infested fruits), followed by kamdhenu keet niyantrak 5% (9. 82%). However, all treatments were statistically superior over untreated check (50.36) (Table 4.22 & Fig 4.26).

Per cent reduction over untreated check for second spray

The data in Table 4.22 & Fig 4.26 showed that, the highest fruit borer reduction was noticed in spinosad 45 SC (82.39%), closely followed by *B. thuringiensis* var. *kurstaki* (80.18%). The next effective treatment was diflubenzuron 25 WP (74.83%), and was on par with novaluron 10 EC (73. 19%). The least fruit borer reduction was recorded in neem oil 0.2% (56.7%), followed by kamdhenu keet niyantrak 5% (62.55%) and neem seed kernel extract 4% (65.51%).

4.4.2 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Pre-treatment count for 13 days after fruit set (First spray)

A day before 13 days after fruit set, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.23 & Fig 4.27).

Seventh day after first spray

The perusal of data in Table 4.23 & Fig 4.27 showed that, the lowest number of infested fruits (0.00) were recorded in spinosad 45 SC and *B. thuringiensis* var. *kurstaki*. The next effective treatments *viz.*, neem seed kernel extract 4%, diflubenzuron 25 WP, novaluron 10 EC and kamdhenu keet niyantrak 5% were recorded with 0.33 infested fruits. The highest infested fruits were recorded in neem oil 0.2% (0.66). However, all treatments were statistically superior over untreated check (6.66).

Fourteenth day after first spray

The data in Table 4.23 & Fig 4.27 represented that, all the treatments were superior over untreated check (7.88). The least fruit borer infestation (0.33) was recorded in spinosad 45 SC and neem seed kernel extract 4%. The treatments *B. thuringiensis* var. *kurstaki*, diflubenzuron 25 WP and novaluron 10 EC were noticed with 0.66 infested fruits. The highest fruit borer infestation was observed in neem oil 0.2% (1.33), followed by kamdhenu keet niyantrak 5% (1.00).

Per cent reduction over untreated check for first spray

The data in Table 4.23 & Fig 4.27 showed that, highest fruit borer reduction was observed in spinosad 45 SC (93.80%), followed by *B. thuringiensis* var. *kurstaki* (90.09%). The next effective treatment was diflubenzuron 25 WP (86.48%), closely followed by novaluron 10 EC (85. 14%). Whereas, treatment neem seed kernel extract 4% (80.12%) was on par with kamdhenu keet niyantrak 5% (80.03%). Least fruit borer reduction was recorded in neem oil 0.2% (77.02%).

Pre-treatment count for 33 days after fruit set (Second spray)

A day before 33 days after fruit set, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.23 & Fig 4.27).

Seventh day after second spray

The perusal of data in Table 4.23 & Fig 4.27 showed that, the least fruit borer infestation was recorded in spinosad 45 SC (2.53), followed by *B. thuringiensis* var. *kurstaki* (3.21). The next effective treatment was novaluron 10 EC (4.08), and was on par with diflubenzuron 25 WP (4.17). The highest fruit borer infestation was recorded in neem oil 0.2% (9.26), followed by kamdhenu keet niyantrak 5% (7. 23) and neem seed kernel extract 4% (6.18). However, all the treatments were superior over the untreated check (28.47).

Fourteenth day after second spray

The data in Table 4.23 & Fig 4.27 represented that, all treatments were superior over untreated check. The lowest number of infested fruits were recorded in spinosad 45 SC (3.46), closely followed by *B. thuringiensis* var. *kurstaki* (4.12). The next best treatment was diflubenzuron 25 WP (5.02), and was on par with novaluron 10 EC (5.19). Whereas, the treatments neem seed kernel extract 4% and kamdhenu keet niyantrak 5% were recorded with 8.20 and 9.05 infested fruits, respectively. The highest number of infested fruits were recorded in neem oil 0.2% (10.06).

Per cent reduction over untreated check for second spray

The highest fruit borer reduction was recorded in spinosad 45 SC (83.68%), followed by *B. thuringiensis* var. *kurstaki* (81.35%). Whereas, the treatment diflubenzuron 25 WP (77.38%) was on par with novaluron 10 EC (76.30%). The least percent reduction fruit borer reduction was recorded in neem oil 0.2% (59.90%), followed by kamdhenu keet niyantrak 5% (63.56%) and neem seed kernel extract 4% (66.56%) (Table 4.23 & Fig 4.27).

Pre-treatment count for 53 days after fruit set (Third spray)

A day before third spray, there was no statistically significant difference between the treatments with respect to mean number of larvae per plant (Table 4.23 &

Fig 4.27).

Seventh day after third spray

The perusal of data in Table 4.23 & Fig 4.27 showed that, the least number of infested fruits were recorded in *B. thuringiensis* var. *kurstaki* (3.65), and was on par with spinosad 45 SC (3.72). The next effective treatment was novaluron 10 EC (4.29), closely followed by diflubenzuron 25 WP (5.56). The highest infested fruits were recorded in neem oil 0.2% (10.00), followed by kamdhenu keet niyantrak 5% (8.27), followed by neem seed kernel extract 4% (7.14). However, all the treatments were superior over the untreated check (45.63).

Fourteenth day after third spray

The data in Table 4.23 & Fig 4.27 represented that, all treatments were significant compared to untreated check (49.68). The lowest number of infested fruits were recorded in spinosad 45 SC (4.08), closely followed by *B. thuringiensis* var. *kurstaki* (5.02). The treatment diflubenzuron 25 WP was recorded with 6.24 infested fruits, followed by novaluron 10 EC (7.67). The highest number of infested fruits were recorded in neem oil 0.2% (11.12), followed by kamdhenu keet niyantrak 5% (10. 30) and neem seed kernel extract 4% (9.73).

Per cent reduction over untreated check for third spray

The perusal of data in Table 4.23 & Fig 4.27 showed that, the highest fruit borer reduction was observed in spinosad 45 SC (80.90%), followed by *B. thuringiensis* var. *kurstaki* (78.37%). The treatment diflubenzuron 25 WP (73.97%) was closely followed by novaluron 10 EC (71.83%). The least fruit borer reduction was noticed in neem oil 0.2% (56.09%), followed by kamdhenu keet niyantrak 5% (60.12%), and neem seed kernel extract 4% (63. 91%).

Table 4.23 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)														
		13 DAF (First spray)					33 DAF (Second spray)					53 DAF (Third spray)				
		PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC
Neem seed kernel extract 4%	400	1.66	0.33	0.33	0.33	80.12	21.50	6.18	8.20	7.19	66.56	23.37	7.14	9.73	8.44	63.91
Bacillus thuringiensis var. *kurstaki*	50	3.33	0.00	0.66	0.33	90.09	19.65	3.21	4.12	3.67	81.35	20.04	3.65	5.02	4.34	78.37
Spinosad 45 SC	4.5	2.66	0.00	0.33	0.17	93.80	18.35	2.53	3.46	3.00	83.68	20.42	3.72	4.08	3.90	80.90
Diflubenzuron 25 WP	3.0	3.66	0.33	0.66	0.50	86.48	20.31	4.17	5.02	4.60	77.38	22.67	5.56	6.24	5.90	73.97
Novaluron 10 EC	1.5	3.33	0.33	0.66	0.50	85.14	19.56	4.08	5.19	4.64	76.30	21.23	4.29	7.67	5.98	71.83
Neem oil 0.2%	200	4.33	0.66	1.33	1.00	77.02	24.09	9.26	10.06	9.66	59.90	24.05	10.00	11.12	10.56	56.09
Kamdhenu keet niyantrak 5%	500	3.33	0.33	1.00	0.67	80.03	22.34	7.23	9.05	8.14	63.56	23.28	8.27	10.30	9.29	60.12
Untreated check	-	3.33	6.66	7.88	7.27	-	26.00	28.47	30.46	29.47	-	36.82	45.63	49.68	47.66	-

PTC: Pre-treatment count; DAF: Days after fruit set; DAS: Days after spraying; ROC: Reduction over control

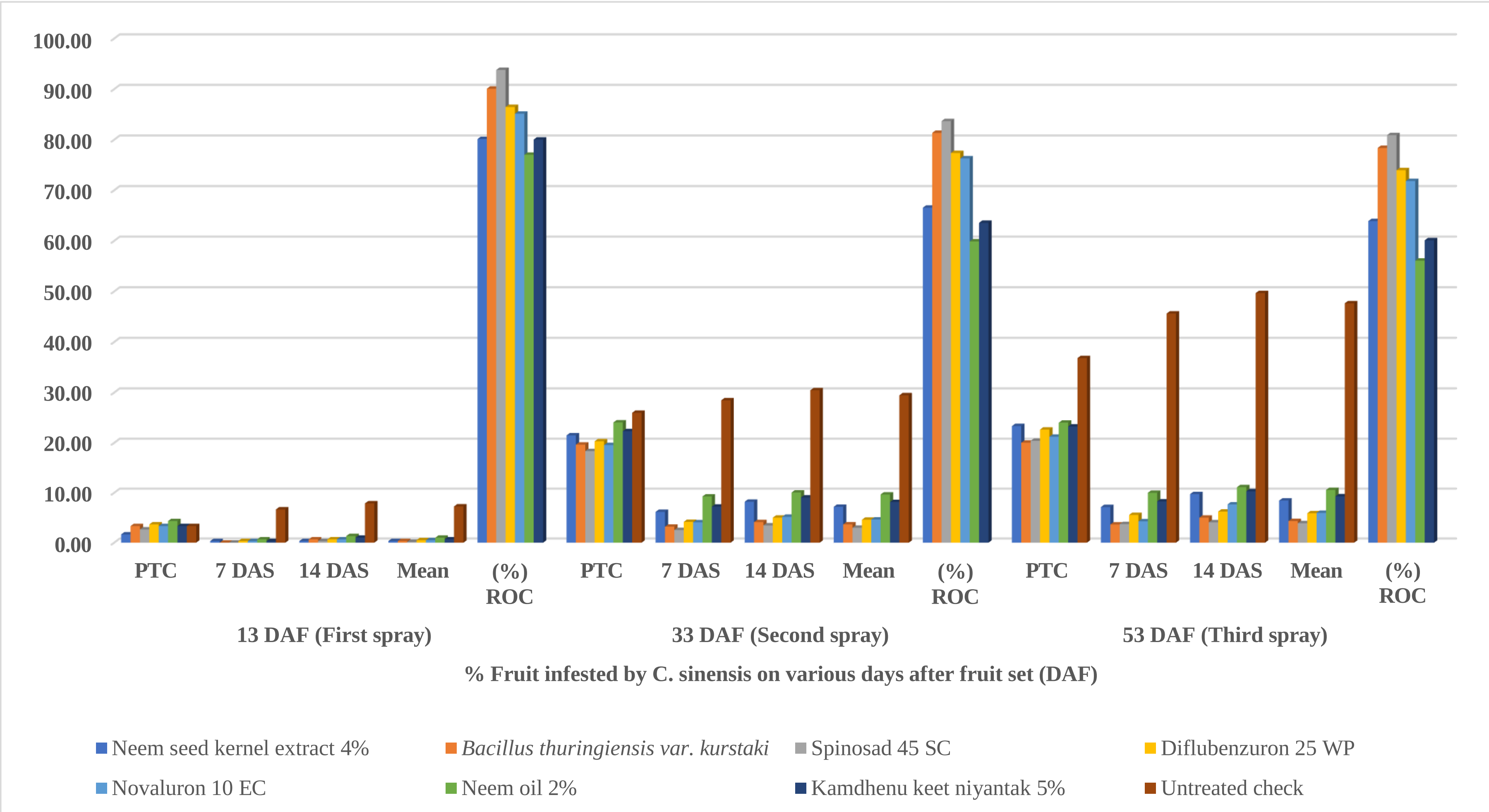


Figure 4.27 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

4.4.3 Pooled mean data on the efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 & 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

13 days after fruit set (First spray)

The pooled mean data in Table 4.24 & Fig 4.28 showed that, spinosad 45 SC was recorded with least number of infested fruits (0.16) over the untreated check (7.13). The next effective treatment was *B. thuringiensis* var. *kurstaki* with (0.24), closely followed by neem seed kernel extract 4% (0.33). Whereas, the treatment diflubenzuron 25 WP was recorded with 0.41 infested fruits, and was on par with kamdhenu keet niyantrak 5% (0.49). The highest number of infested fruits was observed in neem oil 0.2% (0.91), followed by novaluron 10 EC (0.58). However, all treatments were shown significant results over the untreated check (7.13).

33 days after fruit set (Second spray)

The perusal of the pooled mean data in Table 4.24 & Fig 4.28 represented that, the lowest fruit borer infestation was noticed in spinosad 45 SC (3.24), closely followed by *B. thuringiensis* var. *kurstaki* (3.76). Whereas, the treatments novaluron 10 EC and diflubenzuron 25 WP were recorded with similar results *i.e*., 4.45 and 4.73 infested fruits, respectively. The maximum fruit borer infestation was observed in neem oil 0.2% (9.45), followed by kamdhenu keet niyantrak (8.03) and neem seed kernel extract 4% (6.82). However, all treatments were statistically superior compared to untreated check (28.99).

53 days after fruit set (Third spray)

The pooled mean data in Table 4.24 & Fig 4.28 showed that, all treatments were statistically significant over the untreated check (48.27). The treatment Spinosad

Table 4.24 Pooled data on the efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 & 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)								
		13 DAF (First spray)			33 DAF (Second spray)			53 DAF (Third spray)		
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
Neem seed kernel extract 4%	400	0.33	0.33	0.33	6.45	7.19	6.82	8.32	8.44	8.37
Bacillus thuringiensis var. *kurstaki*	50	0.16	0.33	0.24	3.86	3.67	3.76	4.53	4.34	4.43
Spinosad 45 SC	4.5	0.16	0.17	0.16	3.48	3.00	3.24	3.61	3.90	3.75
Diflubenzuron 25 WP	3.0	0.33	0.50	0.41	4.86	4.60	4.73	4.95	5.90	5.42
Novaluron 10 EC	1.5	0.66	0.50	0.58	4.27	4.64	4.45	5.63	5.98	5.80
Neem oil 0.2%	200	0.83	1.00	0.91	9.24	9.66	9.45	10.76	10.56	10.66
Kamdhenu keet niyantrak 5%	500	0.33	0.67	0.49	7.93	8.14	8.03	8.66	9.29	8.97
Untreated check	-	6.99	7.27	7.13	28.51	29.47	28.99	48.89	47.66	48.27
SEm (±)	-	0.67	0.81	0.99	3.36	2.19	0.98	3.11	3.12	1.87
CD (5%)	-	2.02	2.47	2.47	10.19	6.65	2.82	9.43	9.46	5.40

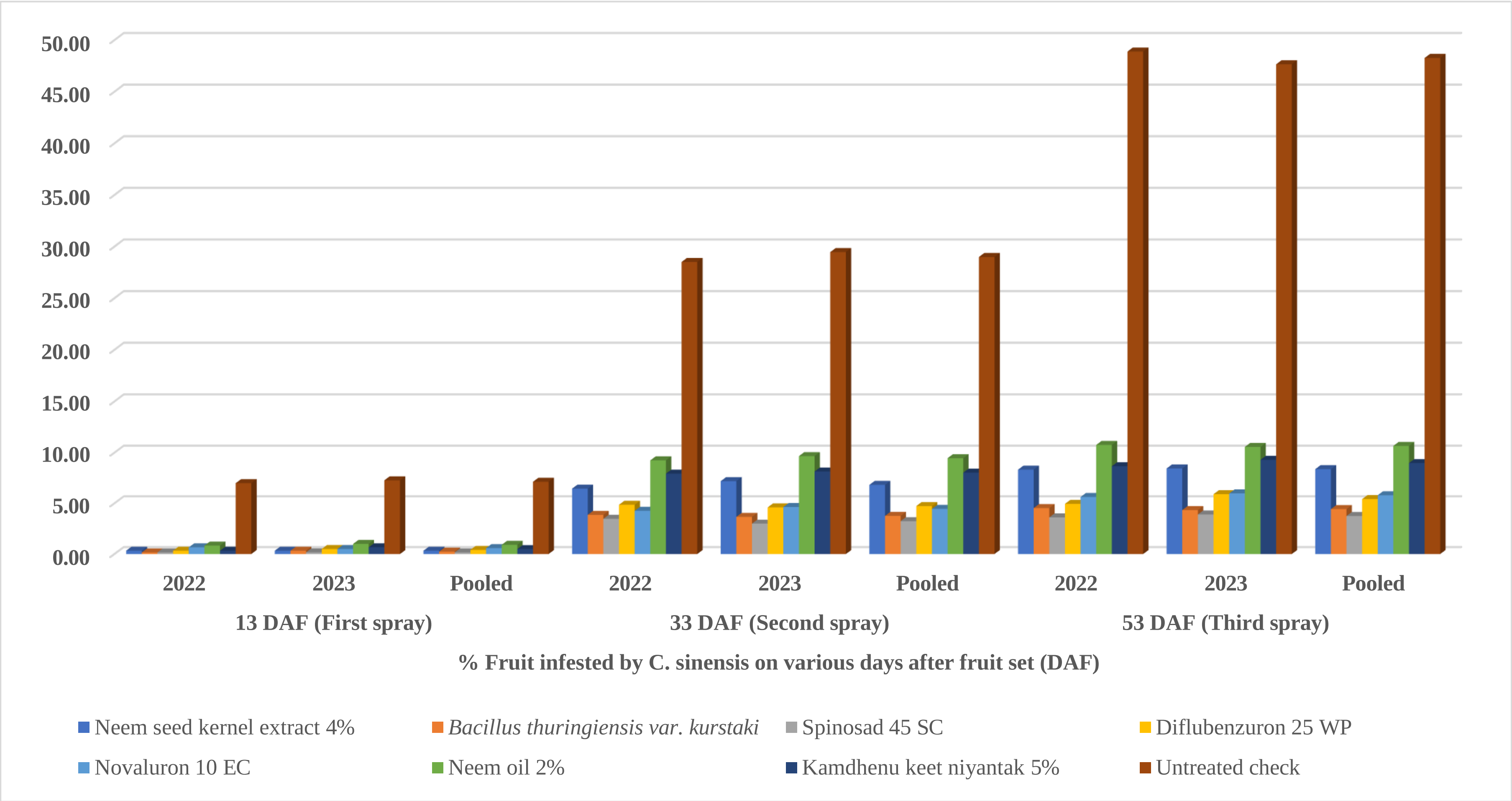


Figure 4.28 Pooled data on the efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 & 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

45 SC was shown least fruit borer infestation (3.75), followed by *B. thuringiensis* var. *kurstaki* (4.43). Whereas, the treatments diflubenzuron 25 WP (5.42) and novaluron 10 EC (5.80) were on par with each other. While, the treatment neem oil 0.2% was recorded with highest fruit borer infestation (10.66), followed by kamdhenu keet niyantrak 5% (8.97) and neem seed kernel extract 4% (8.37).

4.4.4 Pooled data on the efficacy of various insecticides and bio-pesticides on reduction of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 & 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

13 days after fruit set (First spray)

The pooled mean data in Table 4.25 & Fig 4.29 showed that, the highest percent fruit borer reduction (94.15%) was recorded in spinosad 45 SC, followed by *B. thuringiensis* var. *kurstaki* (91.50%). Whereas, the treatment diflubenzuron 25 WP has shown 87.03% reduction, followed by novaluron 10 EC (84.89%). The least percent fruit borer reduction (78.14%) was recorded in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% (80.08%) and neem seed kernel extract 4% (81.81%).

33 days after fruit set (Second spray)

The perusal of the pooled mean data in Table 4.25 & Fig 4.29 showed that, the treatment spinosad 45 SC was recorded highest percent fruit borer reduction (84.42%), followed by *B. thuringiensis* var. *kurstaki* (81.84%). Whereas, the treatments diflubenzuron 25 WP (77.96%) and novaluron 10 EC (77.35%) were on par with each other. The least percent fruit borer reduction (58.57%) was observed in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% and neem seed kernel extract 4% *i.e.*, 64.01% and 67.14%, respectively.

Table 4.25 Pooled data on the efficacy of various insecticides and bio-pesticides on reduction of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 & 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)								
		13 DAF (First spray)			33 DAF (Second spray)			53 DAF (Third spray)		
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
Neem seed kernel extract 4%	400	83.50	80.12	81.81	67.73	66.56	67.14	65.51	63.91	64.71
Bacillus thuringiensis var. *kurstaki*	50	92.92	90.09	91.50	82.33	81.35	81.84	80.18	78.37	79.27
Spinosad 45 SC	4.5	94.50	93.80	94.15	85.17	83.68	84.42	82.39	80.90	81.65
Diflubenzuron 25 WP	3.0	87.59	86.48	87.03	78.54	77.38	77.96	74.83	73.97	74.40
Novaluron 10 EC	1.5	84.64	85.14	84.89	78.40	76.30	77.35	73.19	71.83	72.51
Neem oil 0.2%	200	79.25	77.02	78.14	57.24	59.90	58.57	57.24	56.09	56.67
Kamdhenu keet niyantrak 5%	500	80.12	80.03	80.08	64.46	63.56	64.01	62.80	60.12	61.46
Untreated check	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

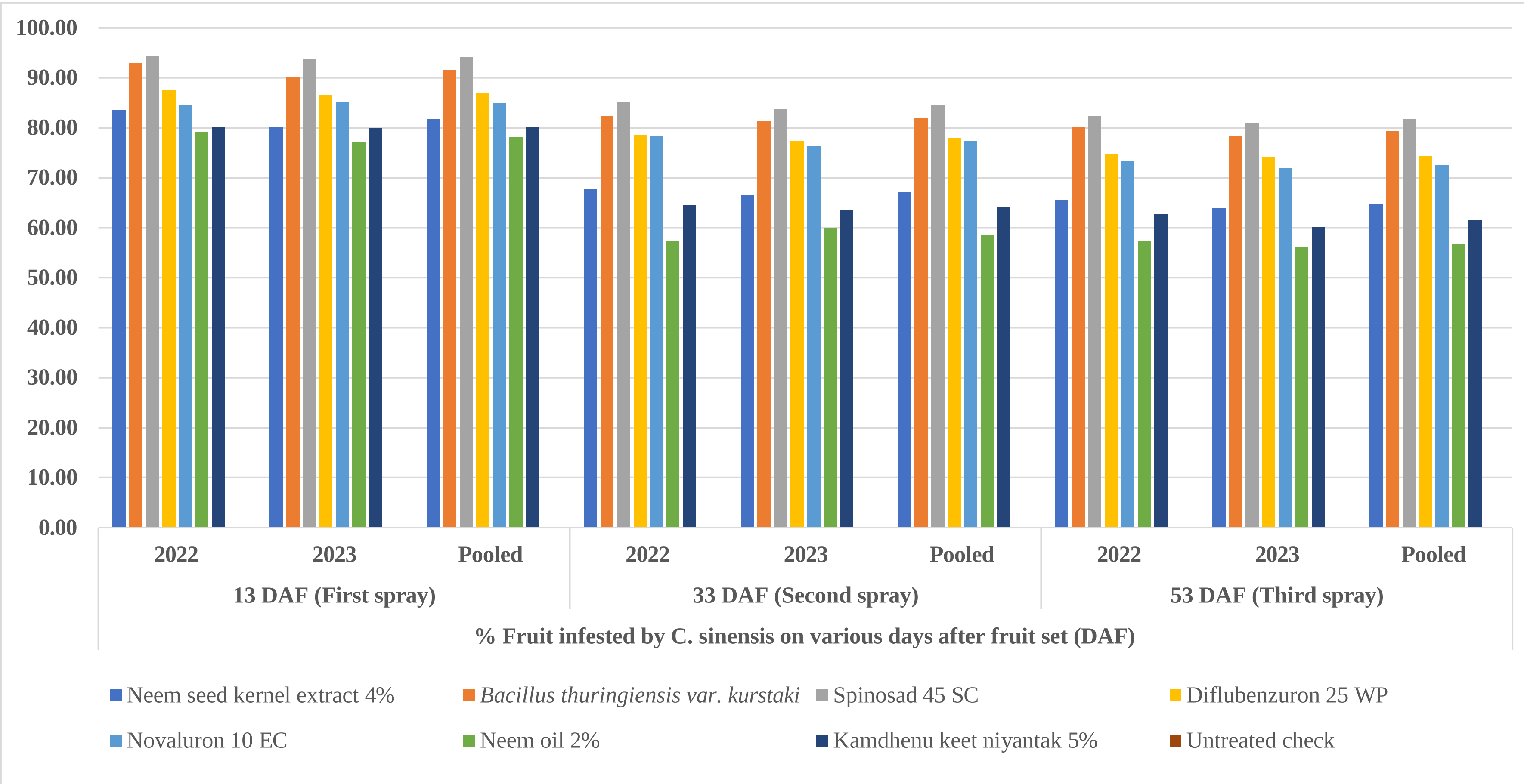


Figure 4.29 Pooled data on the efficacy of various insecticides and bio-pesticides on reduction of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 & 2023 at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland

53 days after fruit set (Third spray)

The pooled data in Table 4.25 & Fig 4.29 showed that, the highest percent fruit borer reduction (81.65%) was recorded in spinosad 45 SC, followed by *B. thuringiensis* var. *kurstaki* (79.27%). Whereas, the treatments diflubenzuron 25 WP was recorded with 74.40%, closely followed by novaluron 10 EC (72.51%). The least percent fruit borer reduction (56.67%) was observed in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% (61.46%) and neem seed kernel extract 4% (64.71%).

4.4.5 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 at Farmers farm, Medziphema, Nagaland

Pre-treatment count for 13 days after fruit set (First spray)

A day before 13 days after fruit set, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.26 & Fig 4.30).

Seventh day after first spray

The data in the Table 4.26 & Fig 4.30. presented that, the lowest number of infested fruits (0.33) were observed in *B. thuringiensis* var. *kurstaki*, spinosad 45 SC, diflubenzuron 25 WP and kamdhenu keet niyantrak 5%. Whereas, the highest infestation was recorded in neem seed kernel extract 4%, novaluron 10 EC and neem oil 0.2% with 0.66 infested fruits. However, all the treatments were shown statistically significant results compared to untreated check (6.68).

Fourteenth day after first spray

The perusal of data in Table 4.26 & Fig 4.30 showed that, all treatments showed superior results over untreated check (8.05 infested fruits). The treatment spinosad 45 SC and novaluron 10 EC recorded least number (0.00) of infested fruits. While, the treatments neem seed kernel extract 4% and *B. thuringiensis* var. *kurstaki* recorded 0.33 infested fruits. The treatments, diflubenzuron 25 WP and kamdhenu keet niyantrak 5% noticed with 0.66 infested fruits. The maximum number of infested fruits (1.00) were observed in neem oil 0.2%.

Per cent reduction over untreated check for first spray

The percent reduction of fruit borer infestation was maximum in spinosad 45 SC (93.80%), followed by *B. thuringiensis* var. *kurstaki* (90.98%). The next effective treatments, diflubenzuron 25 WP (86. 48%) and novaluron 10 EC (85.84%) were on par with each other. The least percent reduction was observed in neem oil 0.2% (77.32%), followed by kamdhenu keet niyantrak 5% (81.39%), and neem seed kernel extract 4% (83.50%) (Table 4.26 & Fig 4.30).

Pre-treatment count for 33 days after fruit set (Second spray)

A day before 33 DAF, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.26 & Fig 4.30).

Seventh day after second spray

The data in Table 4.26 & Fig 4.30 represented that, the minimum number of infested fruits were recorded in spinosad 45 SC (2.46) and *B. thuringiensis* var. *kurstaki* (2.54). The next effective treatment is novaluron 10 EC (3.46), followed by diflubenzuron 25 WP (4.07). The least number of infested fruits were noticed in neem oil 0.2% (9.34) rather compared to kamdhenu keet niyantrak 5% (8.44) and neem seed kernel extract 4% (6.88). Although, all treatments were statistically superior over untreated check (24.47).

Fourteenth day after second spray

The perusal of the data in Table 4.26 & Fig 4.30 showed that, the treatment spinosad 45 SC was recorded with least number of infested fruits (3.21), followed by *B. thuringiensis* var. *kurstaki* (4.04). The treatments, novaluron 10 EC and diflubenzuron 25 WP were noticed with 5.20 and 6.02 infested fruits, respectively. The highest number of infested fruits were recorded in neem oil 0.2% (10.05), followed by kamdhenu keet niyantrak 5% (9.23) and neem seed kernel extract 4% (7.54). However, all treatments shown statistically significant results over untreated check (27.46).

Per cent reduction over untreated check for second spray

The percent reduction of fruit borer infestation was maximum in spinosad 45 SC (85.46%), closely followed by *B. thuringiensis* var. *kurstaki* (84.18%). The next best treatment was novaluron 10 EC (77.19%), followed by diflubenzuron 25 WP (76.45%). The least percent fruit borer reduction was recorded in neem oil 0.2% (58.01%), compared to kamdhenu keet niyantrak 5% (62.61%) and neem seed kernel extract 4% (65.67%) (Table 4.26 & Fig 4.30).

Pre-treatment count for 53 days after fruit set (Third spray)

A day before 53 days after fruit set, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.26 & Fig 4.30).

Seventh day after third spray

The data in the Table 4.26 & Fig 4.30 represented that, least number of infested fruits were recorded in trees with spinosad 45 SC (3.14), closely followed by *B. thuringiensis* var. *kurstaki* (3.28). Whereas, the treatments diflubenzuron 25 WP (5.56) and novaluron EC (5.24) were on par with each other. The maximum infested fruits (9.00) were recorded in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% (8.37) and neem seed kernel extract 4% (7.24).

Table 4.26 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 at Farmers farm, Medziphema, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)														
		13 DAF (First spray)					33 DAF (Second spray)					53 DAF (Third spray)				
		PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC
Neem seed kernel extract 4%	400	3.00	0.66	0.33	0.50	83.50	21.00	6.88	7.54	7.21	65.67	25.63	7.24	10.61	8.93	65.18
Bacillus thuringiensis var. *kurstaki*	50	3.66	0.33	0.33	0.33	90.98	20.79	2.54	4.04	3.29	84.18	22.36	3.28	5.10	4.19	81.26
Spinosad 45 SC	4.5	2.66	0.33	0.00	0.17	93.80	19.50	2.46	3.21	2.84	85.46	21.42	3.14	4.08	3.61	83.15
Diflubenzuron 25 WP	3.0	3.66	0.33	0.66	0.50	86.48	21.42	4.07	6.02	5.05	76.45	22.67	5.56	6.24	5.90	73.97
Novaluron 10 EC	1.5	2.33	0.66	0.00	0.33	85.84	18.98	3.46	5.20	4.33	77.19	22.00	5.24	7.35	6.30	71.39
Neem oil 0.2%	200	3.66	0.66	1.00	0.83	77.32	23.09	9.34	10.05	9.70	58.01	24.21	9.00	11.06	10.03	58.57
Kamdhenu keet niyantrak 5%	500	2.66	0.33	0.66	0.50	81.39	23.63	8.44	9.23	8.84	62.61	25.62	8.37	10.27	9.32	63.62
Untreated check	-	3.33	6.68	8.05	7.37	-	15.24	24.47	27.46	25.97	-	37.85	46.36	50.72	48.54	-

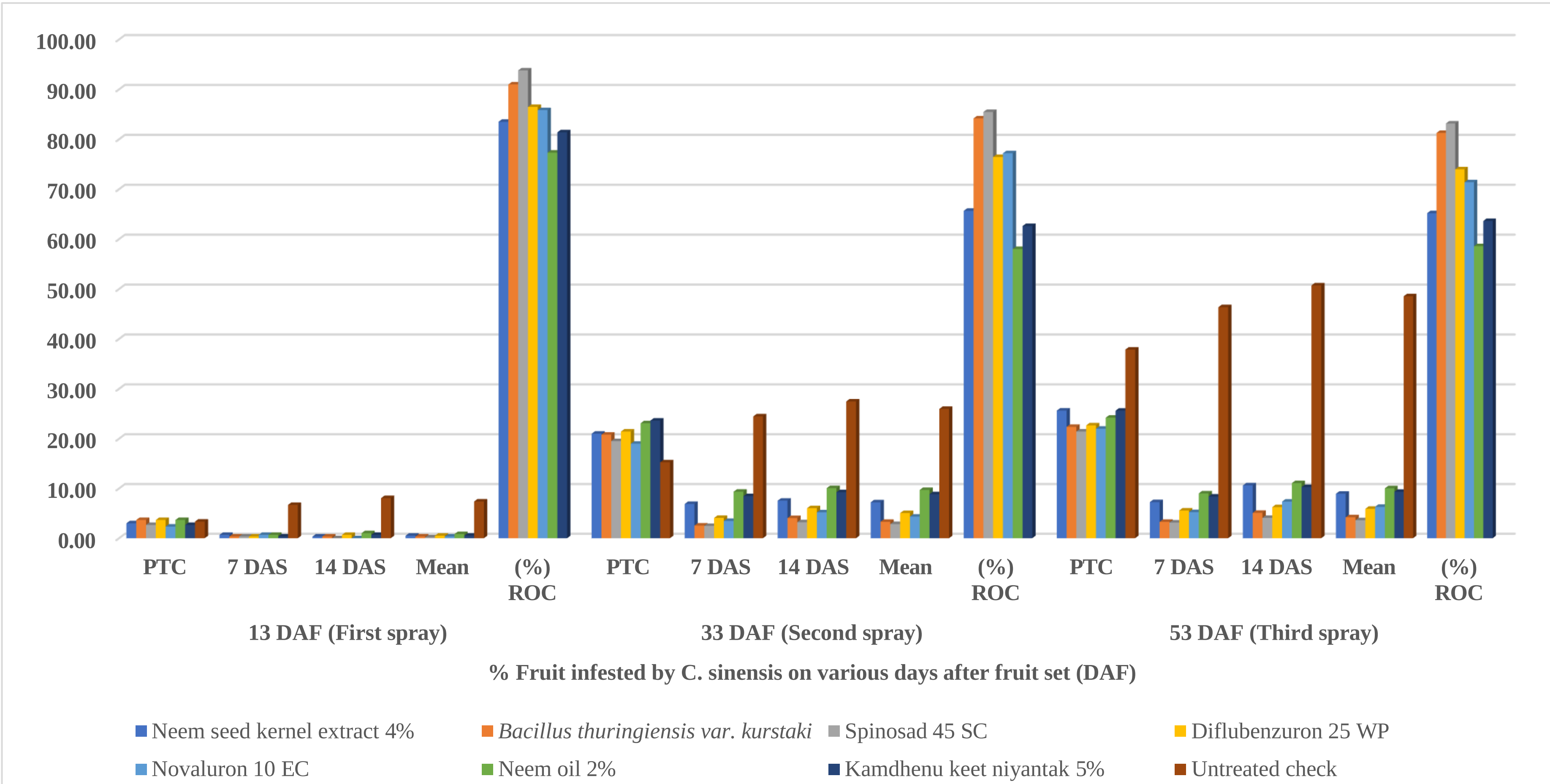


Figure 4.30 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 at Farmers farm, Medziphema, Nagaland

Fourteenth day after second spray

The data in Table 4.26 & Fig 4.30 showed that, the least number of infested fruits (4.08) were recorded in spinosad 45 SC, closely followed by *B. thuringiensis* var. *kurstaki* (5.10). The next effective treatments were diflubenzuron 25 WP and novaluron 10 EC with 6.24 and 7.35 infested fruits, respectively. Whereas, the treatments neem seed kernel extract 4% (10. 61) and kamdhenu keet niyantrak 5% (10. 27) were on par with each other. The treatment neem oil 0.2% was noticed with highest number of infested fruits (11.06). However, all treatments were statistically superior over untreated check (50.72).

Per cent reduction over untreated check for second spray

The perusal of data in Table 4.26 & Fig 4.30 revealed that, maximum percent reduction of fruit borer infestation was recorded in spinosad 45 SC (83.15%), followed by *B. thuringiensis* var. *kurstaki* (81.26%). The next effective treatment was diflubenzuron 25 WP (73.97%), followed by novaluron 10 EC (71. 39%). The least percent reduction of fruit borer infestation was recorded in neem oil 0.2% (58.57%), followed by kamdhenu keet niyantrak 5% (63.62%), and neem seed kernel extract 4% (65.18%).

4.4.6 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Pre-treatment count for 13 days after fruit set

A day before 13 DAF, there was no statistically significant difference between the treatments with respect to mean number of infested fruits (Table 4.27 & Fig 4.31).

Seventh day after first spray

Data in the Table 4.27 & Fig 4.31 represents, the treatments spinosad 45 SC, *B. thuringiensis* var. *kurstaki* and diflubenzuron 25 WP recorded least number (0.00) of infested fruits. Whereas, the treatments novaluron 10 EC and kamdhenu keet niyantrak 5% were noticed with 0.33 infested fruits. The highest number of infested fruits (0.66) were observed in treatments, neem seed kernel extract 4% and neem oil 0.2%. However, all the treatments were statistically superior over the untreated check (7.24 infested fruits).

Fourteenth day after first spray

The perusal of the data in Table 4.27 & Fig 4.31 showed that, all the treatments were statistically significant over the untreated check. The treatment spinosad 45 SC was recorded with lowest number of infested fruits (0.33). The next effective treatments were neem seed kernel extract 4%, *B. thuringiensis* var. *kurstaki*, and diflubenzuron 25 WP noticed with 0.66 infested fruits. Whereas, the treatments novaluron 10 EC, neem oil 0.2%, and kamdhenu keet niyantrak 5% were recorded with highest number of infested fruits (1.00).

Per cent reduction over untreated check for first spray

The data in Table 4.27 & Fig 4.31 represented that, the highest percent reduction (92.92%) of fruit borer infestation was noticed in trees with spinosad 45 SC, followed by *B. thuringiensis* var. *kurstaki* (90.09%). The next effective treatment was noticed in diflubenzuron 25 WP (89.00%) and novaluron 10 EC (84. 64%). Whereas, the treatments neem seed kernel extract 4% and kamdhenu keet niyantrak 5% with 81.97% and 80.03%, respectively. The least percent reduction of fruit borer infestation was noticed in neem oil 0.2% (75.08%).

Pre-treatment count for 33 days after fruit set

A day before 33 days after fruit set, there was no statistically significant difference between the treatments with respect to mean number of infested fruits

(Table 4.27 & Fig 4.31).

Seventh day after second spray

The perusal of the data in Table 4.27 & Fig 4.31 represented that, the treatments, spinosad 45 SC and *B. thuringiensis* var. *kurstaki* were recorded with 2.92 and 3.12 infested fruits. Whereas, the treatment novaluron 10 EC was noticed with 4.87 infested fruits followed by, diflubenzuron 25 WP (5.56). The maximum number of infested fruits (9.87) were reported in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% (7.24) and neem seed kernel extract 4% (6.25). Yet, all the treatments were statistically superior over the untreated check (25.57 infested fruits).

Fourteenth day after second spray

The data in the Table 4.27 & Fig 4.31 showed that, all the treatments were statistically significant compared to untreated check (27.00 infested fruits). The minimum number of infested fruits were recorded in trees with spinosad 45 SC (3. 36 infested fruits), closely followed by *B. thuringiensis* var. *kurstaki* (4.45). The next best treatments were recorded in trees with novaluron 10 EC (5.20 infested fruits) and diflubenzuron 25 WP (6.32 infested fruits). Whereas, the maximum number of infested fruits were noticed in trees with neem oil 0.2% (10.76), followed by kamdhenu keet niyantrak 5% (9.04) and neem seed kernel extract 4% (6.54).

Per cent reduction over untreated check for second spray

The data in Table 4.27 & Fig 4.31 showed that, the maximum percent reduction of fruit borer infestation was found in treatment spinosad 45 SC (84.68%), followed by *B. thuringiensis* var. *kurstaki* (81.79%). The next effective treatment was novaluron 10 EC (74.54%), followed by diflubenzuron 25 WP (72.49%). Whereas, the treatments neem seed kernel extract 4% and kamdhenu keet niyantrak 5% were represented with 70.93% and 64.17%, respectively. The least percent reduction of fruit borer infestation was noticed in neem oil 0.2% (57.23%).

Pre-treatment count for 53 days after fruit set

A day before third spray, there was no statistically significant difference between the treatments with respect to mean number of larvae per plant (Table 4.27 & Fig 4.31).

Seventh day after third spray

The perusal of the data in Table 4.27 & Fig 4.31 represented that, lowest number of infested fruits were recorded in *B. thuringiensis* var. *kurstaki* (3.65), closely followed by spinosad 45 SC (3.24). The next effective treatments, diflubenzuron 25 WP and novaluron 10 EC were noticed with 5.85 and 6.76 infested fruits, respectively. The maximum infested fruits were recorded in trees with neem oil 0.2% (10.21), followed by kamdhenu keet niyantrak 5% (8.75) and neem seed kernel extract 4% (7.53). However, all the treatments shown statistically significant results over the untreated check (47.02 infested fruits).

Fourteenth day after second spray

The data in Table 4.27 & Fig 4.31 showed, the treatment spinosad 45 SC was recorded with least number of infested fruits (4.35), followed by *B. thuringiensis* var. *kurstaki* (5.23). Whereas, the treatments novaluron 10 EC (7.31) and diflubenzuron 25 WP (7.41) were on par with each other. Highest number of infested fruits were observed in trees with neem oil 0.2% (11.76), followed by kamdhenu keet niyantrak 5% (10. 83) and neem seed kernel extract 4% (8. 51). However, all treatments were statistically superior over untreated check (51.72).

Table 4.27 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)														
		13 DAF (First spray)					33 DAF (Second spray)					53 DAF (Third spray)				
		PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC	PTC	7 DAS	14 DAS	Mean	(%) ROC
Neem seed kernel extract 4%	400	3.66	0.66	0.66	0.66	81.97	22.00	6.25	6.54	6.40	70.93	25.12	7.53	9.48	8.51	66.14
Bacillus thuringiensis var. *kurstaki*	50	3.33	0.00	0.66	0.33	90.09	20.79	3.12	4.45	3.79	81.79	22.45	3.36	5.23	4.30	80.87
Spinosad 45 SC	4.5	2.33	0.00	0.33	0.17	92.92	20.50	2.92	3.36	3.14	84.68	21.38	3.24	4.35	3.80	82.25
Diflubenzuron 25 WP	3.0	3.00	0.00	0.66	0.33	89.00	21.59	5.56	6.32	5.94	72.49	23.65	5.85	7.41	6.63	71.97
Novaluron 10 EC	1.5	4.33	0.33	1.00	0.67	84.64	19.78	4.87	5.20	5.04	74.54	23.50	6.76	7.31	7.04	70.06
Neem oil 0.2%	200	3.33	0.66	1.00	0.83	75.08	24.12	9.87	10.76	10.32	57.23	26.21	10.21	11.76	10.99	58.09
Kamdhenu keet niyantrak 5%	500	3.33	0.33	1.00	0.67	80.03	22.72	7.24	9.04	8.14	64.17	26.00	8.75	10.83	9.79	62.35
Untreated check	-	3.66	7.24	8.45	7.85	-	16.65	25.57	27.00	26.29	-	38.54	47.02	51.72	49.37	-

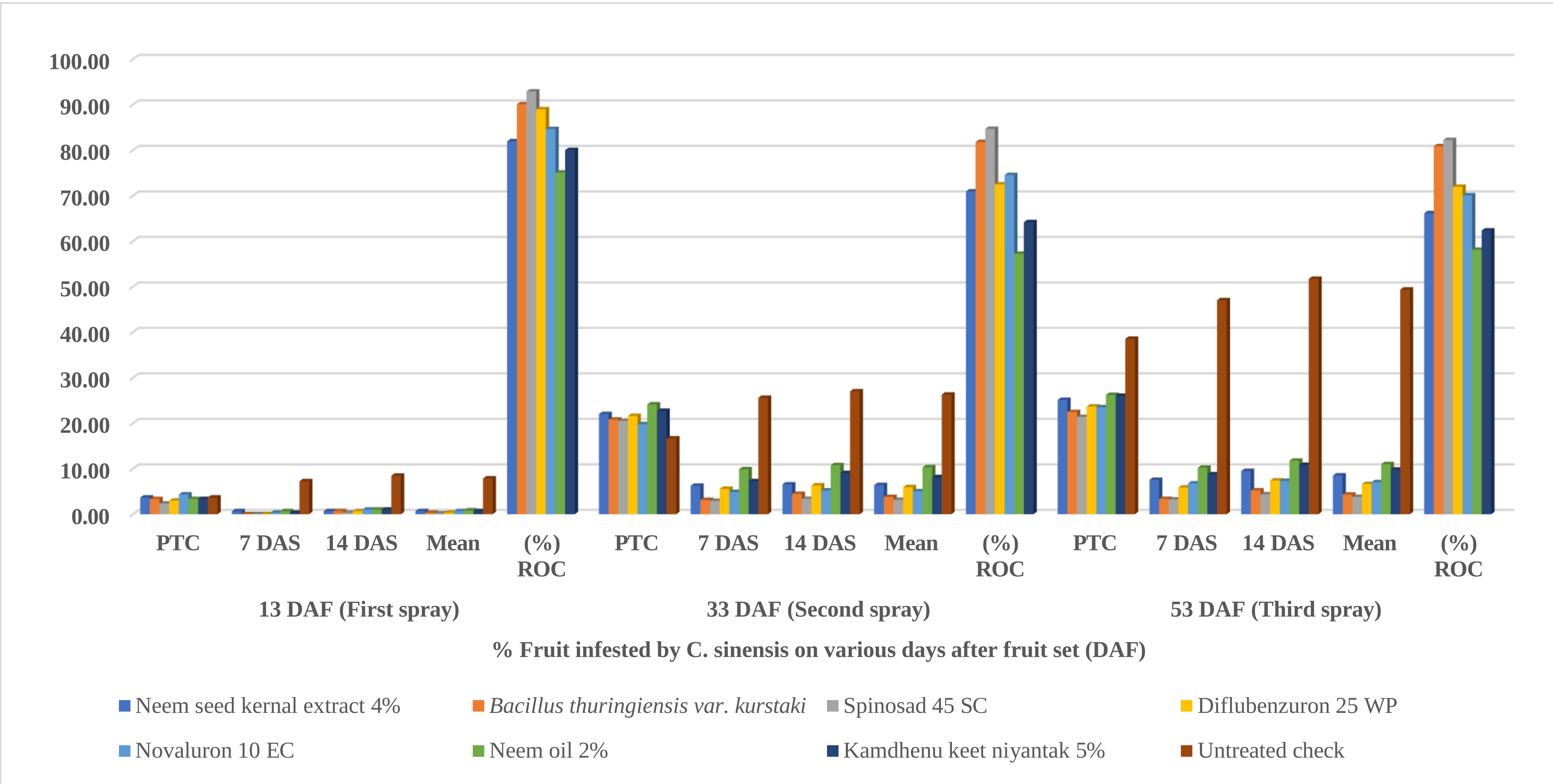


Figure 4.31 Efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2023 at Farmers farm, Medziphema, Nagaland

Per cent reduction over untreated check for third spray

The highest percent reduction of fruit borer infestation was noticed in trees with spinosad 45 SC (82.25%), followed by *B. thuringiensis* var. *kurstaki* (80.87%). The next effective treatment was diflubenzuron 25 WP (71.97%), closely followed by novaluron 10 EC (70.06%). The least percent reduction of fruit borer infestation was recorded in trees with neem oil 0.2% (58.09%), followed by kamdhenu keet niyantrak 5% (62.35%), and neem seed kernel extract 4% (66. 14%) (Table 4.27 & Fig 4.31).

4.4.7 Pooled mean data on the efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 & 2023 at Farmers farm, Medziphema, Nagaland

13 days after fruit set (First spray)

The pooled data in the Table 4.28 & Fig 4.32 represents, treatment spinosad 45 SC was recorded with the least number of infested fruits (0.94), followed by *B. thuringiensis* var. *kurstaki*, diflubenzuron 25 WP, and kamdhenu keet niyantrak 5% with 1.39 infested fruits. The treatments, novaluron 10 EC (1.44) and neem seed kernel extract 4% (1.50) were on par with each other. The highest infested fruits were observed in trees with neem oil 0.2% (1.72). Yet, all the treatments shown significant results over the untreated check (6.24 infested fruits).

33 days after fruit set (Second spray)

The perusal of the pooled data in Table 4.28 & Fig 4.32 showed, the least number of infested fruits were noticed in trees with spinosad 45 SC (8.66), closely followed by *B. thuringiensis* var. *kurstaki* (9.29), novaluron 10 EC (9.58) and diflubenzuron 25 WP (10.83). Whereas, the treatments neem seed kernel extract 4% and kamdhenu keet niyantrak 5% were recorded with 11.70 and 13.38 infested fruits, respectively. The maximum infested fruits were noticed in trees

Table 4.28 Pooled data on the efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 & 2023 at Farmers farm, Medziphema, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)								
		13 DAF (First spray)			33 DAF (Second spray)			53 DAF (Third spray)		
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
Neem seed kernel extract 4%	400	1.33	1.66	1.50	11.81	11.60	11.70	14.49	14.04	14.27
Bacillus thuringiensis var. *kurstaki*	50	1.44	1.33	1.39	9.12	9.45	9.29	10.25	10.35	10.30
Spinosad 45 SC	4.5	1.00	0.89	0.94	8.39	8.93	8.66	9.24	3.90	6.57
Diflubenzuron 25 WP	3.0	1.55	1.22	1.39	10.50	11.16	10.83	11.49	12.30	11.90
Novaluron 10 EC	1.5	1.00	1.89	1.44	9.21	9.95	9.58	11.53	12.52	12.03
Neem oil 0.2%	200	1.77	1.66	1.72	14.16	14.92	14.54	14.76	16.06	15.41
Kamdhenu keet niyantrak 5%	500	1.22	1.55	1.39	13.77	13.00	13.38	14.75	15.19	14.97
Untreated check	-	6.02	6.45	6.24	22.39	23.07	22.73	44.98	45.76	45.37
SEm (±)	-	0.82	0.84	0.99	3.11	3.00	0.98	3.25	3.28	1.87
CD (5%)	-	2.49	2.56	2.56	9.45	9.09	2.83	9.86	9.96	5.40

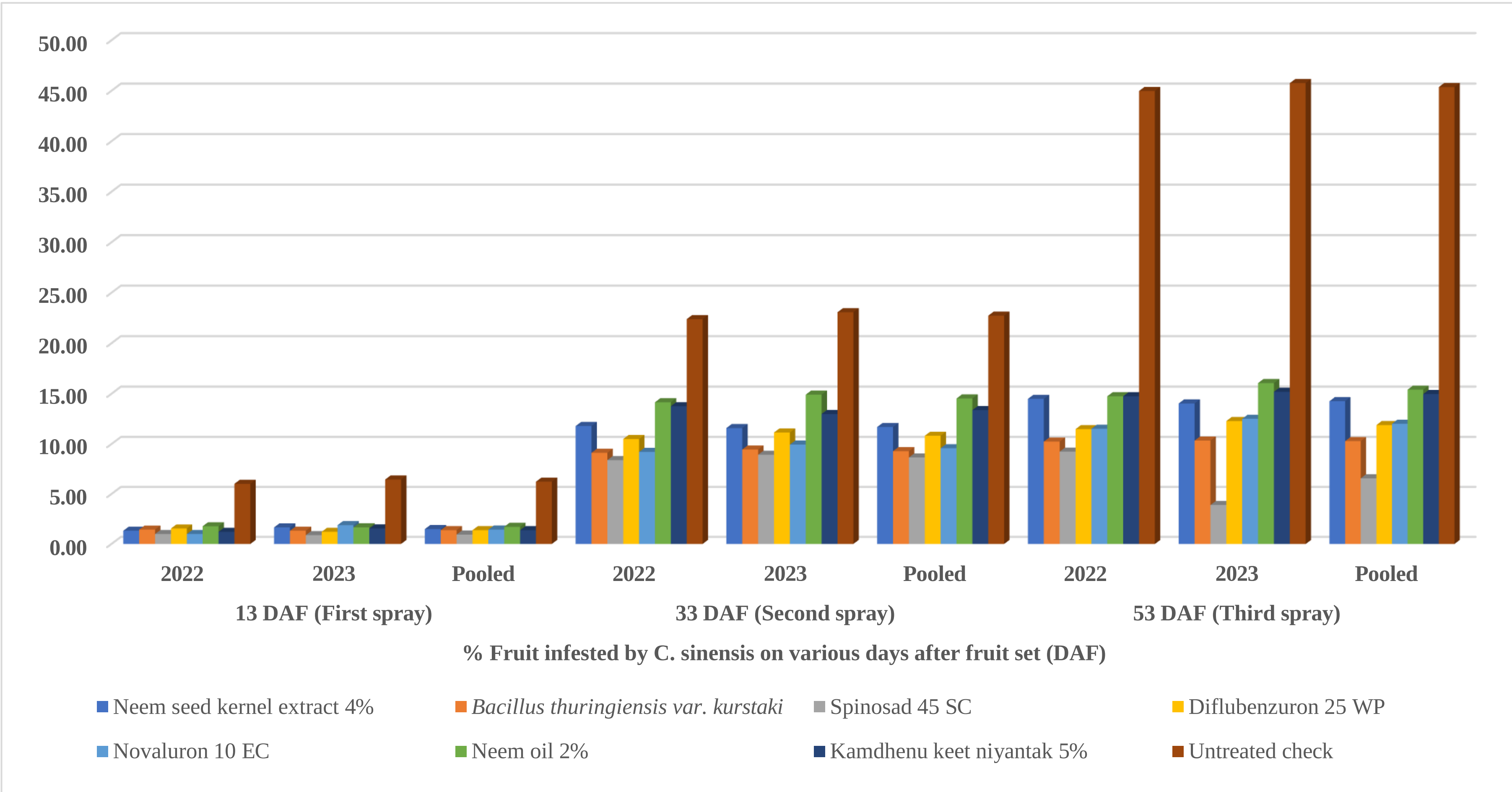


Figure 4.32 Pooled data on the efficacy of various insecticides and bio-pesticides against litchi fruit borer, *C. sinensis* during April-June, 2022 & 2023 at Farmers farm, Medziphema, Nagaland

with neem oil 0.2% (14.54). However, all the treatments were statistically significant compared to untreated check (22.73 infested fruits).

53 days after fruit set (Third spray)

The pooled data in Table 4.28 & Fig 4.32 represented that, all the treatments were statistically significant compared to untreated check (45.37 infested fruits). The treatment Spinosad 45 SC was recorded with least number of infested fruits (6.57), followed by *B. thuringiensis* var. *kurstaki* (10.30 infested fruits). Whereas, the treatments diflubenzuron 25 WP (11.90) and novaluron 10 EC (12.03) were on par with each other. The maximum infested fruits were noticed in neem oil 0.2% (15.41), followed by kamdhenu keet niyantrak 5% (14.97) and neem seed kernel extract 4% (14.27).

4.4.8 Pooled data on the efficacy of various insecticides and bio-pesticides on reduction of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 & 2023 at Farmers farm, Medziphema, Nagaland

13 days after fruit set (First spray)

The pooled data in Table 4.29 & Fig 4.33 showed that, the highest percent reduction of fruit borer (93.36%) over control was noticed in spinosad 45 SC, followed by *B. thuringiensis* var. *kurstaki* (90.54%). Whereas, the treatment diflubenzuron 25 WP was recorded with 87.74% reduction, followed by novaluron 10 EC (85.24%). The least percent reduction (76.20%) was recorded in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% (80.71%) and neem seed kernel extract 4% (82.73%).

33 days after fruit set (Second spray)

The perusal of pooled data in Table 4.29 & Fig 4.33 showed that, treatment spinosad 45 SC was recorded the highest percent reduction (85.07%) of fruit borer, followed by *B. thuringiensis* var. *kurstaki* (82.98%). Whereas, treatments

Table 4.29 Pooled data on the efficacy of various insecticides and bio-pesticides on reduction of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 & 2023 at Farmers farm, Medziphema, Nagaland

Treatments	Dosage (ml or gm/10 lit)	% Fruit infested by *C. sinensis* on various days after fruit set (DAF)								
		13 DAF (First spray)			33 DAF (Second spray)			53 DAF (Third spray)		
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
Neem seed kernel extract 4%	400	83.50	81.97	82.73	65.67	70.93	68.30	65.18	66.14	65.66
Bacillus thuringiensis var. *kurstaki*	50	90.98	90.09	90.54	84.18	81.79	82.98	81.26	80.87	81.06
Spinosad 45 SC	4.5	93.80	92.92	93.36	85.46	84.68	85.07	83.15	82.25	82.70
Diflubenzuron 25 WP	3.0	86.48	89.00	87.74	76.45	72.49	74.47	73.97	71.97	72.97
Novaluron 10 EC	1.5	85.84	84.64	85.24	77.19	74.54	75.87	71.39	70.06	70.73
Neem oil 0.2%	200	77.32	75.08	76.20	58.01	57.23	57.62	58.57	58.09	58.33
Kamdhenu keet niyantrak 5%	500	81.39	80.03	80.71	62.61	64.17	63.39	63.62	62.35	62.98
Untreated check	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

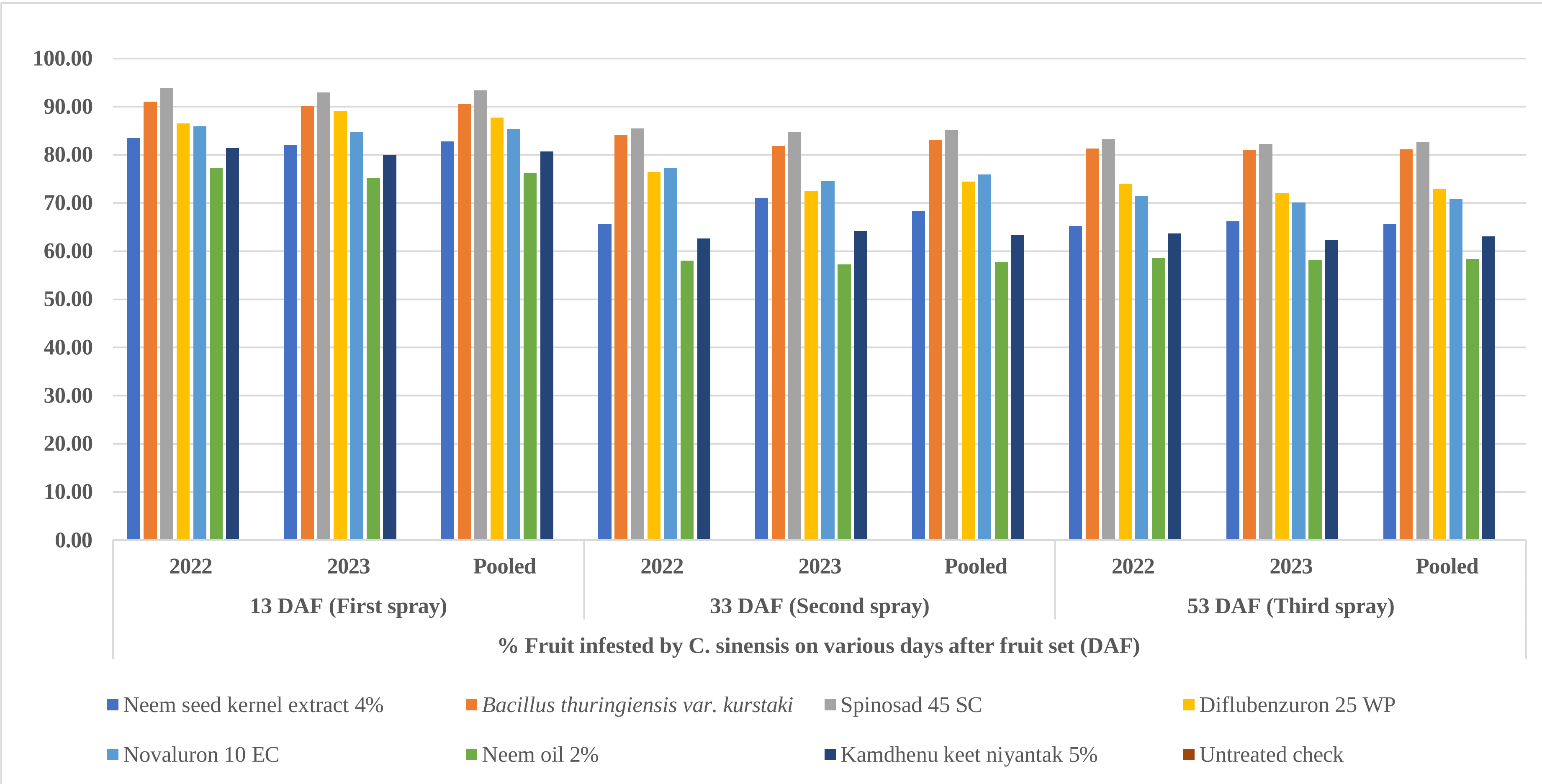


Figure 4.33 Pooled data on the efficacy of various insecticides and bio-pesticides on reduction of litchi fruit borer, *C. sinensis* infestation during April-June, 2022 & 2023 at Farmers farm, Medziphema, Nagaland

novaluron 10 EC (75.87%) and diflubenzuron 25 WP (74.47%) were on par with each other. The least percent reduction (57.62%) was noticed in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% and neem seed kernel extract 4% *i.e*., 63.39% and 68.30%, respectively.

53 days after fruit set (Third spray)

The pooled data in Table 4.29 & Fig 4.33 represented that, the highest percent fruit borer reduction (82.70%) was recorded in the treatment spinosad 45 SC, followed by *B. thuringiensis* var. *kurstaki* (81.06%). Whereas, the treatment diflubenzuron 25 WP was recorded with 72.97%, followed by novaluron 10 EC (70.73%). The least percent fruit borer reduction (58.33%) was noticed in neem oil 0.2%, followed by kamdhenu keet niyantrak 5% (62.98%) and neem seed kernel extract 4% (65.66%).

From the above study, it is concluded that spraying of spinosad 45 SC @ 4.5 ml per 10 lit. and *B. thuringiensis* @ 50 gm per 10 lit. of water found very much effective in managing the litchi fruit borer, *C. sinensis*. It should be applied at least three times at an interval of 15-20 days after the fruit set. Since these insecticides have selective activity against insects than mammals, also may reduce the health risk to humans. The findings of present investigation hold a good promise in management of litchi fruit borer and can significantly increases the fruit yield of litchi.

The present findings are in accordance with Srivastava *et al*. (2021) and Ranjan *et al*. (2019) who reported that, three sprays of spinosad 45 SC @ 1.75ml/5lit. of water at 10-15 days interval was found effective in reducing the fruit borer infestation. This might be due to the disruption of acetylcholine neurotransmission by which, it causes hyperexcitation of the insect nervous system. Our results showed that, *Bacillus thuringiensis* was also found effective against *C. sinensis* with less infestation. Schulte *et al*. (2007) reported that *B.*

thuringiensis was effective against litchi fruit borer, *C. sinensis*.

The results are also in agreement with Srivastava *et al*. (2017) who reported that IGRs like diflubenzuron 25 WP and novaluron 10 EC were also effective against *C. sinensis*. This might be due to ovicidal action as well as inhibition of chitin synthesis of insects which causes abnormal endocuticular deposition and abortive moulting (Mulder and Gijswijt, 1973). However, organic chemicals like neem-based insecticides and cow urine were not found effective against *C. sinensis*. Similar results were also found by Kumar *et al*. (2014a), Ranjan *et al*. (2019) and Upadhyay *et al*. (2020) against *C. sinensis*.

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present investigation entitled "**Study on life cycle of litchi fruit borer(s) and their management"** was carried out at two different locations *viz*., Experimental Research Block, Dept. of Horticulture, School of Agricultural Sciences, Medziphema campus, Nagaland University, and farmers farm, Medziphema, Nagaland under the following objectives: identification of litchi fruit borer(s), to study the life cycle of litchi fruit borer(s), *C. sinensis*, to study the seasonal incidence of litchi fruit borer(s) and their natural enemies and efficacy study of various insecticides and biopesticides against litchi fruit borer(s).

The results thus obtained during the period of investigation are elucidated objective wise in this chapter:

5.1.1 Identification of litchi fruit borer(s)

A total of five identified species were recorded out of 565 specimens collected and reared on litchi fruits. All the collected and reared specimens belong to five genera under four families *viz*., Crambidae, Gracillariidae, Lycaenidae and Tortricidae. The families Crambidae, Gracillariidae, and Lycaenidae were represented by single species each *viz*., *C. punctiferalis*, *C. sinensis* and *D. epijarbus*, respectively. Whereas, Tortricidae was represented by two species, *C. ombrodelta* and *T. zophophanes*. Among these species, *T. zophophanes* was the first record for India feeding on litchi fruits. Among the collected species, *C. sinensis* was found to be predominant with 46.37 per cent followed by *C. ombrodelta* with 32.03 per cent. Other species *i.e*., *D. epijarbus*, *T. zophophanes* and *C. punctiferalis* recorded 10.61, 7.05, and 3.89 per cent, respectively. An illustrated key was prepared for families and species of fruit borers of litchi based on the morphological and genital characters of adults.

The phylogenetic analysis reveals that, the transition between A and G (28.21%) was higher than the transition between T and C (21.33%). There was also a strong AT bias (70.54%). The overall transition/transversion bias is R = 2.01. The intraspecific genetic divergence ranged from 0.00% to 0.14% with overall mean of 0.13%. A minimum intraspecific nucleotide divergence of 0.00% was found in all the species except *T. zophophanes* (0.01%), while a maximum intraspecific nucleotide divergence of 0.04% was found in *C. punctiferalis* and *C. ombrodelta.* The phylogenetic tree analysis showed that, *C. ombrodelta* found as a sister group to the clade of *Cryptophlebia* sp. and *C. illepida* in the family Tortricidae. While, the species, *T. zophophanes* was found as a sister group to the clade *T. leucotreta*. The species, *C. sinensis* found as a sister group to the clade, *C. litchiella*. Besides, the clade (*C. sinensis* + *C. litchiella*) is much closer to the clade, *C. cramerella* under the family Gracillariidae. The species, *C. punctiferalis* and *D. epijarbus* were found in their respective clades of the families Crambidae and Lycaenidae, respectively.

5.1.2 To study the life cycle of litchi fruit borer(s), *Conopomorpha sinensis* Bradley

The biology of *C. sinensis* was studied under laboratory condition. Eggs were laid singly, yellowish orange, flattened and scale like. During the larval period, the larva moulted four times and thus having five larval instars. The first larval instar is transparent, milky white in colour. The second and third instar is creamy white and thick creamy white in colour, respectively. Whereas, fourth and fifth instar larva is yellowish cream and light green in colour, respectively. The pupa is slender, yellowish in colour with prominent eyes, well developed maxillary palpi, antennae, proboscis and legs. Adult smaller in size, greyish brown moth with a yellowish-brown wing apex. The duration of developmental stages such as egg, larval, pre-pupal, pupal, male and female adult period lasts for 3-5, 8-14, 1-3, 4-7, 4-7, and 7-11 days, respectively. The pre-mating, pre-oviposition, and oviposition periods lasts for 2-3, 2-3, and 5-7 days, respectively. The fecundity was 25-43 eggs/female. The total life cycle from egg to adult stage last for 20-36 days in male, whereas 23-40 days in female. Further, the morphometric observations were also made for various life stages.

5.1.3 To study the seasonal incidence of litchi fruit borer and their natural enemies(s)

In the college farm, during 2022 and 2023, the initial infestation *i.e*., 0.33% and 0.17% was first observed at 16th April and 10th April, respectively when the fruits were small, tender, with having no pulp formation. The infestation gradually increased and reached to its peak *i.e*., 47.67% and 48.33% in the last week of May when the fruits were reddish pink coloration leading to maturation. After that, a considerable decrease was observed. From the statistical analysis, it was found that temperature has a profound impact on the larval activity of pest species, followed by relative humidity. Whereas, the rainfall has little influence on the pest activity of the species.

In farmers farm, during 2022 and 2023, the primary phase of infestation *i.e*., 0.50% and 0.17% was first observed at 10th April and 11th April, respectively when the fruits were small, tender, without having any pulp formation. The infestation gradually increased and reached to its peak *i.e*., 36.50% and 42.00% at 5th June and 30th May, respectively when the fruits were reddish pink coloration leading to maturation. After then, an erratic decline was noticed. It is evident from statistical analysis, temperature has a significant impact on the larval activity of the pest species, rather relative humidity. However, the rainfall has little influence on the activity of the pest species.

A total of four natural enemies were recorded preying on litchi fruit borer, *C. sinensis*. Among these, one was a predator, *C. sexmaculata*. Among three parasitoids, one was a chalcid wasp, *B. euploeae* and the other two were unidentified parasitoids of the families Eulophidae and Ichneumonidae.

5.1.4 Efficacy study of various insecticides and biopesticides against litchi fruit borer(s)

Total of eight treatments *viz*., neem seed kernel extract 4% @ 400ml/10lit, *B. thuringiensis* var. *kurstaki* @ 50gm/10lit, spinosad 45 SC @ 4.5 ml/10lit, diflubenzuron 25 WP @ 3 gm/10lit, novaluron 10 EC @ 1.5 ml/10lit, neem oil 0.2% @ 20 ml/10lit, kamdhenu keet niyantak 5% @ 500 ml/10lit and untreated check were evaluated against the litchi fruit borer, *C. sinensis* at Experimental Research Block, Dept. of Horticulture, SAS, Nagaland University, Nagaland and Farmers farm, Medziphema, Nagaland during the months of April-June, 2021-2023.

In the college farm, during 2022 and 2023, the highest percent reduction over control was observed in spinosad 45 SC at 13 DAF (94.15%), 33 DAF (84.42%) and 53 DAF (81.65%), followed by *B. thuringiensis* var. *kurstaki* with 91.50%, 81.84% and 79.27%. The least percent reduction of fruit borer (78.14%, 58.57% and 56.67%) was found in neem oil 0.2% at 13 DAF, 33 DAF and 53 DAF, respectively. In the farmers farm, during 2022 and 2023, the highest percent reduction over control was recorded in spinosad 45 SC at 13 DAF (93.36%), 33 DAF (85.07%) and 53 DAF (82.70%), followed by *B. thuringiensis* var. *kurstaki* with 90.54%, 82.98% and 81.06%. The least percent reduction of fruit borer (76.20%, 57.62% and 58.33%) was found in neem oil 0.2% at 13 DAF, 33 DAF and 53 DAF, respectively.

5.2 Conclusion

From the above-mentioned fact of data, it may be concluded that the different experiments on study of litchi fruit borer(s) were found to provide effective results on identifying the fruit borer pest complex, life cycle studies, seasonal incidence, its natural enemies and management aspects.

- A total of five fruit borer species viz., *C. punctiferalis, C. sinensis, D. epijarbus, C. ombrodelta* and *T. zophophanes* were found infesting litchi fruits in Nagaland. Among these, *C. sinensis* was the most devastating and predominant species (55.57%). All the species exhibited variations with respect to morphological and genital characters and an illustrated key was prepared based on these variations.

- The phylogenetic analysis reveals that, there was a strong AT bias (70. 54%). The minimum intraspecific nucleotide divergence of 0.00% was found in all the species except *T. zophophanes* (0.01%), while the maximum intraspecific nucleotide divergence of 0.04% was found in *C. punctiferalis* and *C. ombrodelta*. Further, all the species were placed in their respective clades *i.e.*, Crambidae, Gracillariidae, Lycaenidae and Tortricidae.

- The adult female moth lays eggs singly, and are yellowish in colour. Larva undergoes four moultings and thus having five larval instars. Pupa is yellowish in colour with well-developed eyes, antennae, proboscis, maxillary and labial palpi, and legs. Adult is small, brown moth with trapezoidal wing apex. Total life cycle completed in 20-40 days. Morphometric studies were studied for various stages of litchi fruit borer.

- In case of seasonal incidence, it was found that fruit borer infestation gradually increased and reaches its peak during the last week of May to first week of June and then decreases gradually. Also, it was found that temperature has a significant impact on the pest larval activity, whereas, relative humidity and rainfall has little influence on the activity of the pest species.

- Among the eight treatments, it was found that spinosad 45 SC @ 4.5ml/10lit. was found much effective in reducing the fruit borer infestation followed by

B. thuringiensis var. *kurstaki* @ 50gm/10 lit. Whereas, neem oil 0.2% @ 20ml/10 lit. was found least effective compared to other treatments.

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