DIVERSITY OF FRUIT FLIES AND THEIR NATURAL ENEMIES ON IMPORTANT CUCURBITS OF NAGALAND

Thesis

Submitted to

NAGALAND UNIVERSITY

in partial fulfillment of requirements for the Degree

of

Doctor of Philosophy

in

Entomology

by

HUIREM DIANA DEVI

Admn. No. Ph – 256/18 Regn. No. Ph.D./ENT/00242

Department of Entomology

School of Agricultural Sciences

Nagaland University, Medziphema Campus – 797 106

Nagaland

2022

Dedicated

To

My Family

DECLARATION

I, Huirem Diana Devi, 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:

(HUIREM DIANA DEVI)

Place:

……………………………………

Supervisor

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

Dr. Pankaj Neog
Associate Professor
Department of Entomology

CERTIFICATE – I

This is to certify that the thesis entitled **"Diversity of fruit flies and their natural enemies on important cucurbits of Nagaland"** submitted to Nagaland University in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy (Agriculture) in Entomology is the record of research work carried out by Ms. HUIREM DIANA DEVI Registration No.Ph.D./ENT/00242 under my personal supervision and guidance.

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

Date:

Place:

....

Dr. PANKAJ NEOG

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 **"Diversity of fruit flies and their natural enemies on important cucurbits of Nagaland"** submitted by HUIREM DIANA DEVI, Admission No. Ph-256/18 Registration No. Ph.D./ENT/00242 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.**

Members	Signature
1.……………………………… (Supervisor & Chairman)	………………………
2. ……………………………… (External examiner)	………………………….
3. ………………………………	………………………….
4. ………………………………	………………………….
5. ………………………………	………………………….
6.………………………………	…………………………

Head
Department of Entomology

Dean
School of Agricultural Sciences and Rural Development

ACKNOWLEDGEMENTS

First and foremost, I give thanks to the Almighty 'God' for His abundant blessings and grace throughout my research programme. Nothing would have been accomplished without His help.

With immense pleasure, I would like to express my deepest gratitude to my supervisor Dr. Pankaj Neog, Associate Professor, Department of Entomology, SAS, Nagaland University for his guidance, support and supervision in all my works, without whose help this thesis would have been incomplete.

I would also like to extend my sincere thanks to the members of Advisory Committee, Dr. G.T. Behere, Principal Scientist. Division of Crop Protection, ICAR- RC for NEH Region, Umiam, Meghalaya, Dr. Hijam Shila Devi, Assi. Prof., Department of Entomology, Dr. N. Tiameren Ao, Prof., Department of Plant Pathology and Dr. S.P. Kanaujia, Prof., Department of Horticulture, NU, SAS, Medziphema Campus for their valuable suggestions and inputs throughout the period of study.

I also express my gratitude to Prof. L. Daiho, Dean, SAS, Nagaland University for his consistent support and for all the assistance the college provided.

I would also like to acknowledge the help of Mr. J. Akato Chishi, STA and the non teaching staffs of the Department of Entomology for their constant valuable help throughout my research work. I am also thankful to Mr. Mhombemo Ngullie, Deputy Librarian and his staff for the assistance and help provided whenever needed.

I also extend my sincere thanks to Dr.Susheel Kumar, Principal Scientist, ICAR, Lamphelpat, Manipur for his enormous help in carrying out my molecular lab work. I would also like to thanks to Dr. Sh. Herojit Department of Agricultural Economics, for helping me out in analysis of data. I am immensely thankful to my friends- Kh. Romio, Naorem chanu Gulleibi, Arensangla, Rumki, Martha, Suraj Yengkhom, Ng Monica and all my seniors and juniors for their constant help, support and encouragement throughout my studies and research work. I will forever be indebted to you all.

The debts of gratitude I owe to my parents, brothers and sisters cannot be comprehended. I thank them for their sacrifice, blessings, unconditional love, support and unfailing patience and for being the strength behind all my endeavours.

Dated:
Place: Medziphema, Nagaland

(Huirem Diana Devi)

CONTENTS

CHAPTER	TITLE	PAGE NO.
1.	INTRODUCTION	1- 5
2.	REVIEW OF LITERATURE	6- 29
	2.1 Species diversity and relative abundance of fruit flies and their natural enemies	
	2.2 Seasonal abundance of fruit flies and their natural enemies	
	2.3 Extent of damage caused by fruit flies on different cucurbits	
	2.4 Evaluation of the effectiveness of different attractants against major fruit flies complex	
	2.5 Development of DNA barcodes of fruit flies and Natural enemy	
3.	MATERIALS AND METHODS	30- 44
	3.1 GENERAL INFORMATIONS	
	3.1.1 Geographical location of the experimental site	
	3.1.2 Climatic conditions and soil status	
	3.2 Experimental details	
	3.2.1 Design and layout of the field	
	3.2.2 Treatment details	
	3.2.3 Preparation of baited trap	
	3.3 Cultivation practices	
	3.3.1 Planting materials	
	3.3.2 Sowing of seeds	
	3.3.3 Manures and fertilizer	
	3.3.4 Thinning	
	3.3.5 Irrigation	
	3.3.6 Weeding	
	3.3.7 Platform	
	3.4 Observation recorded	
	3.4.1 Study on species diversity and relative abundance of fruit flies and their natural enemies in different cucurbits	
	3.4.1.1 Relative abundance	
	3.4.1.2 Diversity indices	
	3.4.1.3 Seasonal abundance of fruit flies and their natural enemies	
	3.4.2 Extent of damage caused by fruit flies in different cucurbit crops	
	3.4.3 Evaluate the effectiveness of different attractants against fruit fly complex	
	3.4.4 Development of DNA barcode of fruit fly and natural enemy	
	3.4.4.1 Collection, preservation and identification of fruit fly and natural enemy	

3.4.4.2 Extraction of DNA
3.4.4.3 Qualitative and Quantitative estimation of DNA
3.4.4.4 Development of DNA barcodes by using COI gene
3.4.4.5 Sequencing of PCR amplicons of COI gene for insect pests and natural enemies of cucurbitaceous crops
3.4.4.6 Bioinformatics analysis

4. RESULTS AND DISCUSSION 45- 129

4.1 Species diversity and relative abundance of fruit flies species
4.1.1 Species Identification
4.1.2 Diversity indices of fruit flies on important cucurbits of Nagaland
4.1.3 Relative abundance of fruit flies species in different cucurbits
4.1.3.1 Cucumber
4.1.3.2 Pumpkin
4.1.3.3 Ash gourd
4.2 Seasonal abundance of fruit flies in different cucurbits and their correlation with abiotic factors
4.2.1 Seasonal abundance of fruit flies in different cucurbits
4.2.1.1 Seasonal abundance of *B. dorsalis* in different cucurbits
4.2.1.2 Seasonal abundance of *Z. tau* in different cucurbits
4.2.1.3 Seasonal abundance of *B. tuberculata* in different cucurbits
4.2.1.4 Seasonal abundance of *Z. cucurbitae* in different cucurbits
4.2.1.5 Seasonal abundance of *B. ruiliensis* in different cucurbits
4.2.2 Correlation between different species of fruit flies with abiotic factors
4.2.2.1 Correlation between different species of fruit flies with abiotic factors in cucumber
4.2.2.2 Correlation between different species of fruit flies with abiotic factors in Pumpkin
4.2.2.3 Correlation between different species of fruit flies with abiotic factors in Ash gourd
4.3. Species diversity and relative abundance of natural enemies of fruit flies
4.3.1 Species Identification
4.3.2 Diversity indices of natural enemies of fruit flies on important cucurbits of Nagaland
4.3.3 Relative abundance of natural enemies of fruit flies in different cucurbit
4.3.3.1 Cucumber
4.3.3.2 Pumpkin
4.3.3.3 Ash gourd
4.4. Seasonal abundance of natural enemies for fruit flies in

different cucurbits and their correlation with abiotic factors
4.4.1 Seasonal abundance of natural enemies of fruit flies in different cucurbits
4.4.1.1 Seasonal abundance of *O. smaragdina* in different cucurbits
4.4.1.2 Seasonal abundance of *Tapinoma* sp. in different cucurbits
4.4.1.3 Seasonal abundance of *P. paracamponota* in different cucurbits
4.4.1.4 Seasonal abundance of *Crematogaster* sp. in different cucurbits
4.4.1.5 Seasonal abundance of *Paederus fuscipes* in different cucurbits
4.4.2 Correlation between different species of natural enemies for fruit flies with abiotic factors
4.4.2.1 Correlation between different species of natural enemies for fruit flies with abiotic factors in cucumber
4.4.2.2 Correlation between different species of natural enemies for fruit flies with abiotic factors in Pumpkin
4.4.2.3 Correlation between different species of natural enemies for fruit flies with abiotic factors in Ash gourd
4.5 Extent of damage caused by fruit fly on different cucurbits
4.6 Evaluation of the effectiveness of different attractants against major fruit fly complex
4.6.1 Effect of different attractants on *B. dorsalis* adult in different cucurbits
4.6.2 Effect of different attractants on *Z. tau* adult in different cucurbits
4.6.3 Effect of different attractants on *B. tuberculata* adult in different cucurbits
4.6.4 Effect of different attractants on adults of *Z. cucurbitae*in different cucurbits
4.6.5 Effect of different attractants on *B. ruiliensis* adult in different cucurbits
4.7 Development of DNA barcodes for different species of fruit flies and their natural enemies of cucurbitaceous crops ecosystem
4.7.1 Extraction of DNA (Deoxyribonucleic acid) from insect
4.7.2 Qualitative and Quantitative estimation of DNA
4.7.3 PCR amplification of COI gene
4.7.4 Sequencing of partial PCR fragment of COI gene
4.7.5 Sequencing analysis
4.7.6 Submission of sequences to NCBI
4.7.7 Blast analysis
4.7.8 Development of DNA barcodes

5. SUMMARY AND CONCLUSION 130 -137

REFERENCES i- xxi

APPENDIX i-vi

LIST OF TABLES

TABLE NO.	TITLE	PAGES
4.1	Diversity of fruit flie's species recorded in different cucurbit crops during 2020 and 2021	47
4.2	Relative abundance (%) of fruit flies species recorded during 2020 and 2021	49
4.3	Seasonal abundance of *B. dorsalis* recorded during May-July 2020 on different cucurbit crops	53
4.4	Seasonal abundance of *B. dorsalis* recorded during May-July 2021 on different cucurbit crops	54
4.5	Seasonal abundance of *Z. tau* recorded during May-July 2020 on different cucurbit crops	57
4.6	Seasonal abundance of *Z. tau* recorded during May-July 2021 on different cucurbit crops	58
4.7	Seasonal abundance of *B. tuberculata* recorded during May-July 2020 on different cucurbit crops	59
4.8	Seasonal abundance of *B. tuberculata* recorded during May-July 2021 on different cucurbit crops	60
4.9	Seasonal abundance of *Z. cucurbitae* recorded during May-July 2020 on different cucurbit crops	61
4.10	Seasonal abundance of *Z. cucurbitae* recorded during May-July 2021 on different cucurbit crops	62
4.11	Seasonal abundance of *B. ruiliensis* recorded during May-July 2020 on different cucurbit crops	66
4.12	Seasonal abundance of *B. ruiliensis* recorded during May-July 2021 on different cucurbit crops	67

4.13	Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2020 on cucumber	68
4.14	Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2021 on cucumber	69
4.15	Correlation coefficient (r) of fruit flies species with abiotic factors recorded during May-July 2020 on pumpkin	71
4.16	Correlation coefficient (r) of fruit flies with abiotic factors recorded during May-July 2021 on pumpkin	72
4.17	Correlation coefficient (r) of fruit flies with abiotic factors recorded during May-July 2020 on Ash gourd	73
4.18	Correlation coefficient (r) of fruit flies with abiotic factors recorded during May-July 2021 on ash gourd	74
4.19	Diversity of fruit fly natural enemies in different cucurbit crops recorded during 2020 and 2021	78
4.20	Relative abundance (%) of fruit flie's natural enemies recorded during 2020 and 2021	80
4.21	Seasonal abundance of *O. smaragdina* recorded during March-July 2020 on different cucurbit crops	84
4.22	Seasonal abundance of *O. smaragdina* recorded during March-July 2021 on different cucurbit crops	85
4.23	Seasonal abundance of *Tapinoma* sp. recorded during March-July 2020 on different cucurbit crops	86
4.24	Seasonal abundance of *Tapinoma* sp. recorded during March-July 2021 on different cucurbit crops	87
4.25	Seasonal abundance of *Polyrhachis* sp. recorded during March-July 2020 on different cucurbit crops	89
4.26	Seasonal abundance of *Polyrhachis* sp. recorded during March-July 2021 on different cucurbit crops	90

4.27	Seasonal abundance of *Crematogaster* sp. recorded during March-July 2020 on different cucurbit crops	92
4.28	Seasonal abundance of *Crematogaster* sp. recorded during March-July 2021 on different cucurbit crops	93
4.29	Seasonal abundance of *P. fuscipes* recorded during March-July 2020 on different cucurbit crops	94
4.30	Seasonal abundance of *P. fuscipes* recorded during March-July 2021 on different cucurbit crops	95
4.31	Correlation coefficient (r) of fruit fly natural enemies recorded with abiotic factors during March- July 2020 on cucumber	97
4.32	Correlation coefficient (r) of fruit fly natural enemies recorded with abiotic factors during March- July 2021 on cucumber	98
4.33	Correlation coefficient (r) of fruit fly natural enemies recorded with abiotic factors during March- July 2020 on Pumpkin	100
4.34	Correlation coefficient (r) of fruit fly natural enemies recorded with abiotic factors during March- July 2021 on Pumpkin	101
4.35	Correlation coefficient (r) of fruit fly natural enemies recorded with abiotic factors during March- July 2020 on Ash gourd	103
4.36	Correlation coefficient (r) of fruit fly natural enemies recorded with abiotic factors during March- July 2021 on Ash gourd	104
4.37	Extent of damage (%) caused by fruit flies on different cucurbits during 2020	107

4.38	Extent of damage (%) caused by fruit flies on different cucurbits during 2021	108
4.39	Effect of different attractants against *B. dorsalis* during 2020 and 2021	111
4.40	Effect of different attractants against *Z. tau* during 2020 and 2021	113
4.41	Effect of different attractants against *B. tuberculata* during 2020 and 2021	116
4.42	Effect of different attractants against *Z. cucurbitae* during 2020 and 2021	117
4.43	Effect of different attractants against *B. ruiliensis* during 2020 and 2021	120
4.44	Qualitative and Quantitative estimation of DNA from fruit flies and their natural enemies	122
4.45	List of identified species along with nucleotide length, protein length and NCBI accession number	125
4.46	NCBI BLASTN search results for different fruit flies species as on 30.06.22	127
4.47	NCBI BLASTN search results for different fruit flie’s natural enemies as 30.06.22	128

LIST OF FIGURES

FIGURE NO.	CAPTION	IN BETWEEN PAGES
3.1	Lay out of experimental field (Cucumber) in Randomized Block Design	33-34
3.2	Lay out of experimental field (pumpkin and ash gourd) in Randomized Block Design	33-34
4.1	Relative abundance (%) of fruit flies species recorded during 2020 and 2021	49-50
4.2	Seasonal abundance of *B. dorsalis* recorded during May-July 2020 on different cucurbit crops	53-54
4.3	Seasonal abundance of *B. dorsalis* recorded during May-July 2021 on different cucurbit crops	54-55
4.4	Seasonal abundance of *Z. tau* recorded during May-July 2020 on different cucurbit crops	57-58
4.5	Seasonal abundance of *Z. tau* recorded during May-July 2021 on different cucurbit crops	58-59
4.6	Seasonal abundance of *B. tuberculata* recorded during May-July 2020 on different cucurbit crops	59-60
4.7	Seasonal abundance of *B. tuberculata* recorded during May-July 2021 on different cucurbit crops	60-61
4.8	Seasonal abundance of *Z. cucurbitae* recorded during May-July 2020 on different cucurbit crops	61- 62
4.9	Seasonal abundance of *Z. cucurbitae* recorded during May-July 2021 on different cucurbit crops	62-63
4.10	Seasonal abundance of *B. ruiliensis* recorded during May-July 2020 on different cucurbit crops	66-67
4.11	Seasonal abundance of *B. ruiliensis* recorded during May-July 2021 on different cucurbit crops	67- 68
4.12	Relative abundance (%) of natural enemies of fruit flies recorded during 2020 and 2021	80-81
4.13	Seasonal abundance of *O. smaragdina* recorded during March-July 2020 on different cucurbit crops	84-85
4.14	Seasonal abundance of *O. smaragdina* recorded during March-July 2021 on different cucurbit crops	85-86

4.15	Seasonal abundance of *Tapinoma* sp. recorded during March-July 2020 on different cucurbit crops	86-87
4.16	Seasonal abundance of *Tapinoma* sp. recorded during March-July 2021 on different cucurbit crops	87-88
4.17	Seasonal abundance of *Polyrhachis* sp.recorded during March-July 2020 on different cucurbit crops	89-90
4.18	Seasonal abundance of *Polyrhachis* sp.recorded during March-July 2021 on different cucurbit crops	90-91
4.19	Seasonal abundance of *Crematogaster* sp. recorded during March-July 2020 on different cucurbit crops	92-93
4.20	Seasonal abundance of *Crematogaster* sp.recorded during March-July 2021 on different cucurbit crops	93-94
4.21	Seasonal abundance of *P. fuscipes* recorded during March-July 2020 on different cucurbit crops	94-95
4.22	Seasonal abundance of *P. fuscipes* recorded during March-July 2021 on different cucurbit crops	95-96
4.23	Extent of damage (%) caused by fruit flies on different cucurbits during 2020 and 2021	108-109
4.24	Effect of different attractants on the population of *B. dorsalis* during 2020 and 2021	111- 112
4.25	Effect of different attractants on the population of *Z. tau* during 2020 and 2021	113-114
4.26	Effect of different attractants on the population of *B. tuberculata* during 2020 ana 2021	116- 117
4.27	Effect of different attractants on the population of *Z. cucurbitae* during 2020 ana 2021	117-118
4.28	Effect of different attractants on the population of *B. ruiliensis* during 2020 ana 2021	120-121

LIST OF PLATES

PLATE NO.	CAPTION	IN BETWEEN PAGES
1	General view of experimental fields	34-35
2	Nature of damaged caused by fruit flies on different cucurbit crops	38-39
3	Different species of fruit fly	45-46
4	Different species of natural enemy of fruit flies	76-77
5	PCR amplification of different fruit flies species and their natural enemies using LepF1 and LepR1 primers on 1.5% agarose gel	121-122
6	Translated image of nucleotide sequences of different species of fruit flies on important cucurbits of Nagaland	126-127
7	Translated image of nucleotide sequences of different species of natural enemies of fruit flies on important cucurbits of Nagaland	127-128

LIST OF APPENDICES

APPENDIX NO.	TITLE	PAGES
A	Nucleotide sequences of different species of fruit flies	**i-iii**
B	Nucleotide sequences of natural enemies of fruit flies	**iv-vi**

LIST OF ABBREVIATIONS

%	Per cent
@	at the rate
^{0}C	Degree Celsius
etc.	*et cetera*
et al.	*Et alli* (and other)
viz.	videlicet (namely)
ha	Hectare
mt	Metric Tonnes
g	Gram
mg	Mili gram
ml	millilitre
Fig.	Figure
SEm	Standard error of mean
RBD	Randomized block design
DNA	Deoxyribonucleic acid
mtDNA	mitochondrial DNA
gDNA	Genomic DNA
AT	Adenine, Thymine
GC	Guanine, Cytosine
COI	Cytochrome oxidase subunit I
Spp/sp.	Species
rRNA	Ribosomal ribonucleic acid
rpm	Revolutions per minute
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
Pvt.ltd	Private limited

Cyt b	Cytochrome b
bp	base pair
ng/ µl	Nanogram per microlitre
SDS	Sodium Dodecyl Sulfate
Mm	milli molar
EDTA	Ethylene-di-amine-tetra acetic acid
TAE	Tris-acetate-EDTA
dNTP	deoxyribonucleoside-triphosphate
M	Molar
µl	Microlitre
µ	Micron
G	Gram
Ng	Nano gram
UV	Ultra Violet
V	Volt
NCBI	National Centre for Biotechnology Information
NBAIR	National Bureau of Agricultural Insect Resources
BLASTN	Basic Local Alignment Search Tool
ORF	Open Reading Frames
IBIn	Insect Barcode Informatica
BOLD	The Barcode of Life DataSystems
MEGA	Molecular Evolutionary Genetic Analysis
ICAR	Indian Council of Agricultural Research
SMW	Standard Meteorological Weeks

ABSTRACT

The study on "**Diversity of fruit flies and their natural enemies on important cucurbits of Nagaland**" was conducted in different experimental farms of School of Agricultural Sciences, Nagaland University, Medziphema campus, during the summer season of 2020-21. Three different cucurbitaceous crops *viz*., cucumber, pumpkin and ash gourd were grown in three different farms. The experiments were laid in Randomized Block Design (RBD) with four treatments including control and replicated five times. The treatments included fruit fly attractants *viz*., methyl eugenol, cue lure, banana poison bait and control (water) which were used in different plastic bottle traps in three different cucurbits to attract and catch different species fruit flies to determine diversity, relative abundance, seasonal abundance and their correlation with abiotic factors. The extent of damage caused by fruit flies and the population of natural enemies of fruit flies were recorded from ecological plots. All the data were recorded at weekly interval.

The fruit flies species recorded from different cucurbitaceous crops were *Bactrocera dorsalis* (Hendel), *Zeugodacus tau* (Walker), *Bactrocera tuberculata* (Bezzi), *Zeugodacus cucurbitae* (Coquillett) and *Bactrocera ruiliensis* (Wang) (Diptera: Tephritidae). Pooled data analyzed for the year 2020 and 2021 revealed that *B. dorsalis* was the most abundant species with 58.29, 56.36 and 50.19% relative abundance, while *B. ruiliensis* was the least abundant species with 2.99, 2.54 and 2.90% relative abundance in cucumber, pumpkin and ash gourd field, respectively. The diversity of fruit flies was relatively low in all the cucurbit fields as the Shannon-Weiner Diversity Index (H'), Simpson Diversity Index (SDI) value was low in all cucurbits field. E_H value was in the range of 0.736 to 0.799 in the three cucurbits.

The seasonal abundance of fruit flies was the highest during the last week of May up to middle of June. In 2020, all the five species of fruit flies

were found to have non-significant correlation with the abiotic factors in the three cucurbit crops. However, in 2021, on cucumber, the population of *B. dorsalis*, *B. tuberculata* and *Z. tau* had shown significant positive correlation with morning relative humidity. Similar results were also obtained in pumpkin and ash gourd field.While, the population of *B. ruiliensis* was found to have significant positive correlation with maximum temperature only in pumpkin field. The natural enemies of fruit flies recorded from different cucurbitaceous crops were *O. smaragdina* (Fabricius), *Tapinoma* sp. (Forel), *Crematogaster* sp. (Emery), *Polyrhachis* sp. (Donisthorpe) (Hymenoptera: Formicidae) and *P. fuscipes* (Curtis) (Coleoptera: Staphylinidae). The pooled data for the year 2020 and 2021 revealed that the natural enemy *O. smaragdina* recorded the highest relative abundance per centage of 44.03, 43.34 and 42.52, while *P. fuscipes* was the least abundant species with 5.30, 4.70 and 5.04% relative abundance in cucumber, pumpkin and ash gourd field, respectively. H', SDI and E_H computed for fruit fly's natural enemies revealed that the H' value was the highest in cucumber (1.37), while SDI value was the highest in pumpkin (0.298). E_H value was in the range of 0.845 to 0.853 in the three cucurbits. The seasonal abundance of natural enemies was the highest during the month of June and July. The correlation studies revealed that the natural enemies of fruit flies had a significant positive relation with minimum temperature, evening relative humidity and rainfall on the three different cucurbits.

Out of the three different fruit fly attractants methyl eugenol was highly effective against *B. dorsalis* (180.90, 137.80 and 130.10 flies/trap in cucumber, pumpkin and ash gourd), *B. tuberculata* (35.60, 41.40 and 43.30 flies/trap) and *B. ruiliensis* (10.00, 8.00 and 9.50 flies/trap), while cue lure was effective against *Z. tau* (49.70, 49.20 and 58.70 flies/trap) and *Z. cucurbitae* (22.80, 21.70 and 25.00 flies/trap). Cucumber was found to be the most susceptible host of fruit flies exhibiting the highest average fruit infestation (42.42% in

2020 and 43.43% in 2021), while the lowest infestation was observed in ash gourd (16.29% in 2020 and 16.82% in 2021).

The DNA barcodes were successfully developed for 10 species of insects (5 species of fruit fly and 5 species of natural enemy) and all the analyzed sequences were deposited to International Gene Bank (NCBI) with accession numbers ON725008 to ON725013, ON724399, ON724947, ON738447 and ON738454.

Key words: Attractants, COI gene, cucurbitaceous crop, DNA barcoding, fruit flies, natural enemies,

CHAPTER I

INTRODUCTION

INTRODUCTION

Cucurbits are members of the Cucurbitaceae family, which has 825 species and 118 genera. The word "corbis," which means "basket or bottle reflecting one of the ways these fruits are used," is most likely the source of the name Cucurbitaceae (Bisognin, 2002). Important cucurbit-growing nations include China, Turkey, India and Iran. China is the world's top producer and India contributes around 5.6 per cent of the overall cucurbit production. About 28 genera and 86 species of Cucurbitaceae have been reported in India and out of which roughly 30 per cent of these genera and 10 per cent of these species are indigenous to the country. The Cucurbitaceae family includes a number of significant food plants, including melon, pumpkin, squash and cucumber, as well as plants that can be used to produce useful goods, such bottle gourds, loofah, ornamental gourds, etc. Many edible squashes are grown in the Asian and Pacific region, which is also regarded as the source of some of them (Nath, 2007). Those having edible fruits were among the first cultivated plants in both the old and new worlds. Cucurbits are primarily found in the tropics. In the North-East region, cucurbits are eaten both as vegetables and fruits and they make up the majority of the vegetables harvests in India and other tropical nations.

According to the Indian Horticulture Database, National Horticulture Board (NHB), the total production of vegetables in million tonnes is 165.22 (2014–15), 176.79 (2015–16), 189.17 (2016–17), 183.17 (2018–2019) and 188.91 (2019–20), whereas the total production of vegetable crops under Nagaland in thousand metric tonnes during 2018–19 is 561.61 (Anonymus, 2019). Since cucurbits are a broad category of vegetables, there are more opportunities to increase total productivity and output. They are widely cultivated using mixed cropping. China has a monopoly on the production of cucumbers, melons and watermelons, whereas India leads the globe in the production of squash, pumpkins and gourds. Despite being grown on a huge scale throughout the world, Asia is regarded as the region that produces the most cucurbit crops, particularly cucumber, gourds, melon, pumpkin and watermelon, followed by Europe.

The fruits of cucurbits are referred to as peppo and provide medical benefits for human health, including blood cleansing, relief from constipation, improved digestion and a plentiful supply of energy. Thus, the nutritional worth and health advantages of cucurbits are well known. These are eaten as immature fruits (such as cucumber, bitter gourd, luffa, bottle gourds, etc.), or mature fruits (such as melon, watermelon, pumpkins, etc) and as young shoots and leaves (e.g., pumpkin, ivy gourd, etc.). These crops are rich in minerals, vitamins and other phytonutrients while being low in calories and fat. Cucurbitacins, which are found in some cucurbitaceous plants like bitter gourd, cucumber, musk melon, etc., are recognised to have therapeutic effects. Others, including Luffa, Cucurbita and others are added as dietary supplements to poultry feed and are increasingly utilised to enhance the protein and vitamins in aqua diets. This family's members, including Momordica, Cucurbita, Cucumis, etc., are also utilized as treatments for animals. Additionally, melon seed oil is a source of biodiesel (Ajuru and Nmom, 2017). Cucurbitaceous plants are an important part of the supply of fresh vegetables as well as an excellent source of minerals, vitamins A, C and carbohydrates. The cucurbits have enormous economic value and are grown all over the world in climates ranging from subtropical to tropical. The cucurbit fruit fly has historically been the most significant insect pest among a number of biotic variables that affect the yield and productivity of cucurbits.

Fruit flies are members of the order Diptera and family Tephritidae. The largest family, Tephritidae, contains more than 4000 species in 500 genera (White and Elson-Harris, 1992). The family is subdivided into the economically significant subfamilies Dacinae and Tryphritinae. Nearly 1000 species of fruit flies from the Tephritidae family have been identified in the oriental region (Kapoor, 1993). In the Indian Subcontinent, there are roughly 325 species of fruit flies, 205 of which are unique to India (Kapoor, 2005). *Bactrocera cucurbitae*, *B. dorsalis* and *B. zonata* are the three most common pest species, whereas *B. correcta*, *B. diversa* and *B. latifrons* are still restricted in where they can be found (Kapoor, 2005). *Bactrocera dorsalis* infests roughly 70 different host plants, while *Bactrocera cucurbitae* attacks 150 host plants (Dhamdhere and Odak, 1975). Fruit flies are significant pests of ornamental plants, fruits and vegetables (Bharathi *et al*., 2004). Depending on the type of cucurbit and the season in various regions of the world, they attack the cucurbit fruits and reduce yield by between 30 and 100% (Dhillon *et al*., 2005). It has been advised to impose domestic and international quarantines on fruit flies to prevent them from entering new ecosystems due to their high potential for damage and quick adaptation and acclimatization.The adult cucurbit fruit fly is a flying, energetic insect that is a light brown color. Maggots typically eat the fruit pulp, but occasionally they also eat the blossoms and stalks. Females typically prefer to pierce the soft, delicate fruit tissues with their needle-like, sharp ovipositors in order to lay their eggs there. A watery-like substance flows from the females' perforation, which later gets slightly concave and changes into a brown resinous deposit. On the skin of the fruit, pseudo-punctures (punctures without eggs) have occasionally also been seen. The maggot developed in the taproots, stems and leaf stalks after the eggs are placed in closed blooms (Weems and Heppner, 2001).

One of the cornerstones to effective fruit fly management in agricultural crops is accurate identification and species characterization. Identification and the traits of these insects frequently present challenges due to the abundance of fruit fly species and the existence of ambiguous traits between species. Morphological characteristics have been utilized in a number of identification techniques for fruit flies. The identification of insects, such as fruit flies, has benefited greatly by the application of conventional morphological features. However, because most morphological traits are homologous and there are unclear species present at the insect family level, the use of morphological characters for the identification of adult fruit fly insects frequently leads to identification errors. Molecular identification has been established through barcode DNA technology, which can study species genetic diversity by using a tiny fragment of DNA. This technique anticipates and complements the limits of morphological identification. A DNA barcode is a condensed DNA sequence from a common genetic locus that can be used to identify different species of insects. By examining the mitochondrial gene that produces cytochrome oxidase I (COI), DNA barcodes have been used to identify animals, including insects (Hebert *et al*., 2003). Out of the various molecular markers available for genetic analysis, molecular DNA barcode markers are regarded as the most advantageous for identifying fruit flies (Blacket *et al*., 2012). In the genus *Bactrocera*, the COI barcode has been successful in identifying the genetic makeup and evolutionary.

Although fruit flies are linked to significant economic losses in cucurbit farms, no systematic study has been started on quantifying these losses and precisely identifying the various fruit fly species in Nagaland. Given the significance of these pests for quarantine, it is crucial to initiate bionomics research on the various species complex in order to prevent losses in cucurbit crops grown in Nagaland by supplying the essential knowledge to enhance safe, economical and ecofriendly management options.

In light of these facts, the following objectives were pursued in the current research on the diversity and abundance of fruit flies on various cucurbits:

1. To study the species diversity and relative abundance of fruit flies and their natural enemies in different cucurbits

2. To determine the extent of damage caused by fruit flies on different cucurbits

3. To evaluate the effectiveness of different attractants against fruit fly complex

4. To develop DNA barcode of different species of fruit flies and their natural enemies

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The cucurbit crops are attacked by several insect pests in field. Among these, the fruit flies (Diptera: Tephritidae) is a serious pest of cucurbit crops causing even up to 100 per cent yield losses in cucurbits (Dhillon *et al*., 2005). Almost all cucurbit crops have been reported to be infested by this pest all over the world.

Therefore, considering the importance of the pest, an attempt has been made in this chapter to review available literatures which are relevant to the objectives of the present study.

2.1 Species diversity and relative abundance of fruit flies and their natural enemies

Kawashita *et al*. (2004) surveyed population of *Bactrocera* spp. in the month of September, 1997 to 1999 in Sri Lanka using Steiner type traps with two kinds of lure (methyl eugenol and cue lure) in one fixed point. Fifteen species of fruit flies belonging to genus *Bactrocera* were captured in total. *B. dorsalis* and *B. kandiensis* were dominant in traps with methyl eugenol and *B. cucurbitae* and *B. nigrofemoralis* were captured mainly by cue lure.

Thomas *et al*. (2005) conducted an experiment in field of bitter gourd near Thrissur (Kerala) and found 151 catches of melon fruit fly (*B. cucurbitae*) by attracting to the cue lure trap in bitter gourd fields over two weeks.

Ekesi and Billah (2007) reported that in coastal Kenya *B. cucurbitae*, *Dacus bivitattus, D. ciliatus* and *D. vertebratus* were the most dominant fruit flies identified in cucurbit crop. They also reported that *B. cucurbitae, D. bivitattus, D. ciliatus* and *D. vertebratus* are significant pests of cucurbit.

Decker and Yeargan (2008) conducted an intensive survey and they reported four families, Araneae, Carabidae, Staphylinidae and Geocoridae as the active predators in squash fields. *Coleomegillam aculata* and *Geocoris punctipes* were the most abundant foliage-inhabiting predators. Direct field observations of predators feeding on squash bugs or their eggs included *G. punctipes*, *Pagasa fusca* and *Nabis* sp. The parasitoids *Trichopoda pennipes* and *Gyron pennsylvanicum* were also noticed.

Ghavami (2008) reported eight species of spider's *viz.*, *Argio peluzona*, *Cyrtophora cicatrosa*, C*hryssoargy rodiformis, Hipossa pantherina, Oxyopes lineatipes, Oxyopes javanus, Peucetia viridana* and *Lycosa pseudoannulata* in snake gourd ecosystem. The predatory potency of web building spiders were estimated in the fields against insects found in the crop fields *viz., Apis florea* (honey bee), *Kallim ainachus* (butterfly), *Plusia orichalcia* (catterpiller), *Leuanodes orbonalis* (moth), *Aphis gossypii* (cotton aphids), *Bemisia tabaci* (whitefly), *Culex quina* (mosquitoe), *Drosophila melanogaster* (fruitfly), *Musca nebulo* (housefly), *Amritodus alkinsoni* (mango leaf hopper), *Macromia magnifica* (dragonfly) and *Epilachna vigintioctopuctata* (beetle). Spiders serve as buffers that limit the exponential growth of pest populations in various ecosystems by virtue of their predatory potency.

Vayssieres *et al*. (2008) reported that *B. cucurbitae* and *D. ciliatus* was the major pest of cucurbit crops and the former was capable of causing heavy damage due to its longer/ higher fecundity level and oviposition time as compared to *D. Ciliates.*

Mohamood *et al*. (2009) studied species composition of fruit flies at Khartoum and Kassala to determine host range. More than 10 species belonging to 3 genera were recorded *viz*., *C. capitata*, *C. cosyra*, *C. quinaria*, *B. invadens*, *D. ciliatus, B. cucurbitae, Dacus* sp., *Paradalopsis incompleta* and *B. longistylus* and two unidentified species. Mango and guava were found attacked by *C. capitata, B. invadens, C. cosyra and C. quinaria*. Grape fruit, orange, mandarin and banana were found infested by *B. invadens*. Lemon and anonna were recorded as new hosts of *B. invadens* at Kassala. Cucumber, water melon, musk melon were found to be infested by *Dacus ciliatus*, *Dacus* sp.and *B. cucurbitae*.

Yong *et al*. (2010) studied the diversity and abundance of Dacinae fruit flies in Malaysia and recorded three species of fruit flies attracted to methyl eugenol traps *viz*., *B. carambolae*, *B. papaya* and *B. umbrosa* and in cue lure traps four species were recorded viz., *B. infesta*, *B. melastomatos*, *B. nigrotibialis*, *B. tau* and *B. nigrotibialis*.

Deguine *et al.* (2012) investigated the biological characteristics (seasonal fluctuation, relative abundance and sex ratio) of communities roosting in corn borders for better control fruit flies (Diptera: Tephritidae) attacking Cucurbitaceae on Reunion Island (21º6 S / 55º36 E) and found that *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) was the least abundant species (27%) compared to *Dacus ciliates* (36%) and *Dacus demmerezi* (37%). Relative abundance of *B. cucurbitae* was the lowest (<18%) in high altitude sites (above 1000 m), whereas *D. demmerezi* was the most prevalent species (>56%). *Dacus ciliatus* showed variable relative abundance (from 18 to 51%) depending on the experimental design (varying in location and in year).

Fernandes *et al.* (2012) evaluated the effectiveness of ants in predation of fruit flies larvae *Anastre phaschiner*, (Diptera: Tephritidae). Eight ant species were responsible for removing 1/4 of the larvae offered. The ants were the most effective genus, removing 93 per cent of the larvae.

Prabhakar *et al*. (2012) studied the diversity of fruit fly in cucurbit fields and surrounding forest areas of Himachal Pradesh, a North-Western Himalayan State of India and recorded *Bactrocera latifrons*, *B. nigrofemoralis* and *Dacus longicornis*, *Dacus sphaeroidalis*, *Cyrtostola limbata* and *Pliomelaenaudham* *purensis* for the first time in Himachal Pradesh in a cucurbit ecosystem. Apart from these, other species *viz.*, *B. tau*, *B. cucurbitae*, *B. dorsalis*, *B. zonata*, *B. scutellaris*, *B. diversa* and *Dioxyna sororcula* were also identified.

Santana *et al*. (2012) noted social wasps (Hymenoptera: Vespidae) as important agents of biological control for *Diaphania hyalinata* and *Diaphania nitidalis* (Lepidoptera: Crambidae) which were the major pests of Cucurbitaceae family. The main predators were Vespidae, followed by Diptera: Syrphidae; Hemiptera: Anthocoridae; Coleoptera: Coccinellidae, Anthicidae; Neuroptera: Chrysopidae and Arachnida: Araneae. These natural enemies were responsible for 98 per cent of the natural mortality of larvae of *D. hyalinata* and *D. nitidalis*.

Vargas *et al*. (2012) reported that due to establishment of *B. cucurbitae* in 1895 resulted in the introduction of the most successful parasitoid, *Psyttalia fletcheri*. Similarly, establishment of *B. dorsalis* in 1945 resulted in the introduction of 32 natural enemies out of which *Fopius arisanus*, *Diachasmimorpha longicaudata* and *Fopius vandenboschi* were the most successful. In 2002, *F. arisanus* was introduced into French Polynesia where *B. dorsalis* had invaded in 1996. Establishment of *D. longicaudata* into the new world has been important to augmentative biological control releases against *Anastrepha* spp. *P. fletcheri* was also reported as an important bioagent in biological control programme of *B. cucurbitae* in Africa.

Ganie *et al.* (2013) studied the species diversity of fruit flies on cucurbits in Kashmir Valley. The study was carried out at six locations; in district Srinagar. The locations were Batmaloo, Shalimar and Dal, while in district Budgam the locations were Chadoora, Narkara and Bugam (Jammu and Kashmir, India) where cucurbit crops, such as cucumber, bottle gourd, ridge gourd and bitter gourd, were selected for the study. With regard to locations, mean fruit fly population was highest (6.09, 4.55, 3.87 and 3.60

flies/trap/week) at Batamaloo and Chadoora (4.73, 3.93, 2.73, and 2.73 flies/trap/week) on cucumber, bottle gourd, ridge gourd and bitter gourd, respectively. The population of fruit flies was significantly correlated with the minimum and maximum temperature. The maximum species diversity of fruit flies was 0.511, recorded in Chadoora. *Bactrocera cucurbitae* (Diptera: Tephritidae) was the most predominant species in both Srinagar and Budgam, followed by *B. dorsalis* and *B. tau,* while *B. scutellaris* was found only in Chadoora.

Taira *et al*. (2013) reported that species of Braconidae and Pteromalidae species were found to be associated with tephritids larvae and *Dorycto braconareolatus* was the most abundant parasitoid in larvae of tephritids infesting both cultivated and wild host fruits.

Badii *et al*. (2015) conducted studies between October, 2011 and September, 2013 to determine the host range and species diversity of pest fruit flies in the northern savannah ecology of Ghana. Fruit samples from 80 potential host plants were collected and incubated for fly emergence; 65 (81.5%) of the plant species were positive to fruit flies. Infestation by *B. invadens* was higher in the cultivated fruits; *Ceratitis cosyra* dominated in most wild fruits. Cucurbitaceae were mainly infested by three species of *Dacus* and *B. cucurbitae,* a specialized Cucurbit feeder.

David *et al*. (2017) reported that two new species of genus *Bactrocera Macquart*, namely *B. brevipunctata* and *B. Furcata* are described from India. *B. aethriobasis*, *B. rubigina*, *B. syzygii* and *B. tuberculata* was recorded for the first time from India. Updated keys to twelve subgenera of *Bactrocera* and Indian species of *Bactrocera* were also provided.

Kishor *et al*. (2018) conducted an extensive survey in Coimbatore and Dharmapuri districts of Tamil Nadu from June, 2017 to May, 2018 to determine the species diversity and relative abundance of fruit flies in gourds viz., snake gourd (*Trichosanthes anguina*), ridge gourd (*Luffa acutangula*) and bitter gourd (*Momardica charantia*) and reported that the diversity of fruit flies in both locations is low (0.04 to 0.06) under gourds because the value of the index ranges from 0 to 1, the greater the value the greater the sample diversity (Simpson, 1949). Since the cucurbits are infested by only two species (*B. cucurbitae* and *D. ciliatus*), resulted in low diversity index value.

Nair *et al*. (2018) conducted an experiment to identify the Dacine fruit fly species present in Tripura, India by using Para-pheromone traps (cue-lure and methyl-eugenol) and food bait traps. Twenty species of Dacine fruit flies have been recorded form this North-Eastern state of India. Among these, 11 species were newly recorded species from Tripura and 6 species, namely *Bactrocera nigrifacia*, *B. rubigina*, *B. tuberculata*, *B. bogorensis, B. vulta and B. apicalis* were the species recorded from India.

Pujar *et al*. (2018) also reported low fruit fly diversity in different cucurbits with H' value ranges from 0.04 to 0.06 under different gourds.

Budiyanti *et al*. (2019) conducted an experiment to investigate the fruit flies' species diversity. In their experiment four kinds of vegetables viz. bitter gourd, cucumber, angled loofah and chili pepper were sampled in the sub districts of Pauh, Kuranji, Lubuk Kilangan and Koto Tangah using purposive sampling. There were three species of fruit flies found in four vegetables in Padang, viz. *B. cucurbitae*, *B. dorsalis* and *Bactrocera* species. The highest diversity of fruit flies based on commodity was found on bitter gourd (0.010). Based on location, the highest species diversity was found in subdistrict of Lubuk Kilangan (0.240).

Apriyadi *et al*. (2021) reported eight species of fruit fly, *B. dorsalis, B. carambolae*, *B. occipitalis*, *Z. caudata*, *B. umbrosa*, *B. neocognata*, *Z. cucurbitae* and *B. albistrigata*. The dominant fruit fly in each treatment in fruit fly fields was *B. dorsalis*. The diversity and species richness of fruit fly in the cucumber field was considered low and uneven.

Varun *et al*. (2022) reported six species of fruit flies that were found to be associated with the cucurbit ecosystem, *viz*., *Zeugodacus cucurbitae*, *Z. tau*, *B. dorsalis*, *B. zonata*, *B. digressa* and *B. correcta*.

2.2 Seasonal abundance of fruit flies and their natural enemies

Monitoring of pest population and their natural enemies is necessary for formulating effective management strategies against any pest. Moreover, seasonal incidence of any insects may vary from place toplace due to variation in climatic conditions. Occurrence of fruit fly species at high population densities is associated with high levels of damage and could lead to high economic losses, so study on the seasonal abundance of fruit flies and their natural enemies in Cucurbit ecosystem and also to know the effect of weather parameters on population dynamics of these insects species is important so that appropriate time of action can be determined for effective management of this pest.

Tan and Serit (1994) stated that the availability of preferred hosts is the variable that mostly influences the size of the population of adults of *B. dorsalis* in Malaysia.

Zaman (1995) reported that the cue-lure baited traps attracted the melon fruit fly, *B. cucurbitae* males from mid-July to mid-November (peaked in August) and from 2nd week of August to the 2nd week of November (peaked in September) for the two years.

Hwang *et al*. (1997) observed the peak incidence of *Bactrocera* spp. from June to September whereas a decrease in the population was observed during winter.

Makhmoor and Singh (1998) reported that the peak population (170.66 males/ trap/ week) of oriental fruit fly was observed in June at Kashmir area in India.

Ishitiaqe *et al.* (1999) reported that population of *Bactrocera* spp. was at peak in the winter crop during September, while in December it was negligible.

Nair and Thomas (1999) observed that the peak population of fruit fly in India is attained during July and August in rainy season and January and February in cold months. Ye (2001) also reported that high abundance of *B. cucurbitae* occurs only from May through November each year in Yunnan Province of China.

Vargas *et al*. (2003) stated that maximum number of *B. cucurbitae* was recorded in the warm months of each year whereas relative humidity was observed as a crucial factor which impacts *B. cucurbitae* incidence.

Dhillon *et al.* (2005) found out that the melon fruit fly, *B. cucurbitae* is distributed widely in temperate, tropical and sub-tropical regions of the world. It has been reported to damage 81 host plants and is a major pest of cucurbitaceous vegetables, particularly the bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*C. melo* var.*momordica*) and snake gourd (*Trichosanthes anguina*). The extent of losses varies between 30 to 100%, depending on the cucurbit species and the season. Its abundance increases when the temperatures fall below 32° C and the relative humidity ranges between 60 to 70%.

Sujit (2005) reported that the population of fruit fly fluctuates throughout the year and the abundance of fruit fly population varies from month to month, season to season, even year to year depending upon various environmental factors.

Ye and Liu (2005) also reported that *B. dorsalis* occurred only from May to November with high abundance in July of each year at Kunming, China.

Mwatawala *et al*. (2006) reported that the population growth of *B. dorsalis* at SUA (Sokoine University of Agriculture) orchards in Tanzania was directly related with the presence of mango and guava.

Mahmood and Mishkatullah (2007) reported that there was increased in the population of *B. cucurbitae* from July to October.

Balog *et al*. (2008) also reported that the abundance of rove beetle was highest in June, July and August while the lowest in spring and autumn.

Kate *et al*. (2009) studied seasonal incidence of fruit fly, *B. cucurbitae* (Coquillet) on cucumber and revealed that infestation of fruit fly commenced during 5th week after germination and increased during next four weeks (6th, 7th, 8th and 9th week after germination) and formed the peak with an infestation of 22.4 per cent. Then, the infestation declined gradually up to 12.00 per cent during last week of April i.e., 12th 18week after germination. The correlation studies revealed that the maximum and minimum temperature had positive correlation with infestation to fruits (r = 0.6667 and 0.3798, respectively). While, morning relative humidity had positive correlation (r = 0.2160) and evening relative humidity had negative correlation (r = -0.1738) with fruit infestation.

Laskar and Chatterjee (2010) studied the incidental pattern of *B. cucurbitae* (Coq.) (Diptera: Tephritidae) throughout the year with a view to formulate a sustainable management technique against the pest. During warm and rainy months (June, July and August at 25-37 ^{o}C) the flies were more active as compared to that of dry and winter (December, January, February 8-23 ^{o}C) months. Significant positive correlation (r) of fly incidence was noted with minimum (r= +0.7596) and maximum temperature (r= +0.7376), whereas temperature gradient correlated negatively (r= -0.4789) with fly incidence. Negative correlation of fly incidence was also recorded with maximum humidity (r= -0.4249) and humidity gradient (r= -0.5481) and positive (r= +0.4366) with the minimum. Rainfall and sunshine hour per day showed positive (r= +0.4367) and negative (r= -0.3123) correlation with the fly incidence respectively.

Nasir *et al*. (2012) conducted an experiment to investigate and relate *Paederus fuscipes* assemblages to cultivated soils and forest parameters of the Punjab, Pakistan during 2008-2009 with six different collecting methods (pitfall traps, light traps, flight intercept traps, Berlese funnel traps, sweep net and visual observation). They had reported that, it was mainly recorded from maize (*Zea maize*) and berseem (*Trifolium alexandrinum*) crops due to presence of soft bodied insects. It preferred damp soil rich in organic matter for egg lying under natural conditions. Maximum population was recorded during March and July-August. Light had attractive effect on it, so maximum activity was observed during night also. Its activity is greatly influenced by light and soil moisture contents.

Ghule *et al.* (2014) reported the incidence pattern of *B. cucurbitae*, *Henosepilachna septima*, *Aulacophora foveicolis* on cucumber. Peak fruit fly larval incidence was recorded during third week of February, 2011 (20.57 maggots/fruit) and during second week of February, 2012 (20.70 maggots/ fruit). Abiotic factors found to be responsible for certain changes in incidence of pests. Fruit fly incidence had positive significant association with maximum (r=+0.870) and minimum (r=+ 0.730) temperature, whereas negatively significant with minimum relative humidity (r=-0.738). Rainfall showed negative correlation with all the incidence of pests during experiment.

Majacunene *et al.* (2014) observed high abundance of *B. dorsalis* from November to March, with peak in January while monitoring population of *B. dorsalis* by methyl eugenol in Manica Province, Mozambique.

Ukey *et al*. (2014) reported that the incidence of *Bactrocera* spp. was noticed from first week of June immediately after the installation of methyl eugenol and cue lure traps. The activity of guava fruit flies was its peak in the month of June and July. Next peak activity was recorded in the month of October and November coinciding with harvesting season of guava. While lowest activity was found in the month of January.

Maharjan *et al*. (2015) recorded the highest number of fruit flies (167.5 male fruit flies /3 traps) in cue-lure trap during the first week of September, which coincided with 85.45 per cent RH and 21.67°C and 25.04°C minimum and maximum temperature, respectively.

Vignesh and Viraktamath (2015) studied population dynamics of melon fruit fly, *B. cucurbitae* on cucumber (*Cucumis sativus*) during *Kharif* and *Rabi* season of 2014 – 2015 at Dharwad and Navalur by using cue-lure traps. Incidence of fruit fly was high (55.67 fruit flies/trap/week) on the crop planted during *kharif* and low (19.67 fruit flies/trap/week) on the crop planted in *Rabi*. The level of fruit infestation by *B. cucurbitae* was 70.90 per cent during *kharif* 2014). Pooled incidence data of melon fruit fly showed significant positive correlation with minimum temperature (r= 0.388*), morning(r= 0.372*) and evening relative humidity (r= 0.427).

Abhilash *et al.* (2017) conducted an experiment on monitoring of melon fruit fly in relation to weather parameter conducted in the farmer’s field at three locations *viz*., Bommankatte, Basavnagangur and Abbalgere using Barrix cue-lure trap during *Rabi* 2016-17. Monitoring of melon fruit fly revealed that the initial incidence of the melon fruit fly population begins from the flowering stage of ridge gourd and peak incidence coincides with the peak fruiting period of the crop. At peak fruiting period highest trap catch of 28.40flies/trap/week were recorded in mid-March (11th standard week of 2017) at Abbalgere. However, at Bommankatte and Basavanagangur peak trap catches of 21.40 and 22.20flies/trap/week were recorded, respectively from ninth standard week of 2017 during peak fruiting period. The incidence of melon fruit fly from three locations showed significant positive correlation with maximum and minimum temperature. Whereas, afternoon relative humidity and rainfall had significant negative correlation with melon fruit fly incidence from all three locations. The incidence of melon fruit fly was influenced to an extent of 83.60, 67.50 and 85.90 percent from the respective locations by all the weather parameters together.

Abro *et al*. (2017) reported the occurrence of *B. cucurbitae* species activities throughout the year. However, maximum number of the fly species was recorded during mid-May to mid-June 2015. They also observed that high temperature significantly positively correlated with the *B. cucurbitae* population while humidity has negative effect on it. When correlation between rainfall and melon fly captures were observed for the studied areas exhibited a temperately positive relationship with the melon fly capture in vegetables agro-ecosystem in district Hyderabad.

Francinaldo *et al*. (2017) reported that a total of 255 specimens of *Paederus* species were collected with highest mean population were recorded mostly during June followed by July.

Khan and Naveed (2017) found a positive correlation between the fruit fly and temperature and a negative correlation with relative humidity.

Hossain *et al*. (2019) reported that the peak population of *Z. cucurbitae* had recorded on March 2017, early in the rainy season and May 2018, in the middle of the rainy summer season.

Wazir *et al*. (2019) reported that the population of *Bactrocera cucurbitae* was highly significant and positively correlated with mean relative morning humidity, relative evening humidity and rainfall but highly negatively correlated with maximum temperature.

Navendu *et al.* (2020) stated that during their study two peaks in the fly population were recorded. One population peak was during last week of March to April and the other one was during September - October.

2.3 Extent of damage caused by fruit flies on different cucurbits

The cucurbits such as cucumber, bitter gourd, sponge gourd, ridge gourd, bottle gourd, snake gourd, ash gour, pointed gourd and pumpkins are some of the major vegetables grown across India. Several biotic factors limit the production and productivity of cucurbits, of which fruit flies (*Bactrocera* spp.) has been the most prominent pest. Depending on the environmental conditions and susceptibility of the crop species, the extent of losses varies between 30 to 100%.

Pradhan (1976) conducted a field experiment on assessment of yield losses caused by cucurbit fruit fly in different cucurbits and reported 28.7-59.2, 24.7-40.0, 27.3-49.3, 19.4-22.1 and 0-26.2% in pumpkin, bitter gourd, bottle gourd, cucumber and sponge gourd, respectively in Nepal.

Rabindranath and Pillai (1986) reported that fruit infestation by melon fruit fly in bitter gourd had been reported to vary from 41 to 89 per cent. The melon fruit fly had been reported to infest 95 per cent of bitter gourd fruits in Papua (New Guinea) and 90 per cent snake gourd and 60 to 87 per cent pumpkin fruits in Solomon Islands (Hollingsworth *et al*., 1997).

According to the reports of Bangladesh Agricultural Research Institute, fruit infestations were 22.48, 41.88 and 67.01% for snake gourd, bitter gourd and musk melon, respectively (Anonymous, 1988).

Kabir *et al*. (1991) reported that yield losses due to fruit infestation varies in different fruits and vegetables and it is minimum in cucumber (19.19%) and maximum in sweet gourd (69.96%).

Gupta and Verma (1992) reported that the fruits infested by fruit flies become rotten, dry up and finally shed up prematurely and also observed that the extent of damage depending on the environmental conditions and susceptibility of the crop species, the extent of losses varies between 30 to 100%.

Amin (1995) reported thatfruit flies were able to cause damaged upto 42.08% in cucumber whereas, Uddin (1996) reported 45.14% infestation.

Hollingsworth *et al*. (1997) conducted a study on melon fruit fly and 95% infestation in bitter gourd fruit in Papua New Guinea and 90% snake gourd and 60 to 87% pumpkin fruit in Solomon Island.

Qureshi *et al*. (2000) reported that local varieties did not show any satisfactory resistance against fruit flies on cucurbits.

Singh *et al*. (2000) studied on the host preferences of the melon fruit fly, *Dacus cucurbitae* by using different cucurbits. They reported that in all cases, percentage of fruit damage by the melon fruit fly was fewer than 50 per cent. However, percentage damage was significantly highest on bitter gourd (31.27 per cent) and water melon (28.55 per cent).

Dhillon *et al*. (2005) found out that the melon fruit fly, *B. cucurbitae* is distributed widely in temperate, tropical and sub-tropical regions of the world. It has been reported to damage 81 host plants and is a major pest of cucurbitaceous vegetables, particularly the bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*C. melo var. momordica*) and snake gourd (*Trichosanthes anguina*). The extent of losses varies between 30 to 100%, depending on the cucurbit species and the season.

Krishna *et al*. (2006) carried out a field experiments at the Indian Institute of Horticultural Research (IIHR), Bangalore (120 58'N; 770 35'E) from June 2002- October 2003. Cucumber, ridge gourd, bitter gourd and pickling cucumbers were raised at monthly interval. Cue lure baited bottle traps were hung to monitor *B. cucurbitae* and other related species. Maximum fruit fly infestation was 77.03 % on bitter gourd (August 2003), 75.65 % on ridge gourd (Nov. 02), 73.83 % on cucumber (October, 02) and 63.31 % on pickling cucumber (October, 02).

Kumar *et al.* (2006) reported 73.83% damage due to melon fruit fly infestation from cucumber crop. Losses of 100% of cucurbit crop harvests have been frequently observed from fruit fly infestation (Philippe *et al*., 2010).

Amin *et al*. (2011) conducted a field experiment with a view to interpret the fruit infestation rate by fruit fly, *B. cucurbitae* on ash gourd, ridge gourd, sweet gourd, bitter gourd and snake gourd. The highest fruit infestation rate was observed in sweet gourd (71.5 per cent) and lowest (21.0 per cent) in ridge gourd and the results were statistically different.

Sapkota *et al*. (2013) conducted an experiment to evaluate the damage caused by cucurbit fruit flies in spring-summer squash. They reported that cucurbit fruit fly preferred young and immature fruits and resulted in a 9.7 per cent loss of female flowers. Out of total fruits set, more than one-fourth (26 per cent) fruits were dropped or damaged just after set and 14.04 per cent fruits were damaged during harvesting stage, giving only 38.8 per cent fruits of marketable quality.

Bhowmik *et al*. (2014) studied on seasonal trends in level of infestation by *Bactrocera cucurbitae* to determine the factors influencing the infestation by melon fruit fly in the field on pointed gourd and bitter gourd in two different seasons i.e., pre- *kharif* and *kharif* during 2012 and 2013. The highest fruit infestation i.e. 51.66 per cent and 58.88 per cent was recorded both during 25th standard week on pointed gourd and 40.14 per cent and 54.71 per cent was recorded during 22nd and 14th standard week on bitter gourd respectively.

Sultana *et al*. (2017) conducted an experiment to study the host preference of cucurbit fruit fly, *Bactrocera cucurbitae* on three different cucurbit crops i.e. bitter gourd, ridge gourd and snake gourd. According to their findings, bitter gourd was the most preferred host causing 40.69 per cent fruit infestation and snake gourd was reported as the least preferred host causing 18.64 per cent fruit infestation.

Budiyanti *et al*. (2019) conducted an experiment to measure the damage caused by fruit flies on vegetables grown in Padang area. The highest percentage of plants infested was on angled loofah (20.34%).

2.4 Evaluation of the effectiveness of different attractants against major fruit fly complex

The fruit flies are pests of international importance that are difficult to be managed as they are polyphagous, multivoltine and have high mobility and fecundity. Only adults are exposed, while eggs and maggots remain protected in the host tissues and pupae are found in the soil. Thus most insecticidal treatments are ineffective. The stage of the pest that can be targeted is the adult insect. Therefore, identification of effective attractants is important to trap the adult fruit flies. So far various studies have been conducted on different attractants to find out the most effective attractants.

Beroza *et al.* (1960) suggested that fruit fly lures and baits have long been employed for monitoring and eradicating insect pests. Cue-lure, a synthetic lure for male melon fruit flies, *D. cucurbitae,* has been used since 1960.

Bateman (1972) said after emergence, the adults need to feed regularly on carbohydrates and water to survive and the females require proteinaceous materials for the development of their gonads. Melon fruit fly can be controlled by using a mixture of insecticide and food attractants, commonly known as baits. This method aims to provide an attractive but, poisoned food source killed flies when they come in contact or feed on the bait.

Tan and Lee (1982) reported that cue lure traps were found to attract *B. tau*, besides *B. cucurbitae*, in peninsular Malaysia where the former was found infesting Bachang, *Mangifera foetida*.

Fang and Chang (1984) found 1:1 mixture of cuelure and methyl eugenol to be more attractive than methyl eugenol alone or a 20:1 mixture of cuelure and dichlorvos.

Wen (1985) reported that cue lure was more effective and persistent attractant of melon fly, than methyl eugenol and protein hydrolysate in Taiwan.

Ramsamy *et al*. (1987) reported that methyl eugenol had no attractive effect on *B. cucurbitae*, but its mixture with cue lure at 3:7 parts by volume, exerted a synergistic effect on the attractant.

Cunningham (1989) reported that methyl eugenol, when used with an insecticide impregnated into a suitable substrate, forms the basis of male annihilation technique and has been successfully used for the eradication and control of several *Bactrocera* species.

Wong *et al*. (1991) found increased response of males with age to cue lure and corresponded to reaching sexual maturity. In India, cue lure proved more effective than tephritid lure (food attractant) in bitter gourd crops in Maharashtra against *B. cucurbitae* (Pawar *et al*., 1991).

Roomi *et al*. (1993) also stated that among the various management used to control fruit flies, methyl eugenol traps stands as the most outstanding alternative as they have both olfactory and phagostimulatory action.

Demilo *et al*. (1994) suggested that methyl eugenol was the most powerful tephritid male lure. The effectiveness of male annihilation was found to be reduced in areas where wild males have consumed methyl eugenol from natural sources.

Zaman (1995) reported that methyl eugenol and cue-lure traps attract *B. cucurbitae* males from mid-July to mid-November.

Bharati *et al.* (2004) studied on the attractiveness of some food baits to melon fruit fly, *B. cucurbitae* his results indicated that banana and soybean hydrolysate were 85–95 per cent more attractive to adult *B. cucurbitae* than fishmeal, beef extract, bread and dog biscuit. Among the fruit pulps, grapes and banana appeared to be more attractive than pineapple. The attractiveness of baits with palm oil lasted longer (up to 5 days) than that of baits without any controlled release (2–3 days). Grapes + beer + palm oil were found to be 37 per cent more attractive than the other admixtures. The fruit flies were attracted towards the baits more intensively between 06:00 and 08:00 h and between 16:00 and 18:00 h.

Muhammad *et al*. (2004) reported that methyl eugenol traps was most attractive and effective for monitoring of fruit flies *B. dorsalis* and *B. zonata*.

Ravikumar (2005) reported that fruit fly diet + sugar + banana was the most superior protein bait with fruit fly capture of 8.00 fruit flies/trap/week in guava and 6.50 fruit flies/trap/week in mango. Ammonium acetate when used at 5 per cent of the bait mixture attracted more females.

Singh (2008) evaluated five baits namely fruit pulps of banana, guava and apple along with jaggery (l0 per cent M:V) and jaggery alone @ 250 ml/bait and aqueous extract of *Shyam* tulsi (*Ocimum sanctum*) (1g crushed leaves in 4 ml water) @ 370 ml/bait for their efficacy in attraction and killing of the *B. dorsalis* in Himachal Pradesh. Fruit pulp of banana attracted maximum number of flies (23.2/week) followed by guava (18.2/week) as compared to other baits which were in ranged between 4.8 to 12.0 flies/week.

Mohamood *et al*. (2009) carried out an experiment to assess the fruit flies field responses to protein hydrolysate (Nulure, Torula yeast, AFFI and GF-120) and male lures (Methyl Eugenol, Terpinyl Acetate, Cuelure and Trimedlure). *B. cucurbitae* were respond positively to Nulure, Torula yeast, AFFI and GF-120. *B. invadens*was found to be respond to Methyl Eugenol while *C. capitata, C. cosyra* and *C. quinaria* were attracted to Terpinyl Acetate and *C. capitata* alone was attracted to Trimedlure.

Satarkar *et al*. (2009) studied on spatial distribution of *Bactrocera* fruit flies in the Goa region (West Coast of India) using several dispersion parameters between April 2006 and March 2008 in three ecological zones, viz., coastal, midland and upland. These zones are 10, 25 and 50 km to the east from the coastline of the Arabian Sea at an elevation of 15, 70 and 100 m above mean sea level, respectively. The dispersion parameters revealed that the population of all the fruit fly species attracted to methyl eugenol-baited traps, *viz*., *B. dorsalis*, *B. caryeae*, *B. zonata*, *B. affinis* and *B. correcta*, followed the negative binomial distribution pattern and was highly aggregated or clumped.

Ferrar (2010) reported that methyl eugenol attracts males of many *Bactrocera* species, but not members of the sub-genus *Bactrocera* (*Zeugodacus*) which includes the melon fruit fly *viz*., *B. cucurbitae*, *B. caudate* and *B. tau*.

Khan *et al*. (2010) reported that cue-lure was more effective in higher male catches of *B. cucurbitae* (171.82/trap/week) as compared to methyl eugenol (81.69/trap/week).

Pandey *et al*. (2010) reported that among different traps, banana based poison bait trap containing banana (1 kg) + carbofuron (10 g) + yeast (10 g) +

citric acid (5 g) consistently showed significant superiority in terms of higher catches of fruit fly throughout the cropping season.

Chaudhary and Patel (2012) while conducting experiments with different doses (0.05, 0.10, 0.15, 0.20 and 0.25 ml) of cue-lure to standardize the optimum dose to suppress population of melon fly, *B. cucurbitae* in cucurbit crops observed that 0.25 ml dose of cue-lure remained active for 32 weeks while, the remaining doses attracted male flies only upto 30 weeks.

Pal *et al*. (2014) reared a total of four species of fruit fly from infected flower head of bottle gourd and *B. dorsalis* reared from guava and mango fruits as well as trapped in methyl eugenol baited traps. *B. zonata* was not reared but only trapped in methyl eugenol baited traps. *B. cucurbitae* reared from all growing cucurbits, mango and apple except guava fruits and trapped in cue-lure baited traps. *B. zonata* with 36.7% and 32.3% and *B. cucurbitae* with 37.5% and 38.7% were predominant in respective lure during 2011 and 2012, respectively.

2.5 Development of DNA barcodes of fruit flies and Natural enemy

DNA barcoding, a tool of DNA-based taxonomy is in current use to identify known and unknown species on the basis of the pattern of nucleotide arrangement in a fragment of DNA of a particular species. Several researchers have suggested the use of DNA barcoding in taxonomy as a method to achieve rapid species descriptions in the context of the current biodiversity crisis.

In DNA barcoding a short standardized DNA sequence (in insects, a 658 bp fragment of the mitochondrial cytochrome c oxidase (COX I) gene) is used to identify and assign unknown specimens to species besides facilitating the discovery of new species. This tool is widely accepted all over the globe from hard-core taxonomists' to graduate molecular biologists.

Hebert *et al*. (2003) stated that insects are the most abundant of all life on earth and have evolved into a tremendous range of different forms. With millions of species and their different life stages, correct identification becomes a challenge for taxonomy. DNA based identification by using mitochondrial gene Cytochrome oxidase subunit I (COI) helps in resolving the problem. A Cytochrome oxidaseI (COI) based identification system can provide a decent, time-saving, cost-effective and accessible solution to the modern problem of species identification as the number of taxonomists and other identification experts has drastically dropped (Jinbo *et al*., 2011).

Will and Rubinoff (2004) stated that DNA barcodes are suitable for species level identification and can be considered an appropriate use of new technology to solve the problem of identifying and classifying the world's biodiversity.

Gariepy *et al.* (2007) stated that advances in DNA-sequencing technologies have enabled researchers studying about arthropod pests by means of simple, cost-effective and rapid DNA analyses. The molecular approaches provide powerful tools to identify species and investigate phylogenetic relationships in insects.

Hulcr *et al*. (2007) stated that the Cytochrome oxidaseI (COI) gene is most widely applied as a molecular barcode for the identification of species of animal species with very high accuracy.

Delomen *et al*. (2013) carried out morphometric analysis and DNA barcoding using the 5’ region of the mitochondrial cytochrome c oxidase subunit 1 (mtCOI) to distinguish the fruit flies *Bactrocera occipitalis* and *B. philippinensis*. For DNA barcoding, genomic DNA was extracted from specimens assigned with every rating (0-2 = *B. occipitalis*, 3 = intermediate/ hybrid, 4-6 = *B. philippinensis*). Using customized primers, mtCOI was amplified, sequenced and analyzed. A phylogenetic tree was constructed using the Neighbour Joining method. mtCOI clustering did not support morphological ratings, with *B. occipitalis*, hybrid and *B. philippinensis* samples grouped together. Low bootstrap values at certain branches suggested the lack of phylogenetic differentiation among morphological species delineations. Pairwise distances of consensus sequences ranged from zero to 0.033, which were lower than the standard threshold of 0.5 per cent utilized for species delineation in fruit flies. Therefore DNA barcoding failed to delineate *B. occipitalis* and *B. philippinensis*.

Smit *et al*. (2013) executed a feasibility test of molecular identification of European fruit flies (Diptera: Tephritidae) based on COI barcode sequences. A dataset containing 555 sequences of 135 in group species from three subfamilies and 42 genera and one single out group species has been analysed. 73.3 per cent of all included species could be identified based on their COI barcode gene, based on similarity and distances. The low success rate is caused by singletons as well as some problematic groups: several species groups within the genus Terellia and especially the genus Urophora. With slightly more than 100 sequences - almost 20 per cent of the total genus alone constitutes the larger part of the failure for molecular identification for this dataset. Deleting the singletons and Urophora results in a success-rate of 87.1 per cent of all queries and 93.23 percent of the not discarded queries as correctly identified. They demonstrated that the success of DNA barcoding for identification purposes strongly depends on the contents of the database used to BLAST.

Lalrinfeli (2015) developed twenty six DNA barcodes of insect pest and natural enemies in cole crop ecosystem of mid hills of Meghalaya and corrected the identities of many insect species which were identified incorrectly by previous studies and reported few species which were not reported earlier from North Eastern region of India.

Manger (2015) used standard barcoding region of COI gene and identified, characterized and documented ten species of fruit flies of the genus *Bactrocera* from fruit and vegetable ecosystem including fruit flies of cucurbitaceous crops. Before this study there was very little information available on species composition of *Bactrocera* in mid hills of Meghalaya. By using DNA barcoding technology, the scientist identified and reported *Bactrocera aethriobasis* for the first time from India.

Wang *et al.* (2015) stated that the insect mitochondrial genome (mito genome) consists of a circular, two-stranded genome of 14,000-19,000 bp length, which contains 37genes, including 13 protein coding genes (PCGs). Among 13 protein coding genes, a fragment of the COI gene has been selected as the standard barcoding region for animals.

Behere *et al*. (2016) sated that in recent years, DNA-based methods have been used to identify natural enemies of pest species where morphological differentiation is problematic (Jenkins *et al*., 2012).

Kunprom and Pramual (2016) conducted an experiment to determine the genetic variation in fruit flies (Diptera: Tephritidae) and to evaluate the effectiveness of the mitochondrial cytochrome *c* oxidase subunit I (COI) barcoding region for species-level identification. *Bactrocera correcta* was found in the most diverse host plants when they collect 12 fruit flies species from 24 host plant species belonging to 13 families. A total of 123 COI sequences were obtained from these fruit fly species. DNA barcoding identification analysis based on the best close match method shows a good performance, with 94.4 per cent of specimens correctly identified. However, many specimens (3.6 per cent) had ambiguous identification, mostly due to intra- and inter specific overlap between members of the *B. dorsalis* complex. A phylogenetic tree based on the mitochondrial barcode sequences showed that all species, except for the members of the *B. dorsalis* complex, were monophyletic with strong support. Within *B. correcta* and *B. tuberculata*, divergent lineages were recorded and according to this result they suggested that these species need further taxonomic reexamination.

Yong (2016) studied the complete mitochondrial genome and their phylogenetic implications of three *Bactrocera* fruit flies *viz*., *B. latifrons*, *B. melastomatos* and *B. umbrosa.*

Lengkong *et al*. (2017) analyzed the genetic diversity of mitochondrial COI genes in several types of *Bactrocera* associated with fruit and vegetable crops in Minahasa Regency and *Drosophila* sp. as an out group. Experimental results showed that in the five species of *Bactrocera* there were an average difference of 75 nitrogen bases. The smallest amount of nitrogenous bases, as many as five nitrogen bases, were found in *B. albistrigata* and *B. fraunfeldi*, whereas the largest difference of 104 nitrogen bases were found in *B. umbrosa* and *B. tau*. Genetic diversity analysis revealed 12.4 per cent genetic differences among *Bactrocera* spp. Insects, the smallest manifestation (0.8 per cent) between *B. albistrigata* and *B. fraunfeldi* and the greatest diversity between *B. umbrosa* and *B. tau* (15.8 per cent).

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present research entitled "**Diversity of fruit flies and their natural enemies on important cucurbits of Nagaland**" was carried out at different experimental farms of School of Agricultural Sciences, Nagaland University, Medziphema campus, during summer season of 2020-21. The details of the materials used and methods employed during the research are mentioned below.

3.1 GENERAL INFORMATIONS

3.1.1 Geographical location of the experimental site

The experimental site is located at 25^0 45'53" N latitude and 93^0 53'04" E longitudes at an elevation of 310 meters above mean sea level.

3.1.2 Climatic conditions and soil status

The experimental field lies in humid sub-tropical region with an average rainfall range from 2000-2500 mm annually. The temperature ranges from 21^0C- 32^0C during summer and winter temperature varies from 10- 15^0C and rarely goes below 8^0C in winter due to high humidity. The texture of the soil is sandy loam with acidic in nature, pH ranges from 4.5 to 6.5.

3.2 Experimental details

3.2.1 Design and layout of the field

Three different cucurbit crops *viz.*, cucumber, pumpkin and ash gourd were grown in three different experimental farms of SASRD, Nagaland University, Medziphema Campus by following recommended package of practices. The experiment was laid in Randomized Block Design (RBD) with four treatments replicated five times. Five ecological plots were maintained at 5m away from the main field to determine the extent four treatments replicated five times. Five ecological plots were maintained at 5m away from the main field to determine the extent of damage caused by fruit flies in three different cucurbit crops and also to collect natural enemy of fruit flies for determination of species diversity and relative abundance and seasonal incidence/ abundance. Field layout for different cucurbit crops were mentioned below-

1. Crop	Cucumber
Variety	Local
Experimental designs	Randomized Block Design (RBD)
Number of treatments	04
Number of replication	05
Size of plot	2.4 m x 2 m
Spacing between replication	1 m
Row to row spacing	1 m
Plant to plant spacing	60 cm
Number of plants per plot	8
Total number of plot	20
Gross area	204 m^2 (12 m x 17 m)
2. Crop	Pumpkin
Variety	Local
Experimental designs	Randomized Block Design (RBD)
Number of treatments	04
Number of replication	05
Size of plot	3 m x 4 m
Spacing between replication	1 m
Row to row spacing	2 m
Plant to plant spacing	1 m
Number of plants per plot	6

Total number of plot	20
Gross area	400 m^2 (20 m x 20 m)

3. Crop	Ash gourd
Variety	Local
Experimental designs	Randomized Block Design (RBD)
Number of treatments	04
Number of replication	05
Size of plot	3 m x 4 m
Spacing between replication	1 m
Row to row spacing	2 m
Plant to plant spacing	1 m
Number of plants per plot	6
Total number of plot	20
Gross area	400 m^2 (20 m x 20 m)

3.2.2 Treatment details

T_1	Methyl eugenol@ 40 ml + 60 ml ethyl alcohol+ 20 g malathion i.e., in the ratio of 4:6:2
T_2	Cue lure @ 40 ml + 60 ml ethyl alcohol+ 20 g malathion i.e. in the ratio of 4: 6: 2
T_3	Banana poison bait @ 1 kg rotten banana + 10 g carbofuran + 5 g yeast + 5 g citric acid
T_0	Control

3.2.3 Preparation of baited trap

Four different fruit fly bottle traps were set up in different plots of three different cucurbits fields at initiation of flowering. This trap was made up of transparent mineral water bottle of 1L capacity with four holes of 20 mm size on four sides. A cotton wad impregnated with the treatment was placed inside the trap in a loop made of iron wire. Impregnation of these chemicals was carried out at weekly intervals. Preparation procedure was followed from the booklet by Latha and Sathyanarayana, National Institute of Plant Health Management (NIPHM), Hyderabad (2015).

3.3 Cultivation practices

3.3.1 Planting materials

Seeds for all the above mentioned crops were purchased from Dimapur new market.

3.3.2 Sowing of seeds

Direct sowing of seeds was done @ 2-3 seeds per pit.

3.3.3 Manures and fertilizer

About 20 t/ha well decomposed FYM was applied to the pit 15 days before sowing. 100: 60: 60 Kg/ha N: P: K was applied. Fifty per cent of nitrogen and entire amount of phosphorous and potassium was applied in the pit at the time of sowing as basal dose and remaining quantity of nitrogen was applied at 30 days after sowing as a top dressing.

3.3.4 Thinning

Thinning was done 15 days after germination.

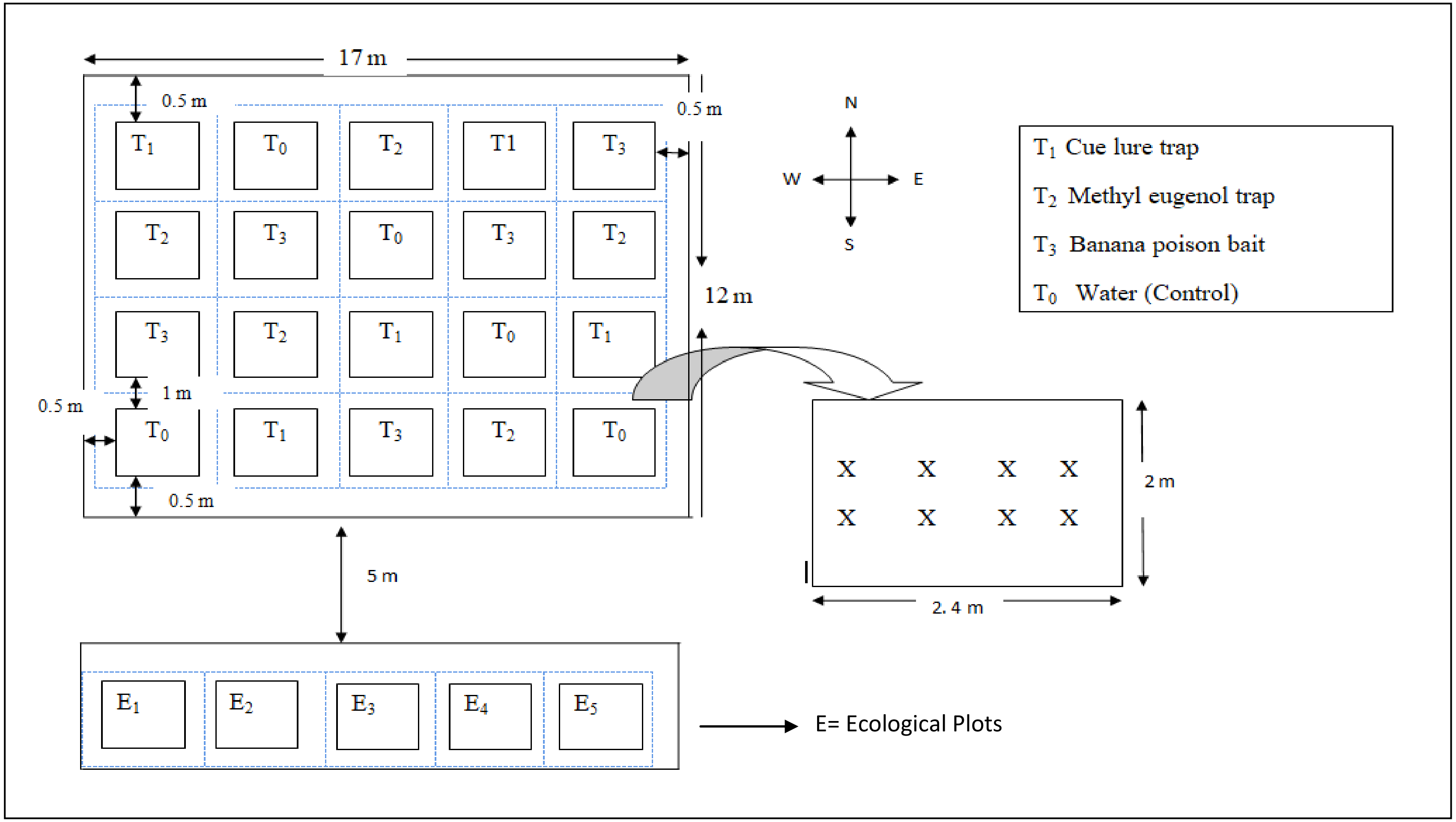


Fig 3.1 Lay out of experimental field (Cucumber) in Randomized Block Design

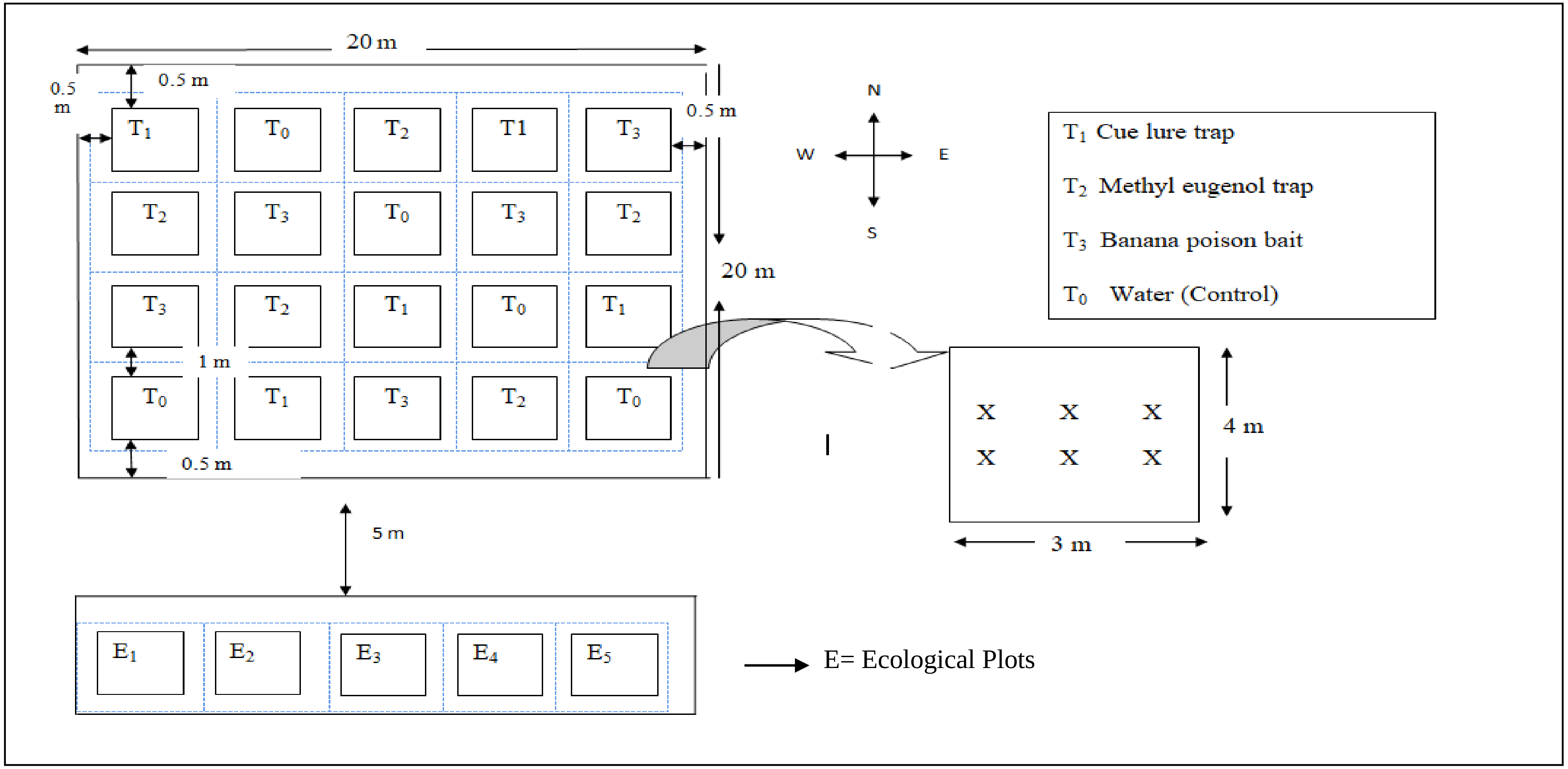


Fig 3.2 Lay out of experimental field (pumpkin and ash gourd) in Randomized Block Design

3.3.5 Irrigation

Irrigation was done daily during evening hours.

3.3.6 Weeding

Weeding was carried out at 15- 20 DAS, followed by second weeding at 20- 25 days after the first weeding.

3.3.7 Platform

Kniffin system of trailing was adopted for allowing the vines to creep. A platform of split bamboos along with coconut ropes was established at 20 days after transplanting in each plot.

3.4 Observation recorded

3.4.1 Study on the species diversity and relative abundance of fruit flies and their natural enemies in different cucurbits

The fruit fly adults attracted in different traps were collected at weekly interval and brought to the laboratory where their numbers were counted, identified species wise and the individual specimens were preserved in different glass vial (8x0.75 cm^2) containing 95 per cent alcohol.

Different natural enemies were collected from 5 ecological plots of three different cucurbit fields and direct counting method was done to record their population from 5 vines per plot. Collection of data was started from the first day of seedling emergence till harvest at weekly intervals.

The relative abundance and diversity indices of both fruit fly and their natural enemies in three different cucurbit crops were worked out by using the following formula-

a. Cucumber Field **b. Ash gourd Field**

c. Pumpkin Field

Plate 1: General view of experimental fields

3.4.1.1 Relative abundance (Preston, F. W. 1948):

$$\text{Relative abundance } = \frac{\text{Number of individual of one species}}{\text{Number of individuals of all species}} \times 100$$

3.4.1.2 Diversity indices

(a) Shannon-Weiner Index (H')

Shannon-Weiner Index is a very widely used diversity index for comparing diversity between various habitats (Clarke and Warwick, 2001). Shannon- Weiner Index assumes that individuals are randomly sampled from an independently large population and all the species are represented in the sample.

A value near 4.6 would indicate that the numbers of individuals are evenly distributed between all the species. Mathematically, Shannon-Weiner Index is:

$$H' = \frac{s}{i=1} - \sum \text{Pi} \log 2 \text{ pi}$$

Where,

Pi = The proportion of individuals of i^{th} species in a whole community (n_i/N)

s = The number of species

(b) Simpson Diversity Index (SDI)

Simpson Diversity Index measures the probability that two individuals randomly selected from a sample will belong to the same species (Simpson, 1949). The value of this index ranges between 0 and 1 which indicates greater the value, greater the sample diversity.

$$SDI = \sum[n_i (n_i-1)]/N(N-1)$$

Where,

n_i = The total number of individuals of a particular species

N = The total number of individuals of all species

(c) Species Evenness (E_H)

Evenness is a measure of the relative abundance of different species making up the richness of an area. This evenness is an important component of diversity indices (Leinster and Cobbold, 2012) and expresses evenly distribution of the individuals among different species.

The value of E_H ranges from 0 to 1 with 1 indicating maximum evenness. Mathematically,

$$E_H = H'/H'_{max} = H'/\ln S$$

Where,

H' = The number derived from the Shannon diversity Index

H'_{max} = The maximum possible value of H'

S = The total number of species

3.4.1.3 Seasonal abundance of fruit flies and their natural enemies

The abundance of different species of fruit flies as observed from different traps in different cucurbits at weekly interval on different Standard Meteorological week (SMW) was correlated with the abiotic factors like maximum and minimum temperature, morning and evening relative humidity and rainfall using SPSS 16.0 software.

Similarly, the abundance of different species of fruit flies' natural enemies recorded from ecological plots of different cucurbits (5 vines/plot) on different SMW was also correlated with the abiotic factors to determine any significant relationship between them.

The different weather parameters during the study period were collected from ICAR NEH Region, Nagaland Centre, Jharnapani.

3.4.2 Extent of damage caused by fruit flies in different cucurbit crops

To determine the extent of damage caused by fruit flies in different cucurbit crops five vines per plot were selected randomly and the number of damaged fruits and total number of fruits were counted from the selected vines at weekly intervals. The percent fruit infestation was worked out by using the following formula (Iqbal 2018):

$$\text{Per cent fruit infestation} = \frac{\text{Number of infested fruit}}{\text{Total Number of fruits}} \times 100$$

3.4.3 Evaluation of the effectiveness of different attractants against fruit fly complex

Three different attractants like methyl eugenol, cue lure and Banana poison bait (Pandy *et al*., 2008) were evaluated against different species of fruit flies in three different cucurbit following Randomized Block design (RBD) with five replications as mentioned in 3.2. The numbers of different species of fruit flies attracted/trapped in each trap were recorded on weekly interval and the effectiveness of attractants was compared with control (traps without attractant).

The data then recorded were tabulated and further subjected to suitable statistical analysis of variance.

3.4.4 Development of DNA barcode of fruit fly and natural enemy

The laboratory work for this research was conducted in the Molecular Entomology Laboratory, Division of Crop Protection, ICAR Research Complex for North Eastern Hills (NEH) Region, Umiam, Meghalaya.

3.4.4.1 Collection, preservation and identification of fruit fly and natural enemy

Different species of Fruit flies and their natural enemies collected in glass vials were carried to the laboratory and individual specimen were kept in different vial containing 95 per cent alcohol and then stored at -20 ^{0}C before the extraction of DNA.

3.4.4.2 Extraction of DNA

DNA was extracted from all the species of fruit flies and their natural enemies by using Qiagen Dneasy Blood and Tissue kit (Behere *et al*., 2016). The preserved specimens were taken out from the vials with the help of sterilized forceps and air dried on blotting paper at room temperature for an hour for evaporation of ethanol. This was followed by extraction of DNA from whole insect.

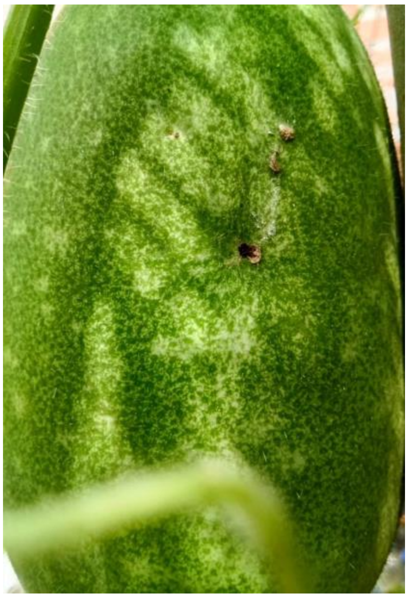
<u>Materials and equipments used</u>

- Insect sample
- Latex or Nitrile gloves
- Eppendorf Tubes (1.5ml)
- Micro pestle
- Micro pipettors and tips
- Digital Heat Block
- Vortex machine
- Micro-centrifuge (Eppendorf, Model No.:5430)

a. Damaged caused by Fruit flies on cucumber

b. Damaged caused by fruit flies on immature pumpkin fruit

c. Damaged caused by fruit flies on matured pumpkin



d. Damaged caused by Fruit flies on ash gourd

Plate 2: Nature of damaged caused by fruit flies on different cucurbit crops

- Refrigerator (-4 and -20°C)

<u>QiagenDNeasy Kit protocol</u> (Behere *et al*., 2016)

Total genomic DNA of insect specimens was extracted by using QiagenDNeasy Blood and Tissue Kit by following manufacturer's instructions. The details of the protocol are as follows:

1. Air dried specimens (whole insect) were placed in 1.5 ml micro centrifuge tubes and closed tightly. The tubes were further dipped into liquid nitrogen (-196°C) for few seconds, in order to soften the specimens, then crushed by using sterilized micro pestles.
2. 180µl of ATL buffer followed by 20µl of Proteinase K was added in each tubes and vortexed vigorously.
3. The tubes were incubated on Digital Heat Block at 56°C, until completely lysed.
4. The samples were taken outfrom the heat block and vortexed for 15 seconds.
5. 200µl of AL buffer and 96-100% of ethanol was added to the samples and mixed it thoroughly by vortex.
6. Then the sample tubes were centrifuged for 1 minute at 8000 rpm.
7. The mixture was pipeted into a DNeasy mini spin columns, containing 2 ml collection tubes.
8. The spin columns was placed in a new 2 ml collection tubes and 500µl of AW 1 buffer was added and centrifuged for 1 minute at 6000 x g (8000 rpm).
9. The flow- through and collection tubes were discarded.
10. The spin columns was again placed in a new 2 ml collection tubes and 500µl of AW 2 buffer was added and centrifuged for 3 minute at 20,000 x g (14,000 rpm).
11. The flow- through and collection tubes were again discarded.

12. The spin columns were transferred to a new 1.5 ml micro centrifuge tubes.
13. 100µl AE buffer were added for elution, incubated for 1 minute at room temperature.
14. The supernatants were decanted and the pellets were air dried at room temperature. The dried pellets were re-suspended or dissolved in 1X TE buffer
15. The sample tubes were finally centrifuged for 1 minute at >6000 X g (8000rpm) and stored in -20°C for further use.

3.4.4.3 Qualitative and Quantitative estimation of DNA

The quality and quantity of extracted DNA was determined by using Nanodrop. From every sample 1µl of DNA was used and the DNA was measured at 260/280 wavelength in nanodrop. 1X TE buffer was used as blank control for estimation of quality and quantity of DNA in nanodrop. After the estimation, the DNA samples were stored at -20°C for further use.

3.4.4.4 Development of DNA barcodes by using COI gene (Behere *et al*., 2016)

(a) PCR amplification

Materials and Equipments used

- Sterile disposable micro-centrifuge tubes (1.5ml and 200µl capacity)
- Pipettes and tips
- Micro centrifuge
- Vortex machine
- PCR machine (Eppendorf Master Cycler Nexus Gradient)
- Latex or Nitrile gloves

Reagents used

- Diluted DNA
- PCR Master mix (2X)

- Molecular biology grade water
- Primers

For COI gene based barcoding, two pairs of primers have been considered as standard barcoding primers for insect DNA barcoding work. The details of the primers are as follows:

Primers	Sequence (5'-3')	Primer length	Reference
LepF1	ATTCAACCAATCATAAAGATATTGG	25bp	(Folmer, 1994)
LepR1	TAAACTTCTGGATGTCCAAAAAATCA	26bp	
LCO	GGTCAACAAATCATAAAGATATTGG	25bp	(Hebert *et al.*, 2004)
HCO	TAAACTTCTGGATGTCCAAAAAATCA	26bp	

PCR reaction was carried out using LCO (Forward) and HCO (Reverse) primer. The samples which failed to amplify with LCO/HCO were further amplified with LepF1 (Forward) and LepR1 (Reverse) primer.

(b) PCR reaction mixture

PCR amplifications were carried out in the thermal cycler (Eppendorf, India) to test the amplifications of all the samples with two standard DNA barcoding primers. The reaction mixture contain 2µl of gDNA (~40-50 ng), 0.5µl each of forward and reverse primers, 5µl of ready to use EmeraldAmp® MAX PCR Master Mix (2x) (Takara) and 2µl of molecular biology grade water. This premix master mix has composition of 5µl of 2mM dNTPs, 1.5 µlof 50 mM $MgCl_2$, 0.25µl of 5U *Taq*DNA polymerase and 5µl of 10X PCR buffer.

(c) PCR cycles

PCR profile consist of initial denaturation at 94°C for 2 minutes, followed by 5 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 40 seconds and extension for 1 minute at 72°C, again followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 51°C for 40 seconds and extension for 1 minute at 72°C. A final extension was allowed for 10 minutes at 72°C and samples were allowed to hold at 10°C in PCR machine after completion of all the cycles and then stored in -20°C for further use.

(d) Gel electrophoresis and documentation

Materials and Equipments used

- Gel Documentation system (Care stream Gel Logic 212 Pro)
- Digital Weighing balance
- Microwave oven
- Conical flask
- Pipettors and tips
- Gel electrophoresis unit (Tank, Power pack and combs)

Reagents used

- Agar Powder
- 1X TAE Buffer
- Ethidium Bromide
- 6X loading dye
- 100bp DNA ladder

Procedure

1. The amplified 10 µl of PCR products were subjected to electrophoresis on agarose gel.
2. The gel was prepared by adding 1.5g of agarose in 100ml 1X TAE buffer in a wide mouthed conical flask.

3. The mixture was heated in a microwave oven for few minutes, until the agarose melted. Then the flask was gently removed from the oven and kept into a water bath to cool down.
4. After which, 2µl of ethidium bromide was added to stain the gel. The gel solution was mixed thoroughly before pouring into the tank.
5. The amplified PCR products (10µl) were loaded serially into the wells along with 100bp DNA ladder (4µl) as a molecular marker in the first well and the control loaded into the last well, in order to see any contamination in the PCR product.
6. The samples were allowed to run at 160V for 20-30 minutes.
7. After completion, the gel was visualized under UV trans-illuminator and gel was documented in gel documentation system (Care stream Gel Logic 212 Pro). The presence or absence of amplification for each of the sample was recorded.

3.4.4.5 Sequencing of PCR amplicons of COI gene for insect pests and natural enemies of cucurbitaceous crops

For sequencing, PCR reactions were carried out with universal LCO/HCO primer and samples which failed to amplify with LCO/HCO primer were amplified with LepF1/LepR1 primers for sequencing. A total volume 50µl of PCR reaction was carried out. The PCR profile is similar as described in the previous section. After completion of PCR amplification, 10µl of each PCR product were used for gel electrophoresis and documentation. The remaining 40µl of post PCR product of each species was transferred into 1.5ml sterilized eppendorf tubes and the tubes were packed properly and sent for sequencing in frozen condition to Eurofins Genomics India Pvt. Ltd, Bangalore. Sequencing was performed for all the samples from both the ends (5’ and 3’).

3.4.4.6 Bioinformatics Analysis

The molecular biology software STADEN Package was used for Nucleotide sequence analysis/assembling (Staden *et al.*, 2000). The messy 5’ and 3’ end of the sequences were trimmed with the aid of this software. All sequences were also checked manually within the software for accuracy.

Thereafter, BLASTN search in online portal of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) was conducted for identity and homology of all the analyzed sequences. The Blast was performed for individual species, against nucleotide collections within NCBI data base. The query (species) showing 99-100% homology was considered as similar species and in such cases molecular identification was established without any ambiguity. The top three search results for each species were recorded for all the species. In case of absence of matching database in NCBI, the species identity was established based on taxonomic identification only.

The representative sequence of partial COI gene of each species identified in this study was deposited to NCBI and accession numbers of all the submitted sequences were also obtained. The sequences were prepared for submission in the software Sequin.

The DNA barcodes for all the insect pests and natural enemies identified in cucurbitaceous crops ecosystem were generated using web based software (http://biorad-ads.com/.DNABarcodeWeb/.).

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The data collected during the present investigation on "**Diversity of fruit flies and their natural enemies on important cucurbits of Nagaland**" are presented in tables and illustrated with figures in this chapter. This part of the thesis presents the findings, interpretation and discussion of the results of the study with appropriate tables and illustrations.

4.1 Species diversity and relative abundance of fruit flies species

4.1.1 Species identification

Fruit fly species recorded during the present study are presented in Table 4.1 and (Plate 3). The five fruit fly species that have been identified during the year 2020 and 2021 were *Bactrocera dorsalis*, *Zeugodacus tau*, *Bactrocera tuberculata*, *Zeugodacus cucurbitae* and *Bactrocera ruiliensis*. *Bactrocera ruiliensis* was reported for the first time from Nagaland.

4.1.2 Diversity indices of fruit flies on important cucurbits of Nagaland

The diversity indices data collected from two years experimental trials (Table 4.1) revealed that, in the year 2020 the Simpson's Diversity Index (SDI) computed for fruit flies was the highest in cucumber with 0.415 followed by pumpkin with 0.390 and was the least in ash gourd with 0.330. While, Shannon-Weiner Index (H') was highest in ash gourd field (1.30) followed by pumpkin (1.19) and lowest value was obtained from cucumber (1.14). The even distribution of species in three different cucurbit fields was indicated by species evenness which showed that all the species were distributed evenly in all the cucurbit fields as all the values obtained from different field were near 1 with 0.711 in cucumber, 0.742 in pumpkin and 0.807 in ash gourd. Similarly, in 2021, the Sipmson's Diversity Index (SDI) value was highest in cucumber (0.379) followed by pumpkin (0.374) and lowest in ash gourd (0.341). However, Shannon-Weiner Index (H') was highest in ash gourd (1.27) followed by cucumber (1.23) and was least in pumpkin (1.21). The maximum species evenness pattern was showed in ash gourd with 0.791 which was followed by cucumber with 0.762 and lowest in pumpkin with 0.755.

Pooled data (Table 4.1) showed that highest Simpson diversity index (SDI) was in cucumber (0.397) followed by pumpkin (0.381) and least in ash gourd (0.336). However, Shannon- Weiner Diversity Index (H') was highest in ash gourd (1.29) followed by pumpkin (1.21) and cucumber (1.18). The value of species evenness was maximum in ash gourd (0.799) followed by pumpkin with 0.749 and cucumber with 0.736.

4.1.3 Relative abundance of fruit flies species in different cucurbits

4.1.3.1 Cucumber

The data (Table 4.2 and Fig 4.1) from two years experimental trials revealed that in the year 2020 the relative abundance of different fruit flies species was recorded highest for *B. dorsalis* (59.87%) followed by *Z. tau* (19.71%), *B. tuberculata* (11.24%), *Z. cucurbitae* (6.84%) and *B. ruiliensis* (2.35%). Similar result was obtained in 2021, with highest relative abundance percentage obtained for *B. dorsalis* (56.54) which was followed by *Z. tau* with 18.97, *B. tuberculata* with 12.49, *Z. cucurbitae* with 8.31 and *B. ruiliensis* with 3.69.

Pooled data (Table 4.2 and Fig 4.1) revealed that *B. dorsalis* have the highest relative abundance percentage with 58.29 which was followed by Z. tau with 19.35, *B. tuberculata* with 11.83, *Z. cucurbitae* with 7.53 and *B. ruiliensis* with 2.99.

Table 4.1 Diversity of fruit flies species recorded in different cucurbit crops during 2020 and 2021

Species	First year (2020)			Second year (2021)			Pooled		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
Bactrocera dorsalis	1604.00	1375.00	1233.00	1362.00	1271.00	1185.00	1483.00	1323.00	1209.00
Zeugodacus tau	528.00	471.00	568.00	457.00	456.00	536.00	492.50	463.50	552.00
Bactrocera tuberculata	301.00	301.00	385.00	301.00	346.00	349.00	301.00	323.50	367.00
Zeugodacus cucurbitae	183.00	182.00	226.00	200.00	174.00	197.00	191.50	178.00	211.50
Bactrocera ruiliensis	63.00	67.00	76.00	89.00	52.00	64.00	76.00	59.50	70.00
Total number of species	2679.00	2396.00	2488.00	2409.00	2299.00	2331.00	2544.00	2347.50	2409.50
Shannon-weiner diversity index (H')	1.14	1.19	1.30	1.23	1.21	1.27	1.18	1.21	1.29
Simpson diversity index (SDI)	0.415	0.390	0.330	0.379	0.374	0.341	0.397	0.381	0.336
Evenness of species (E_H)	0.711	0.742	0.807	0.762	0.755	0.791	0.736	0.749	0.799

*Population based on 20 traps

4.1.3.2 Pumpkin

The data (Table 4.2 and Fig 4.1) from two years experimental trials revealed that *B. dorsalis* was the most abundant species as compared to the other four species of fruit flies. The relative abundance percentage of *B. dorsalis* in the year 2020 was observed to be 57.39 followed by *Z. tau* with 19.66, *B. tuberculata* with 12.56, *Z. cucurbitae* with 7.59 and *B. ruiliensis* with 2.79. In the year 2021, the relative abundance of *B. dorsalis* was 55.28% which were followed by *Z. tau* with 19.84%, *B. tuberculata* with 15.05%, *Z. cucurbitae* with 7.56% and *B. ruiliensis* with 2.27%.

Pooled data (Table 4.2 and Fig 4.1) reveals that *B. dorsalis* have the highest relative abundance with 56.36% which was followed by *Z. tau* with 19.74%, *B. tuberculata* with 13.78%, *Z. cucurbitae* with 7.58% and *B. ruiliensis* with 2.54%.

4.1.3.3 Ash gourd

The data from two years experimental trials (Table 4.2 and Fig 4.1) revealed that *B. dorsalis* was the most abundant species as compared to the other four species of fruit flies. The relative abundance percentage of *B. dorsalis* in the year 2020 was observed to be 49.56 followed by *Z. tau* with 22.84, *B. tuberculata* with 15.47, *Z. cucurbitae* with 9.08 and *B. ruiliensis* with 3.05. In the year 2021, the relative abundance of *B. dorsalis* was 50.84% which was followed by *Z. tau* with 22.99%, *B. tuberculata* with 14.97%, *Z. cucurbitae* with 8.45% and *B. ruiliensis* with 2.75%.

Pooled data (Table 4.2 and Fig 4.1) revealed that *B. dorsalis* have the highest relative abundance percentage with 50.19 which was followed by *Z. tau* with 22.90, *B. tuberculata* with 15.23, *Z. cucurbitae* with 8.78 and *B. ruiliensis* with 2.90.

a. ***Bactrocera dorsalis***

b. ***Zeugodacus tau***

c. ***Bactrocera tuberculata***

d. ***Zeugodacus cucurbitae***

e. ***Bactrocera ruiliensis***

Plate 3: Different species of fruit fly

Table 4.2 Relative abundance (%) of fruit flies species recorded during 2020 and 2021

Species	Relative abundance (%)								
	2020			2021			Pooled		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
B. dorsalis	59.87	57.39	49.56	56.54	55.28	50.84	58.29	56.36	50.19
Z. tau	19.71	19.66	22.84	18.97	19.84	22.99	19.35	19.74	22.90
B. tuberculata	11.24	12.56	15.47	12.49	15.05	14.97	11.83	13.78	15.23
Z. cucurbitae	6.84	7.59	9.08	8.31	7.56	8.45	7.53	7.58	8.78
B. ruiliensis	2.35	2.79	3.05	3.69	2.27	2.75	2.99	2.54	2.90

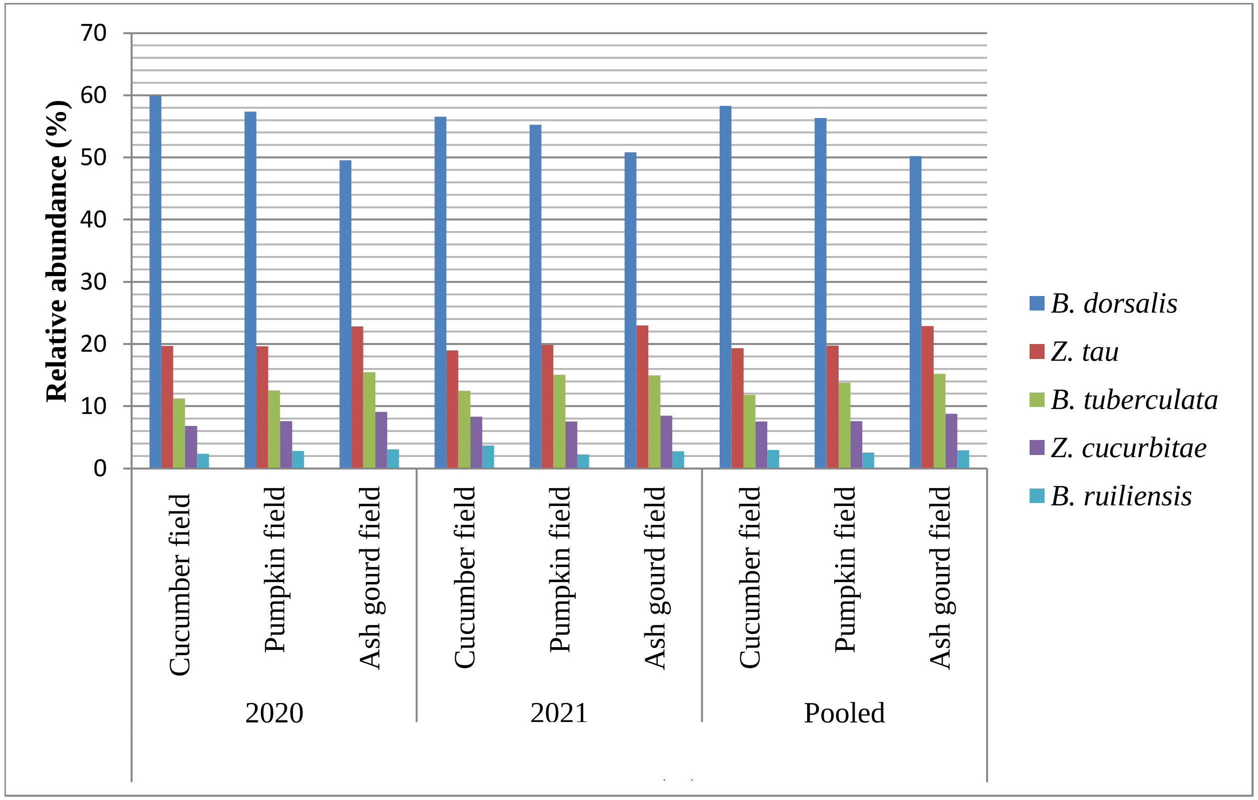


Fig 4.1 Relative abundance (%) of fruit flies species recorded during 2020 and 2021

From the pooled data it can be illustrated that the diversity of fruit fly in different cucurbit fields was categorized as low as the value of H’ was in the range of 1.18 to 1.29 which is less than 4.6. The value obtained for Simpson diversity index (SDI) was also low under different cucurbit crops as the value of the index ranges from 0.336 to 0.397 which is also less than 1. The low value of H’ and SDI from the three cucurbits field was because only five species of fruit flies were observed *viz*., *B. dorsalis*, *Z. tau*, *B. tuberculata*, Z. *cucurbitae* and *B. ruiliensis* resulting in low diversity index value. These findings are in agreement with the findings of Kishor *et al*. (2018) who reported that the diversity of fruit flies in Coimbatore and Dharmapuri districts of Tamil Nadu were low (0.04 to 0.06) under gourds, since the cucurbits were infested by only two species (*B. cucurbitae* and *Dacus ciliates*). The species evenness value was in the range of 0.736 to 0.799 in all the different cucurbit fields, it means that the individuals are distributed equally and the community is more even in the distribution of individuals. Similar results were recorded during the experiments laid by Ganie *et al*. (2013). They observed low fruit fly diversity in cucurbits with H’ value ranges between 0.255-0.511 and evenness index value of 0.846-0.977. Pujar *et al*. (2018) also reported low fruit fly diversity in different cucurbits with H’ value ranges from 0.04 to 0.06 under different gourds. However, Budiyanti *et al*. (2019) stated that the index value of species evenness of fruit flies on vegetables in Padang was E_H= 0.058 in cucumber plantation which are not in agreement with the present findings.

The results from the pooled data of per cent relative abundance revealed that *B. dorsalis* was the dominant species in cucurbit crops with relative abundance ranges from 50.19 to 58.29%. This finding are in line with Hancock *et al*. (2000) who reported that *B. dorsalis* is the main pest on 51 plant families including cucumber, pumpkin and ash gourd. Similarly, the present findings are in conformity with Apriyadi *et al*. (2021) who reported eight species of fruit fly *viz., B. dorsalis, B. carambolae, B. occipitalis, Z. caudata, B. umbrosa,* *B. neocognata, Z. cucurbitae* and *B. albistrigata* from cucumber field and out of the eight species, *B. dorsalis* was the most dominant species. Varun *et al*. (2022) also observed six species of fruit flies being associating with the cucurbit ecosystem, *viz., Zeugodacus cucurbitae, Z. tau, Bactrocera dorsalis, B. zonata, B. digressa and B. correcta.* Whereas, Kishor *et al*. (2018) reported two species of fruit flies from three different gourd i.e., *B. cucurbitae and Dacus ciliates* and *B. cucurbitae* was the dominant species in all the three gourds.

4.2 Seasonal abundance of fruit flies in different cucurbits and their correlation with abiotic factors

4.2.1 Seasonal abundance of fruit flies in different cucurbits

Fruit fly seasonal abundance was monitored using different attractant traps to detect the presence and abundance of different existing fruit fly species in selected cucurbits. The seasonal fluctuation of trap catches of fruit flies was observed at weekly intervals. It was evident from table 4.1 during 2020 and 2021 that five species of fruit fly *viz*., *B. dorsalis*, *Zeugodacus tau*, *B. tuberculata*, *Z. cucurbitae* and *B. ruiliensis* were observed at the different experimental field. Their abundance in different cucurbits field were discussed in the following subheadings.

4.2.1.1 Seasonal abundance of *B. dorsalis* in different cucurbits

The results (Table 4.3 and Fig 4.2) of the present investigation revealed that in 2020, the incidence of *B. dorsalis* appeared initially at 18 SMW i.e., on 3rd May. The peak incidence of *B. dorsalis* was recorded on 14th June (24 SMW) on all the cucurbits representing mean population of 18.65, 16.50 and 15.40 flies/trap, respectively on cucumber, pumpkin and ash gourd and the lowest mean population was observed on 5th July (27 SMW) on all the cucurbits with mean population of 2.05, 1.70 and 1.35 flies/trap, respectively on cucumber, pumpkin and ash gourd.

In 2021 (Table 4.4 and Fig 4.3) the population of *B. dorsalis* appeared initially at 18 SMW i.e., on 2nd May. The peak incidence of *B. dorsalis* was recorded on 20th June (25 SMW) in cucumber and pumpkin with a mean population of 13.45 and 15.50 flies/trap, respectively, while in ash gourd the highest population was recorded on 13th June (24 SMW) with a mean of 15.40 flies/trap. After this their population decreases gradually reaching the lowest on 4th July (27 SMW) with a mean of 1.35, 1.05 and 1.15 flies/trap in cucumber, pumpkin and ash gourd, respectively.

4.2.1.2 Seasonal abundance of *Z. tau* in different cucurbits

In 2020, *Z. tau* was also found to be active from the 1st week of observation i.e., 18 SMW till the harvest time. In the Table 4.5 and Fig 4.4, it was observed that the peak incidence of *Z. tau* was on last week of May (22 SMW) with a mean catches of 4.60, 4.40 and 6.25 flies/trap on cucumber, pumpkin and ash gourd, respectively. After this their population decreases and the lowest population was observed on 26 SMW with a mean population of 1.00 and 0.85 flies/trap in cucumber and pumpkin but in ash gourd the lowest population was recorded on 27 SMW with 0.75 flies/trap.

Table 4.3 Seasonal abundance of *B. dorsalis* recorded during May-July 2020 on different cucurbit crops

SMW	Temperature (^oC)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	30.07	20.30	90.00	60.00	20.05	2.10	1.70	1.35
19	32.22	23.90	87.00	56.00	22.60	2.55	2.30	1.95
20	32.00	21.50	92.00	61.00	4.10	3.50	3.40	3.00
21	30.50	22.90	92.00	79.00	38.60	3.50	3.05	2.75
22	30.10	21.20	92.00	63.00	74.00	4.10	3.55	3.15
23	31.90	22.70	94.00	68.00	16.50	10.00	8.60	7.40
24	33.20	24.70	92.00	80.00	67.10	18.65	16.50	15.40
25	33.10	24.30	92.00	71.00	111.90	15.75	14.00	12.95
26	31.80	23.90	92.00	73.00	53.60	15.45	11.65	10.40
27	32.90	24.60	94.00	78.00	79.50	2.05	1.70	1.35
28	32.90	24.80	93.00	71.00	29.70	2.55	2.30	1.95

*Mean of 20 traps

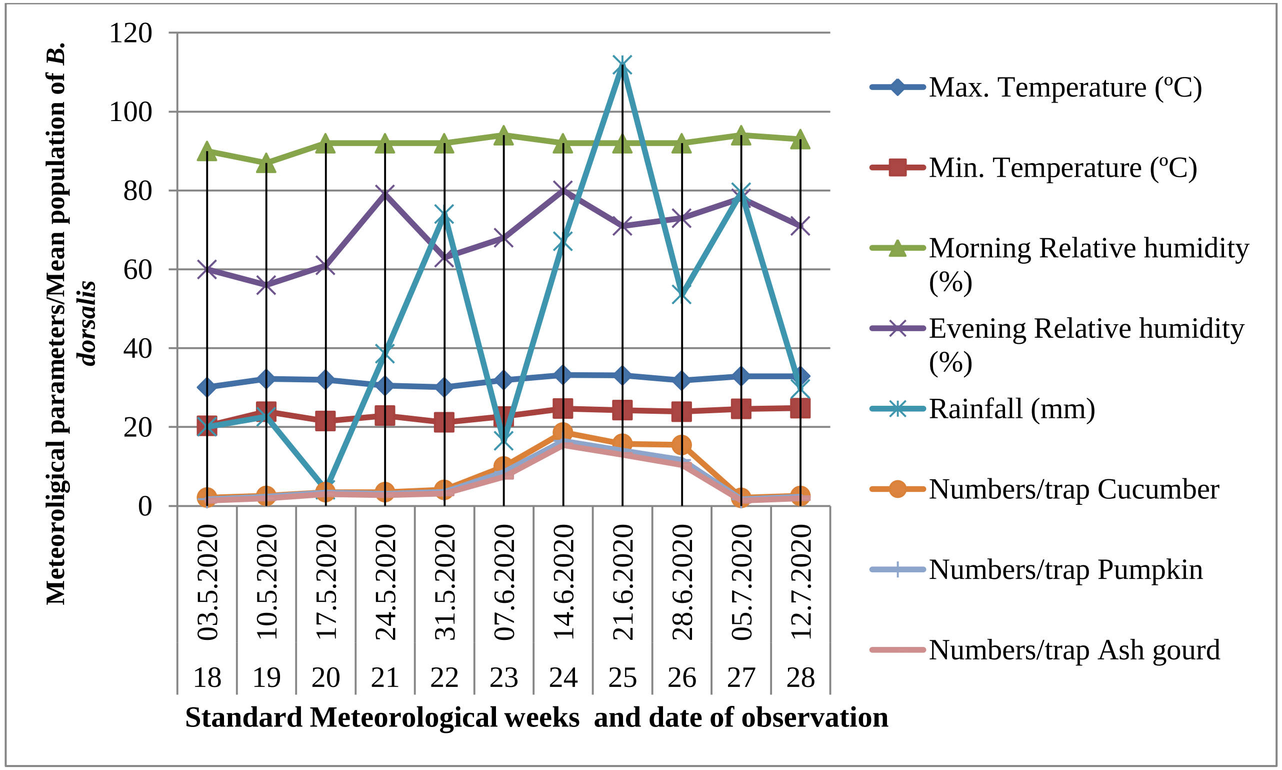


Fig 4.2 Seasonal abundance of *B. dorsalis* recorded during May-July 2020 on different cucurbit crops

Table 4.4 Seasonal abundance of *B. dorsalis* recorded during May-July 2021 on different cucurbit crops

SMW	Temperature (^{o}C)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	32.20	20.05	85.00	49.00	31.10	1.35	1.05	1.15
19	30.03	20.06	89.00	62.00	19.40	2.70	1.95	1.65
20	31.70	21.60	91.00	58.00	3.20	3.15	2.45	3.00
21	35.60	23.90	92.00	60.00	31.10	6.35	3.20	2.75
22	33.10	22.90	91.00	61.00	17.40	8.00	3.55	3.10
23	33.60	23.60	92.00	63.00	39.10	9.45	8.00	7.55
24	33.00	24.80	95.57	75.00	19.50	10.30	12.55	15.40
25	33.00	24.50	93.00	67.00	43.30	13.45	15.50	12.95
26	33.00	25.00	93.00	69.00	37.60	9.30	12.30	8.90
27	33.20	24.70	89.00	73.00	19.20	1.35	1.05	1.15
28	32.40	24.70	93.00	70.00	105.70	2.70	1.95	1.65

*Mean of 20 traps

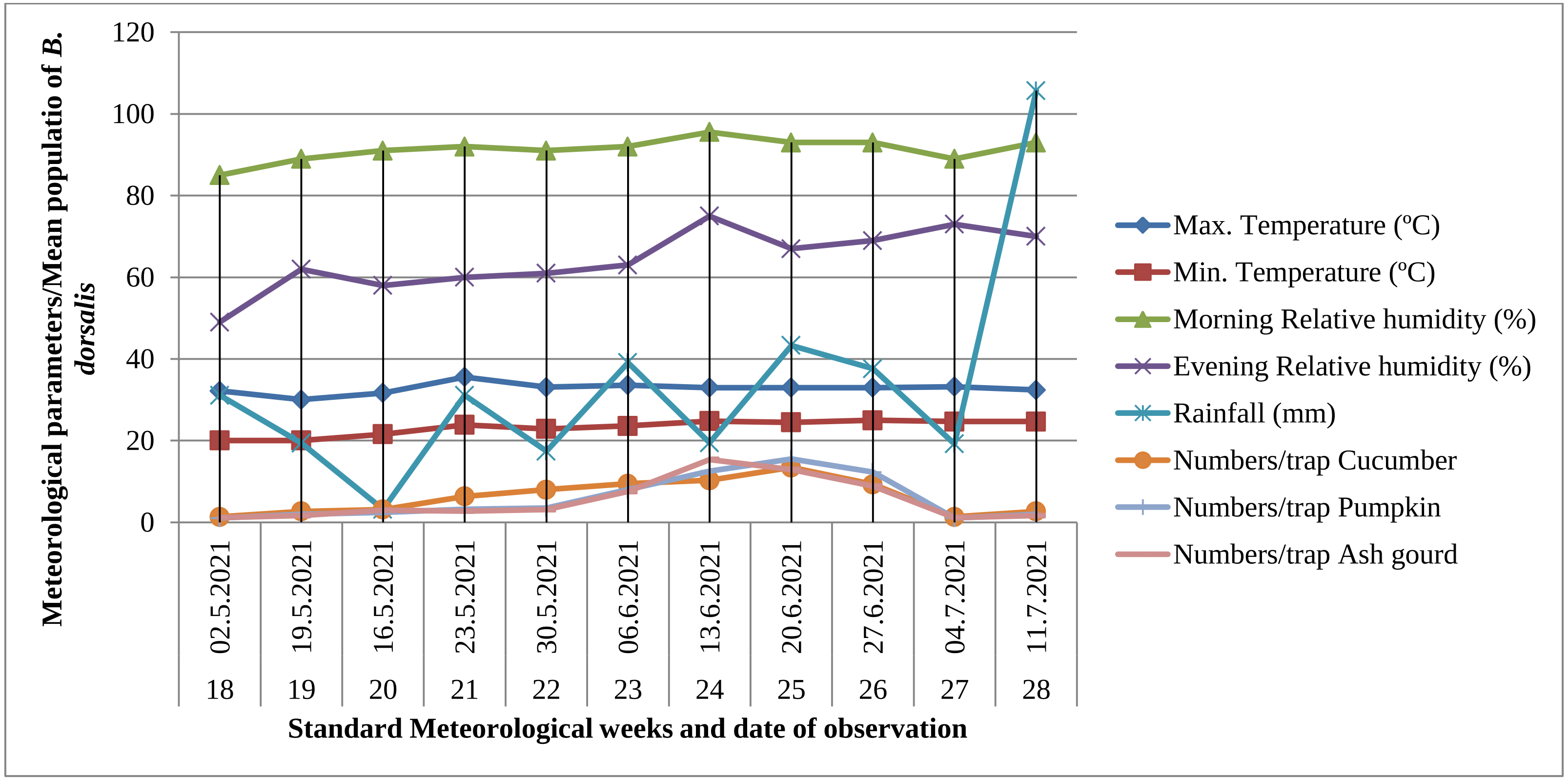


Fig 4.3 Seasonal abundance of *B. dorsalis* recorded during May-July 2021 on different cucurbit crops

In 2021 (Table 4.6 and Fig 4.5), the population of *Z. tau* started noticing from 18 SW (2nd May 2021) till 28 SMW (11th July 2021). It revealed that the peak incidence of *Z. tau* was recorded on 6th June (23 SMW) with mean population of 3.85 flies/trap in cucumber, while in pumpkin and ash gourd peak incidence was observed on 30th May (22 SMW) with mean population of 5.45 and 7.30 flies/trap respectively. Thereafter decreasing trend was observed reaching its lowest population on 4th July (27 SMW) with mean population of 0.80, 0.45 and 0.80 flies/trap in cucumber, pumpkin and ash gourd, respectively.

4.2.1.3 Seasonal abundance of *B. tuberculata* in different cucurbits

The weekly mean number of *B. tuberculata* during 2020 presented in Table 4.7 and grapgically depicted in Fig 4.6 indicated that the population fluctuated over the growing season of crops, ranging from zero population on 1st week (18 SMW) of May to maximum of 7.00 flies/trap during 1st week (23 SMW) of June. The first trap catch was observed on 17th May (20 SMW) with mean population of 0.35 flies/trap in cucumber, while in pumpkin and ash gourd on 3rd May with 0.20 and 0.30 flies/trap, respectively. After that their population increases gradually reaching its peak on 7th June (23 SMW) with mean population of 6.45, 6.40 and 7.00 flies/trap in cucumber, pumpkin and ash gourd, respectively. Lowest population of *B. tuberculata* was recorded on 28th July (26 SMW) in cucumber with 1.30 flies/trap and in pumpkin and ash gourd on 5th July (27 SMW) with mean population of 0.20 and 0.30 flies/trap, respectively.

In 2021 *B. tuberculata* was recorded from first week of taking observation i.e., 2nd May 2021 (18 SMW) with mean population of 0.20, 0.25 and 0.15 flies/trap in cucumber, pumpkin and ash gourd, respectively (Table 4.8 and Fig 4.7). After that their population increased gradually reaching its peak on 13th June (24 SMW) with 3.20 flies/trap in cucumber but in pumpkin and ash gourd on 6th June (23 SMW) with 5.40 and 6.80 flies/trap, respectively. The lowest population was recorded on 4th July (27 SMW) in cucumber, pumpkin and ash gourd with mean population of 0.20, 0.25 and 0.15 flies/trap respectively.

4.2.1.4 Seasonal abundance of *Z. cucurbitae* in different cucurbits

The seasonal incidence of *Z. cucurbitae* during 2020 is being presented in Table 4.9 and Fig 4.8. Experimental findings revealed that the mean number of flies captured in traps was in the range of 0.20 to 2.30 flies/trap. Highest incidence was on 31st May (i.e., 22 SMW) with mean population of 2.30 flies/trap in cucumber and pumpkin but 2.65 flies/trap in ash gourd. Thereafter, the trap catches showed declining trend and lowest population was recorded on 3rd May (18 SMW) and 5th July (27 SMW) with mean population of 0.20, 0.25 and 0.45 flies/trap in cucumber, pumpkin and ash gourd, respectively.

The seasonal incidence of *Z. cucurbitae* during 2021 is being presented in Table 4.10 and illustrated in Fig 4.9. The data thus recorded depicts that the mean number of flies captured in traps was in the range of 0.20 to 2.50 flies/trap. Highest incidence was on 6th June (23 SMW) in cucumber with 1.85 flies/trap while in pumpkin and ash gourd on 30th May (i.e., 22 SMW) with mean population of 2.15 and 2.50 flies/trap, respectively. Lowest population was recorded on 9th May (19 SMW) and 11th July (28 SMW) with mean population of 0.30 and 0.25 flies/trap in cucumber and ash gourd, respectively and 0.20 flies/trap from pumpkin on 2nd May (18 SMW) and 4th July (27 SMW).

Table 4.5 Seasonal abundance of *Z. tau* recorded during May-July 2020 on different cucurbit crops

SMW	Temperature (^oC)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	30.07	20.30	90.00	60.00	20.05	1.55	1.10	1.00
19	32.22	23.90	87.00	56.00	22.60	1.70	1.50	1.70
20	32.00	21.50	92.00	61.00	4.10	2.30	2.25	2.80
21	30.50	22.90	92.00	79.00	38.60	4.50	4.00	4.70
22	30.10	21.20	92.00	63.00	74.00	4.60	4.40	6.25
23	31.90	22.70	94.00	68.00	16.50	2.95	3.10	3.75
24	33.20	24.70	92.00	80.00	67.10	2.60	2.35	2.60
25	33.10	24.30	92.00	71.00	111.90	2.10	1.50	2.05
26	31.80	23.90	92.00	73.00	53.60	1.00	0.85	1.10
27	32.90	24.60	94.00	78.00	79.50	1.40	1.00	0.75
28	32.90	24.80	93.00	71.00	29.70	1.70	1.50	1.70

*Mean of 20 traps

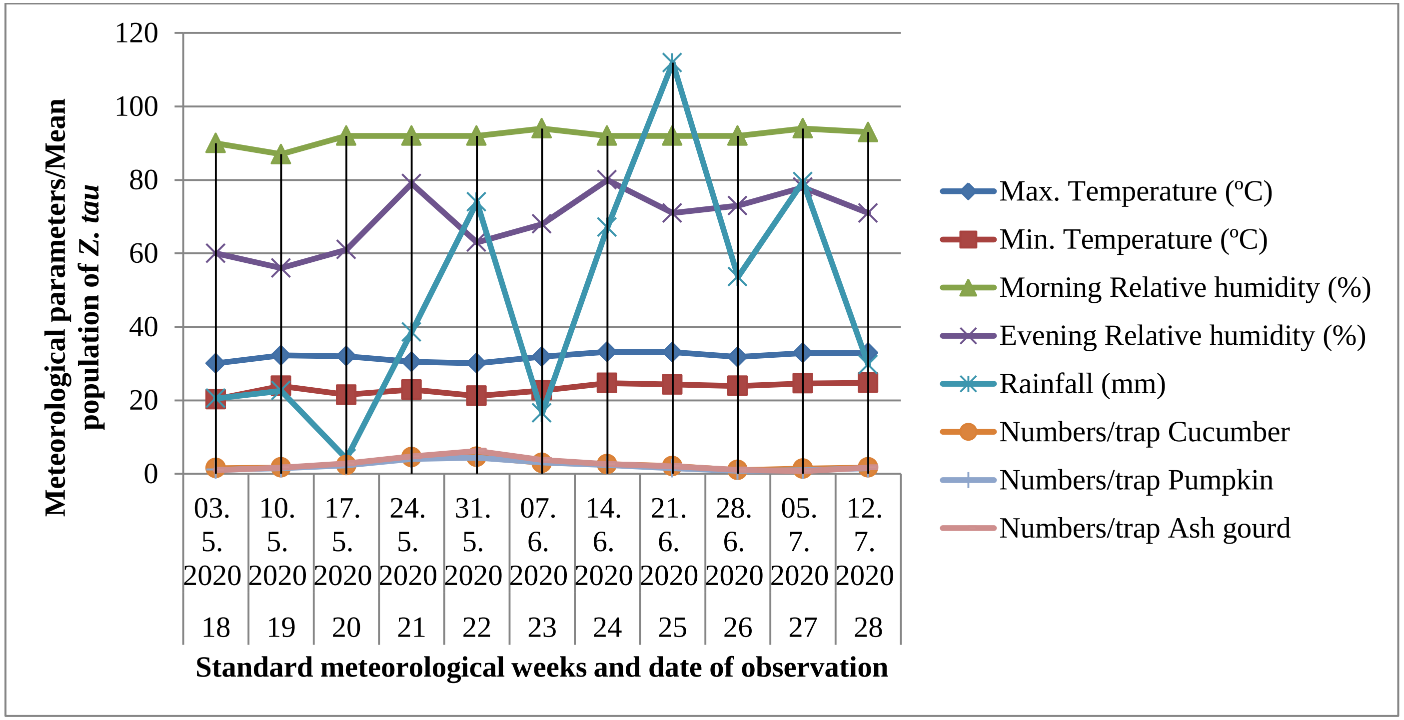


Fig 4.4 Seasonal abundance of *Z. tau* recorded during May-July 2020 on different cucurbit crops

Table 4.6 Seasonal abundance of *Z. tau* recorded during May-July 2021 on different cucurbit crops

SMW	Temperature (^oC)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	32.20	20.05	85.00	49.00	31.10	0.80	0.45	0.80
19	30.03	20.06	89.00	62.00	19.40	1.15	0.80	1.20
20	31.70	21.60	91.00	58.00	3.20	1.50	1.55	2.15
21	35.60	23.90	92.00	60.00	31.10	1.90	2.90	3.60
22	33.10	22.90	91.00	61.00	17.40	3.45	5.45	7.30
23	33.60	23.60	92.00	63.00	39.10	3.85	3.95	3.55
24	33.00	24.80	95.57	75.00	19.50	3.05	2.85	2.75
25	33.00	24.50	93.00	67.00	43.30	2.75	2.20	2.10
26	33.00	25.00	93.00	69.00	37.60	2.45	1.40	1.35
27	33.20	24.70	89.00	73.00	19.20	0.80	0.45	0.80
28	32.40	24.70	93.00	70.00	105.70	1.15	0.80	1.20

*Mean of 20 traps

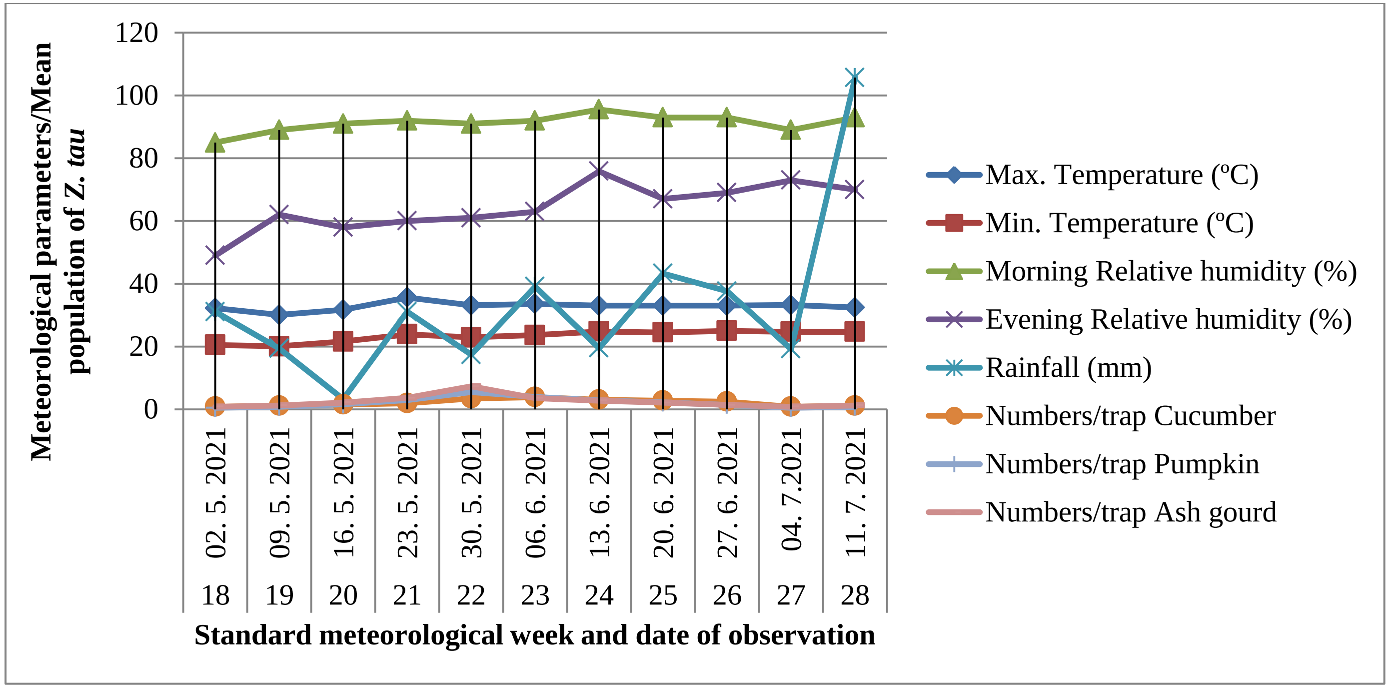


Fig 4.5 Seasonal abundance of *Z. tau* recorded during May-July 2021 on different cucurbit crops

Table 4.7 Seasonal abundance of *B. tuberculata* recorded during May-July 2020 on different cucurbit crops

Standard Meteorological weeks	Temperature (^oC)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	30.07	20.30	90.00	60.00	20.05	0.00	0.20	0.30
19	32.22	23.90	87.00	56.00	22.60	0.00	0.25	0.55
20	32.00	21.50	92.00	61.00	4.10	0.35	0.45	1.00
21	30.50	22.90	92.00	79.00	38.60	0.80	0.90	1.30
22	30.10	21.20	92.00	63.00	74.00	1.75	1.90	2.35
23	31.90	22.70	94.00	68.00	16.50	6.45	6.40	7.00
24	33.20	24.70	92.00	80.00	67.10	2.35	2.00	2.75
25	33.10	24.30	92.00	71.00	111.90	2.05	1.65	2.00
26	31.80	23.90	92.00	73.00	53.60	1.30	0.85	1.15
27	32.90	24.60	94.00	78.00	79.50	0.00	0.20	0.30
28	32.90	24.80	93.00	71.00	29.70	0.00	0.25	0.55

*Mean of 20 traps

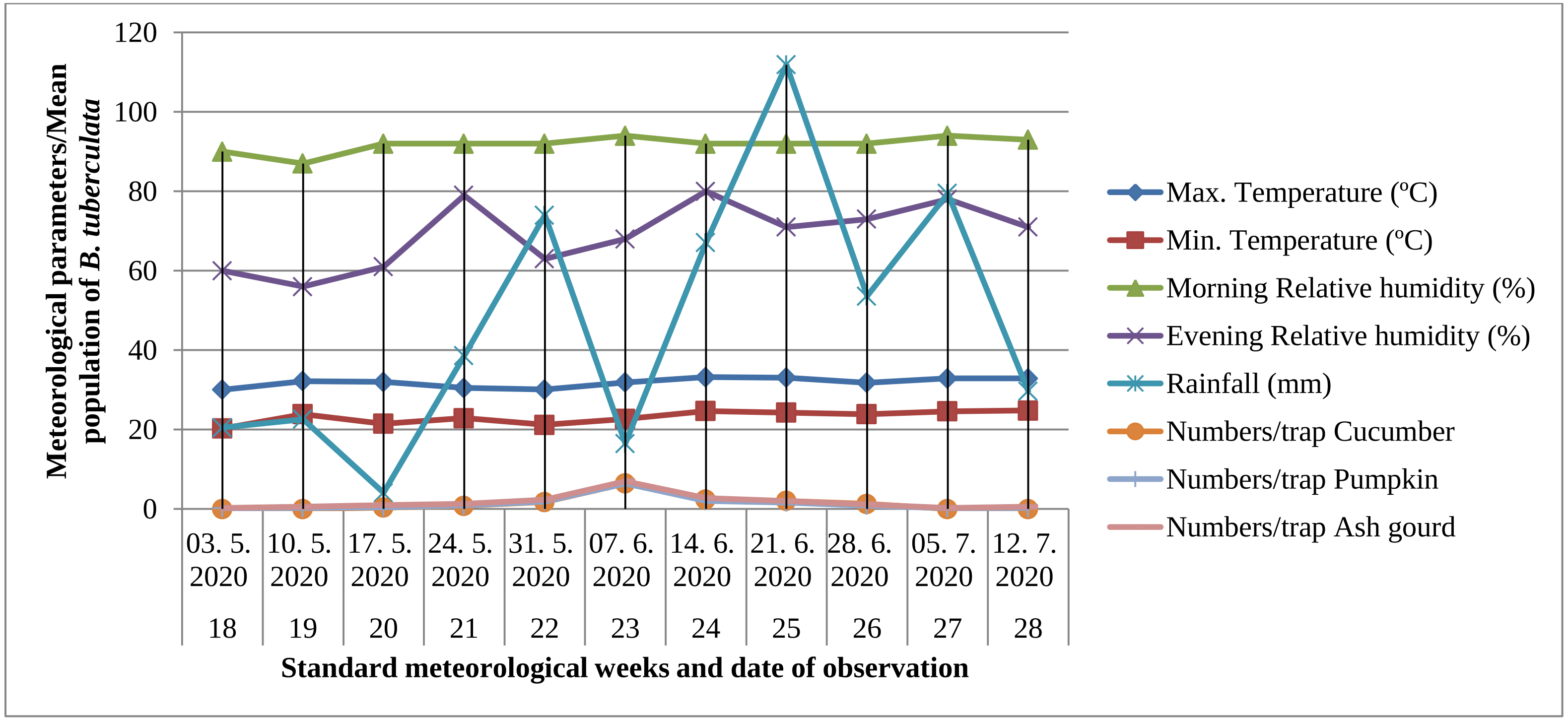


Fig 4.6 Seasonal abundance of *B. tuberculata* recorded during May-July 2020 on different cucurbit crops

Table 4.8 Seasonal abundance of *B. tuberculata* recorded during May-July 2021 on different cucurbit crops

SMW	Temperature (^oC)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	32.20	20.05	85.00	49.00	31.10	0.20	0.25	0.15
19	30.03	20.06	89.00	62.00	19.40	0.30	0.45	0.40
20	31.70	21.60	91.00	58.00	3.20	0.65	0.85	0.85
21	35.60	23.90	92.00	60.00	31.10	1.35	1.05	1.15
22	33.10	22.90	91.00	61.00	17.40	1.80	3.65	2.10
23	33.60	23.60	92.00	63.00	39.10	2.65	5.40	6.80
24	33.00	24.80	95.57	75.00	19.50	3.20	2.25	2.60
25	33.00	24.50	93.00	67.00	43.30	2.25	2.00	1.85
26	33.00	25.00	93.00	69.00	37.60	2.15	0.80	1.00
27	33.20	24.70	89.00	73.00	19.20	0.20	0.25	0.15
28	32.40	24.70	93.00	70.00	105.70	0.30	0.45	0.40

*Mean of 20 traps

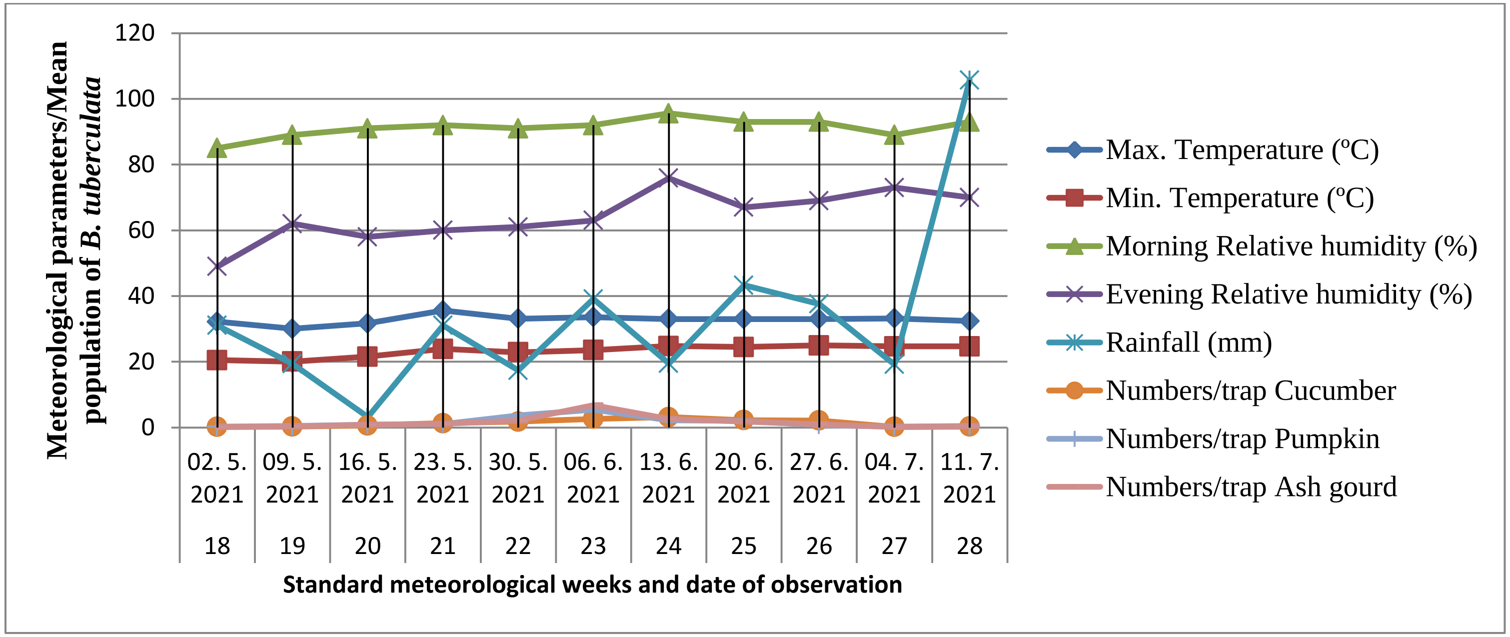


Fig 4.7 Seasonal abundance of *B. tuberculata* recorded during May-July 2021 on different cucurbit crops

Table 4.9 Seasonal abundance of *Z. cucurbitae* recorded during May-July 2020 on different cucurbit crops

SMW	Temperature (^{o}C)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	30.07	20.30	90.00	60.00	20.05	0.20	0.25	0.45
19	32.22	23.90	87.00	56.00	22.60	0.30	0.35	0.55
20	32.00	21.50	92.00	61.00	4.10	0.60	0.60	0.70
21	30.50	22.90	92.00	79.00	38.60	1.30	1.30	1.45
22	30.10	21.20	92.00	63.00	74.00	2.30	2.30	2.65
23	31.90	22.70	94.00	68.00	16.50	1.60	1.35	1.85
24	33.20	24.70	92.00	80.00	67.10	1.05	1.05	1.15
25	33.10	24.30	92.00	71.00	111.90	0.70	0.80	0.95
26	31.80	23.90	92.00	73.00	53.60	0.60	0.50	0.55
27	32.90	24.60	94.00	78.00	79.50	0.20	0.25	0.45
28	32.90	24.80	93.00	71.00	29.70	0.30	0.35	0.55

*Mean of 20 traps

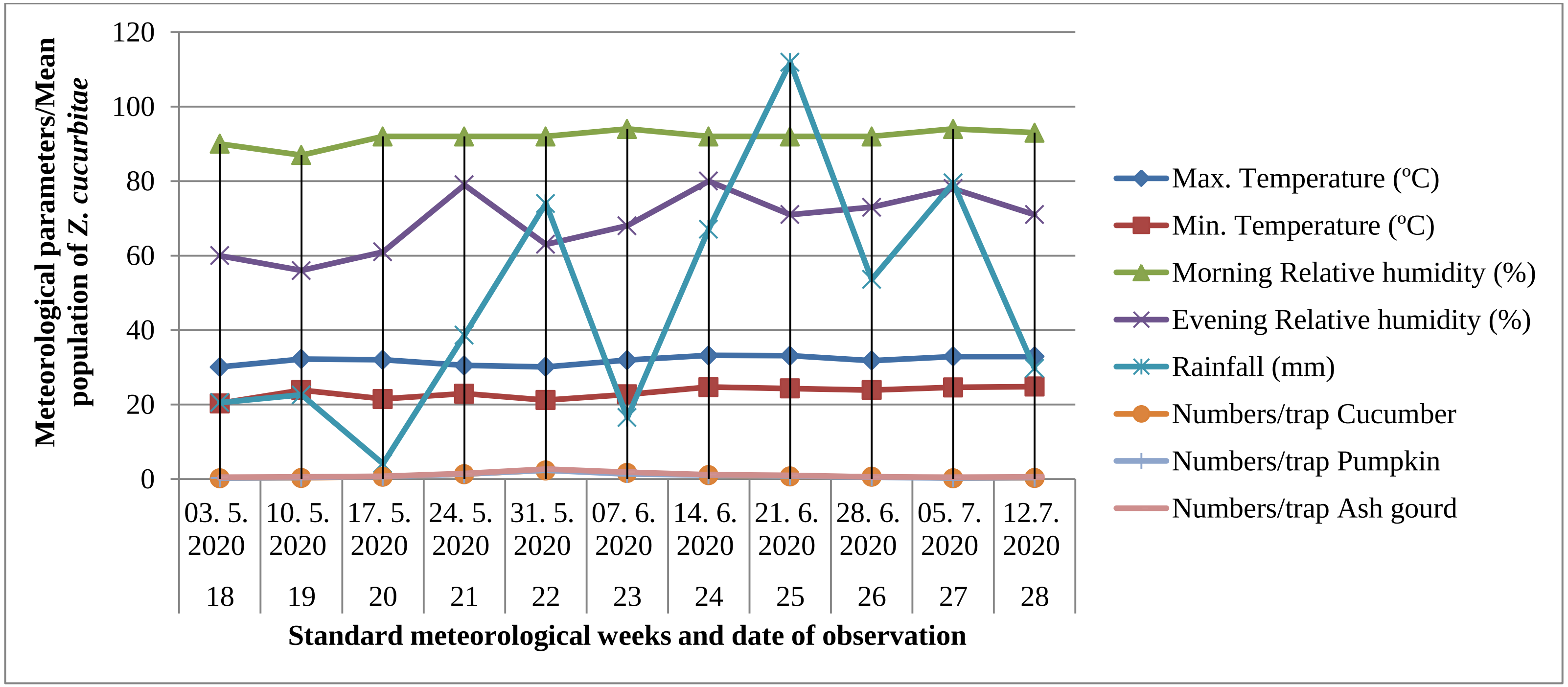


Fig 4.8 Seasonal abundance of *Z. cucurbitae* recorded during May-July 2020 on different cucurbit crops

Table 4.10 Seasonal abundance of *Z. cucurbitae* recorded during May-July 2021 on different cucurbit crops

SMW	Temperature (^{o}C)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	32.20	20.05	85.00	49.00	31.10	0.35	0.20	0.35
19	30.03	20.06	89.00	62.00	19.40	0.30	0.25	0.25
20	31.70	21.60	91.00	58.00	3.20	0.35	0.60	0.70
21	35.60	23.90	92.00	60.00	31.10	0.85	1.35	1.45
22	33.10	22.90	91.00	61.00	17.40	1.15	2.15	2.50
23	33.60	23.60	92.00	63.00	39.10	1.85	1.60	1.75
24	33.00	24.80	95.57	75.00	19.50	1.65	0.90	1.00
25	33.00	24.50	93.00	67.00	43.30	1.55	0.75	0.70
26	33.00	25.00	93.00	69.00	37.60	1.30	0.45	0.55
27	33.20	24.70	89.00	73.00	19.20	0.35	0.20	0.35
28	32.40	24.70	93.00	70.00	105.70	0.30	0.25	0.25

*Mean of 20 traps

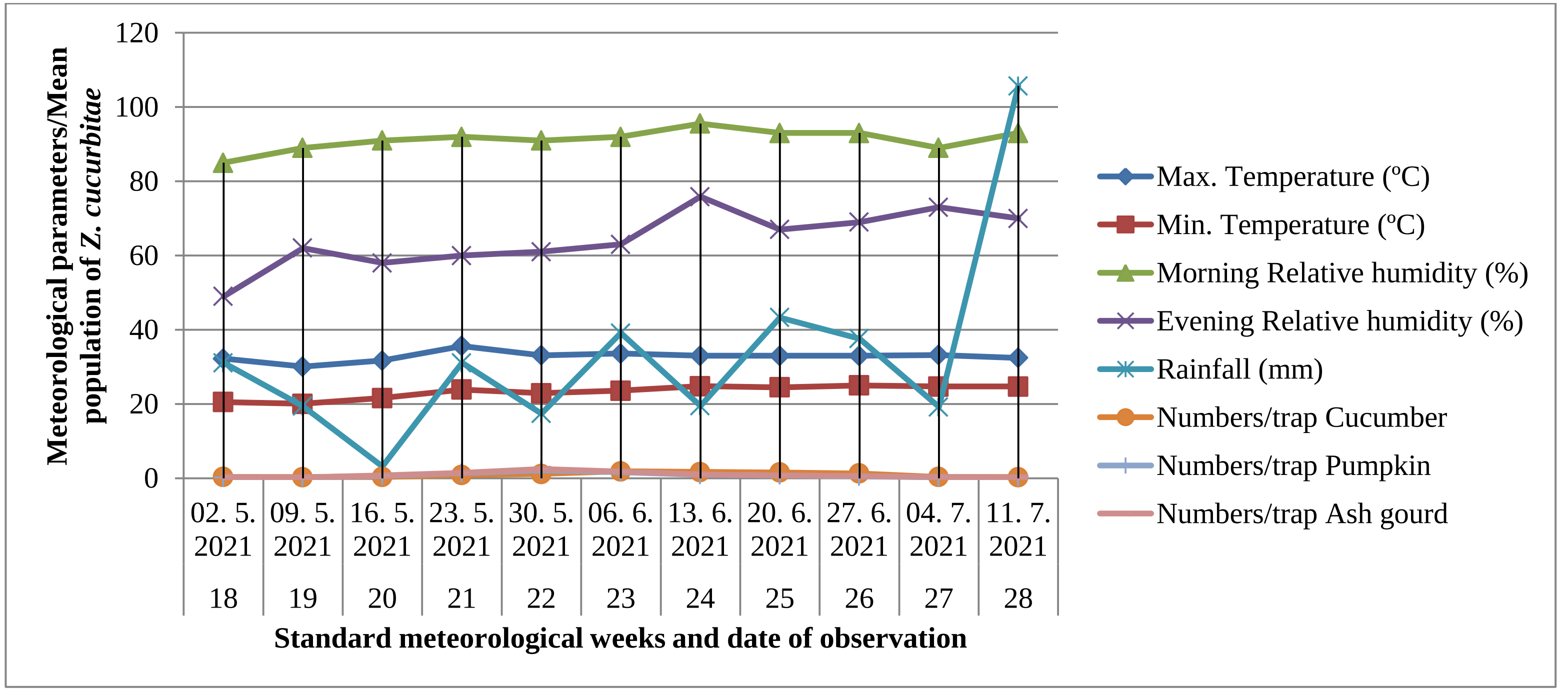


Fig 4.9 Seasonal abundance of *Z. cucurbitae* recorded during May-July 2021 on different cucurbit crops

4.2.1.5 Seasonal abundance of *B. ruiliensis* in different cucurbits

Data presented in Table 4.11 and Fig 4.10, it was found that *B. ruiliensis* species during 2020 was the smallest in number in comparison to other four species trapped. It was also found that *B. ruiliensis* was first observed on 19th SMW in cucumber and 20th SMW in pumpkin and ash gourd. The peak incidence of *B. ruiliensis* was recorded on 22 SMW with 0.70 and 1.10 flies/trap in cucumber and pumpkin, respectively, while in the ash gourd highest population was recorded on 23 SMW (7th June) with 1.20 flies/trap. Thereafter, in the following weeks declining trend was observed in all the fields of cucurbits crop reaching its lowest population on 12th July (28 SMW) with 0.1 flies/trap in cucumber and zero population was recorded on 27th SMW i.e., on 5th July in pumpkin and ash gourd.

(Table 4.12 and Fig 4.11) It was found that *B. ruiliensis* species during 2021 was the smallest in number of total flies/trap in comparison to other four species trapped. It was also found that *B. ruiliensis* was first observed on 20 SMW in cucumber and ash gourd, while in pumpkin on 21 SMW. The peak incidence of *B. ruiliensis* was recorded on 23 SMW with 1.05 and 0.85 flies/trap in cucumber and pumpkin, respectively, while in ash gourd highest population was recorded on 21 SMW (23rd May) with 0.75 flies/trap. Thereafter, in the following weeks declining trend was observed in all the fields of cucurbits crop with zero population was recorded on 27 SMW i.e., on 4th July in cucumber and ash gourd, while in pumpkin on 28 SMW i.e., on 11th July.

As per the present investigation, the abundance of all the five species of fruit flies *viz*., *B. dorsalis*, *Z. tau*, *B. tuberculata*, *Z. cucurbitae* and *B. ruiliensis* reached its maximum in the month of May and June. Similar findings were reported by Vayssieres *et al*. (2015) who observed that *B. dorsalis* was most abundant during the rainy season and peaked at June. Makhmoor and Singh (1998) also reported that the peak population (170.66 males/trap/week) of oriental fruit fly was observed in June at Kashmir area in India. Majacunene *et al*. (2014) however observed high abundance of *B. dorsalis* from November to March, with peak in January while monitoring population of *B. dorsalis* by methyl eugenol in Manica Province, Mozambique. Tan and Serit (1994) concluded that the availability of preferred hosts is the variable that mostly influences the size of the population of adults of *B. dorsalis* in Malaysia. Mwatawala *et al*. (2006) has reported that the population growth of *B. dorsalis* at SUA (Sokoine University of Agriculture) orchards in Tanzania was directly related with the presence of mango and guava. Ye and Liu (2005) also reported that *B. dorsalis* occurred only from May to November with high abundance in July of each year at Kunming, China. Ukey *et al*. (2014) reported that the incidence of *B*actrocera spp. was noticed from first week of June immediately after the installation of methyl eugenol and cue lure traps. The activity of guava fruit flies was its peak in the month of June and July. Next peak activity was recorded in the month of October and November coinciding with harvesting season of guava. They also observed lowest activity in the month of January.

The present finding is also in line with the work of Abro *et al*. (2017) who reported that the occurrence of *B. cucurbitae* species activities throughout the year. However, maximum number of the fly species was recorded during mid-May to mid-June. Hossain *et al*. (2019) reported that the peak population of *Z. cucurbitae* occured on March, 2017 (early in the rainy season) and May 2018 (middle of the rainy summer season). Ye (2001) also reported that high abundance of *B. cucurbitae* occurs only from May upto November each year in Yunnan Province of China. However, our experimental results were not in agreement with the findings of Lee *et al*. (1992) and Mahmood and

Mishkatullah (2007) who reported increased population of *B. cucurbitae* from July to October.

In the present investigation the peak activity of all the five fruit fly species in three cucurbits (cucumber, pumpkin and ash gourd) occurred during last week of May upto middle of June which might be due to presence of maximum tender fruits in the field for oviposition by fruit flies species as the females prefer tender fruits with soft skin for oviposition.

4.2.2 Correlation between different species of fruit flies with abiotic factors

The correlation study was conducted during two seasons i.e., 2020 and 2021in three different cucurbit crops *viz*., cucumber, pumpkin and ash gourd between the different species of fruit fly and abiotic factors such as maximum and minimum temperature, relative humidity and rainfall. The results thus obtained are discussed in the following sub headings.

4.2.2.1 Correlation between different species of fruit flies with abiotic factors in cucumber

In the present investigation, the correlation (Table 4.13) of *Bactrocera dorsalis* population with abiotic factors for the year 2020 did not show any significant relationship. However in the year 2021 (Table 4.14), the population of *B. dorsalis* showed positive significant relationship with morning relative humidity (r = 0.739**). In case of *Z. tau* has revealed a non-significant correlation with all the abiotic factors from cucumber field in the year 2020, but in 2021 the population of *Z. tau* were also found to have positive significant relationship with morning relative humidity (r = 0.650*). Furthermore, the cor-

Table 4.11 Seasonal abundance of *B. ruiliensis* recorded during May-July 2020 on different cucurbit crops

SMW	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	30.07	20.30	90.00	60.00	20.05	0.00	0.00	0.00
19	32.22	23.90	87.00	56.00	22.60	0.25	0.00	0.00
20	32.00	21.50	92.00	61.00	4.10	0.35	0.40	0.10
21	30.50	22.90	92.00	79.00	38.60	0.45	0.70	0.50
22	30.10	21.20	92.00	63.00	74.00	0.70	1.10	0.85
23	31.90	22.70	94.00	68.00	16.50	0.40	0.65	1.20
24	33.20	24.70	92.00	80.00	67.10	0.20	0.20	0.55
25	33.10	24.30	92.00	71.00	111.90	0.25	0.15	0.40
26	31.80	23.90	92.00	73.00	53.60	0.20	0.15	0.20
27	32.90	24.60	94.00	78.00	79.50	0.25	0.00	0.00
28	32.90	24.80	93.00	71.00	29.70	0.10	0.00	0.00

*Mean of 20 traps

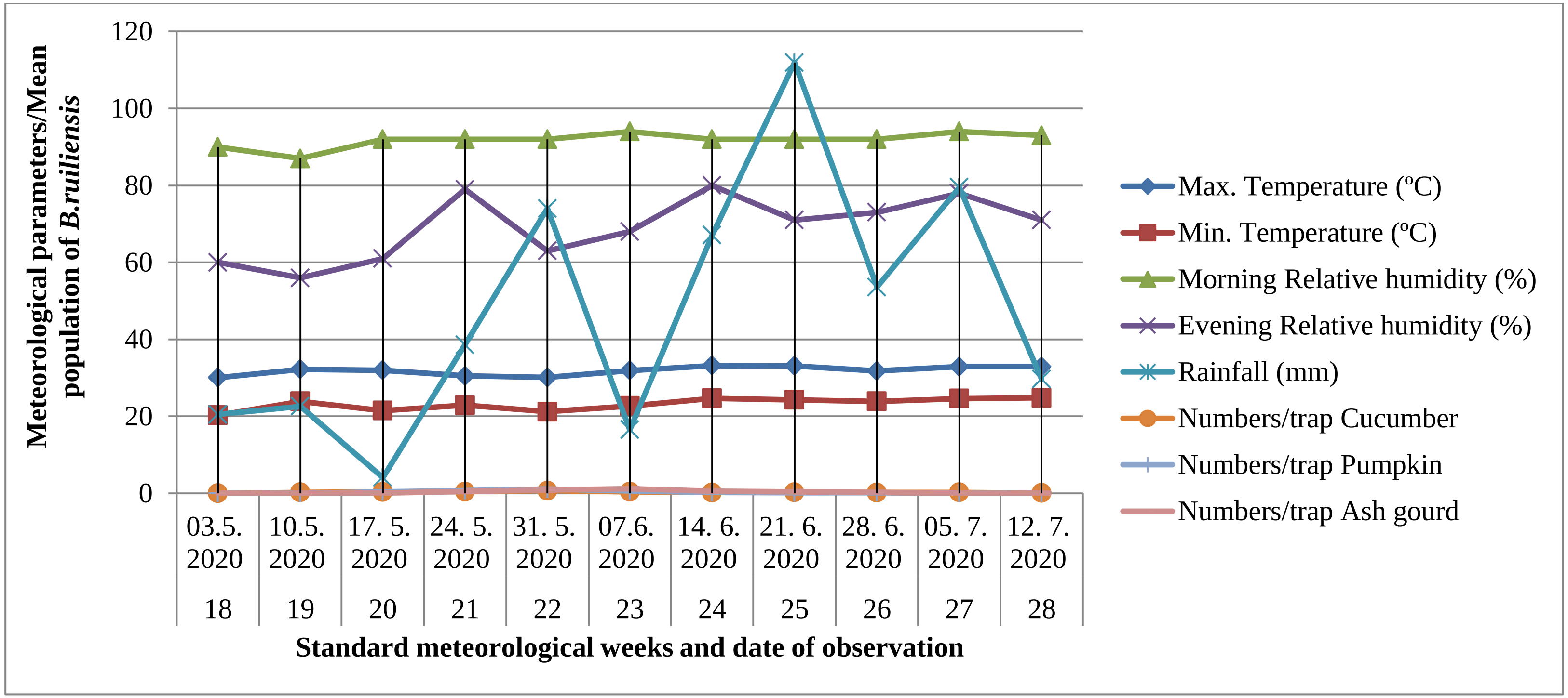


Fig 4.10 Seasonal abundance of *B. ruiliensis* recorded during May-July 2020 on different cucurbit crops

Table 4.12 Seasonal abundance of *B. ruiliensis* recorded during May-July 2021 on different cucurbit crops

SMW	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	*Numbers/trap		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
18	32.20	20.05	85.00	49.00	31.10	0.00	0.00	0.00
19	30.03	20.06	89.00	62.00	19.40	0.00	0.00	0.00
20	31.70	21.60	91.00	58.00	3.20	0.25	0.00	0.55
21	35.60	23.90	92.00	60.00	31.10	0.55	0.50	0.75
22	33.10	22.90	91.00	61.00	17.40	0.85	0.55	0.70
23	33.60	23.60	92.00	63.00	39.10	1.05	0.85	0.35
24	33.00	24.80	95.57	75.00	19.50	0.90	0.30	0.35
25	33.00	24.50	93.00	67.00	43.30	0.50	0.20	0.30
26	33.00	25.00	93.00	69.00	37.60	0.35	0.10	0.20
27	33.20	24.70	89.00	73.00	19.20	0.00	0.10	0.00
28	32.40	24.70	93.00	70.00	105.70	0.00	0.00	0.00

*Mean of 20 traps

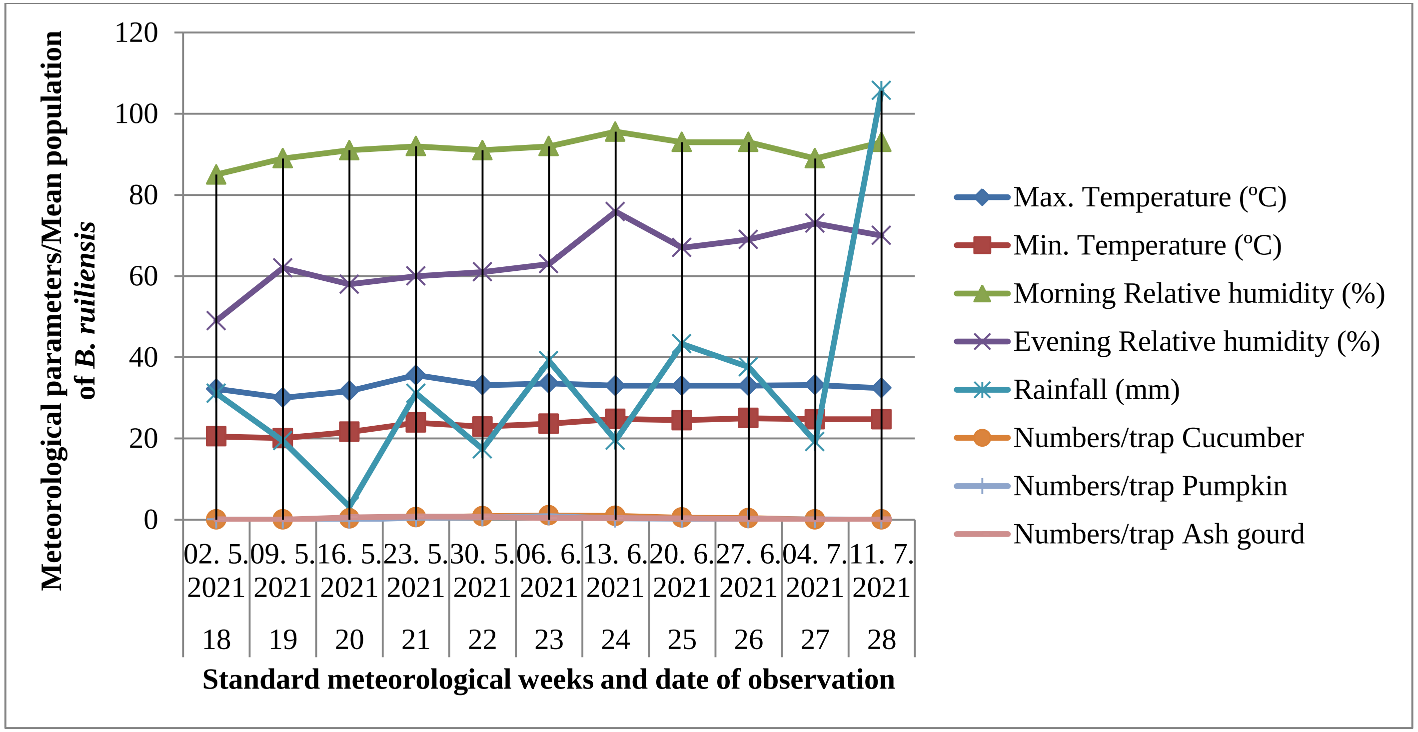


Fig 4.11 Seasonal abundance of *B. ruiliensis* recorded during May-July 2021 on different cucurbit crops

Table 4.13 Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2020 on cucumber

Fruit flies species	Pearson's correlation coefficient(r)				
	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
B. dorsalis	0.399	0.376	0.221	0.423	0.459
Z. tau	-0.463	-0.347	0.156	0.090	0.043
B. tuberculata	0.021	-0.013	0.421	0.251	0.204
Z. cucurbitae	-0.352	-0.252	0.297	0.138	0.160
B. ruiliensis	-0.067	0.027	0.258	0.123	0.211

Note: * = Significant at 5% level of significance

** = significant at 1% level of significance

Table 4.14 Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2021 on cucumber

Fruit flies species	**Pearson's correlation coefficient (r)**				
	Temperature (°C)		**Relative humidity (%)**		**Rainfall (mm)**
	Max.	**Min.**	**Morning**	**Evening**	
B. dorsalis	0.407	0.520	0.739**	0.328	-0.017
Z. tau	0.385	0.403	0.650*	0.219	-0.107
B. tuberculata	0.456	0.513	0.736**	0.329	-0.129
Z. cucurbitae	0.489	0.519	0.633*	0.309	-0.082
B. ruiliensis	0.539	0.388	0.632*	0.136	-0.259

Note: * = Significant at 5% level of significance

** = significant at 1% level of significance

relation of *B. tuberculata* in relation with abiotic factors has revealed a non-significant correlation with all the abiotic factors during the year 2020, while in 2021 their population showed significant positive relation with morning relative humidity (r = 0.736**). Similarly, in 2020 the population of *Z. cucurbitae* had showed non-significant relationship with all the abiotic factors, whereas in 2021 significant positive relationship was found with morning relative humidity (r = 0.633*). The correlation of *B. ruiliensis* with all the abiotic factors had shown a non-significant relationship in 2020 whereas, in the year 2021 positive significant correlation relationship was showed with morning relative humidity (r = 0.632*).

4.2.2.2 Correlation between different species of fruit flies with abiotic factors in Pumpkin

The correlation study during 2020 and 2021 between the different species of fruit fly and abiotic factors such as maximum and minimum temperature, relative humidity and rainfall are expressed in Table 4.15 and 4.16. The data thus recorded showed that the population of all the five fruit fly species had a non-significant relationship with abiotic factors during the year 2020. However, in the year 2021, the population of *B. dorsalis* exhibited a positive significant co-relation with morning relative humidity (r= 0.726*).

4.2.2.3 Correlation between different species of fruit flies with abiotic factors in Ash gourd

In ash gourd field during the year 2020 and 2021 (Table 4.17 and 4.18) the population of all the five species of fruit flies *viz*., *B. dorsalis*, *Z. tau*, *B. tuberculata*, *Z. cucurbitae* and *B. ruiliensis* was found to have non-significant correlation with all the abiotic factors, except for *B. dorsalis* in 2021 where their population was found to have a significant positive correlation with morning relative humidity (r= 0.745**).

Table 4.15 Correlation coefficient (r) of fruit flies species with abiotic factors recorded during May-July 2020 on pumpkin

Fruit flies species	**Pearson's correlation coefficient(r)**				
	Temperature (°C)		**Relative humidity (%)**		**Rainfall (mm)**
	Max.	**Min.**	**Morning**	**Evening**	
B. dorsalis	0.426	0.379	0.219	0.415	0.460
Z. tau	-0.438	-0.349	0.174	0.040	-0.072
B. tuberculata	0.006	-0.039	0.415	0.167	0.107
Z. cucurbitae	-0.357	-0.255	0.264	0.129	0.219
B. ruiliensis	-0.422	-0.381	0.328	0.096	0.047

Note: * = Significant at 5% level of significance

** = significant at 1% level of significance

Table 4.16 Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2021 on pumpkin

Fruit flies species	Pearson's correlation coefficient(r)				
	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
B. dorsalis	0.248	0.550	0.726*	0.448	0.018
Z. tau	0.479	0.259	0.516	0.029	-0.199
B. tuberculata	0.345	0.254	0.475	0.086	-0.118
Z. cucurbitae	0.553	0.216	0.418	-0.065	-0.229
B. ruiliensis	0.725*	0.461	0.432	0.186	-0.158

Note: * = Significant at 5% level of significance

** = significant at 1% level of significance

Table 4.17 Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2020 on Ash gourd

Fruit flies species	Pearson's correlation coefficient (r)				
	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
B. dorsalis	0.425	0.381	0.211	0.419	0.468
Z. tau	-0.430	-0.351	0.147	-0.022	-0.021
B. tuberculata	0.036	-0.029	0.393	0.150	-0.058
Z. cucurbitae	-0.381	-0.289	0.275	0.069	0.175
B. ruiliensis	-0.177	-0.132	0.390	0.260	0.228

Note: * = Significant at 5% level of significance

** = significant at 1% level of significance

Table 4.18 Correlation coefficient (r) of fruit flies species with abiotic factors during May-July 2021 on ash gourd

Fruit flies species	**Pearson's correlation coefficient (r)**				
	Temperature (°C)		**Relative humidity (%)**		**Rainfall (mm)**
	Max.	**Min.**	**Morning**	**Evening**	
B. dorsalis	0.231	0.524	0.745**	0.468	-0.059
Z. tau	0.433	0.136	0.372	-0.083	-0.239
B. tuberculata	0.363	0.303	0.542	0.143	-0.069
Z. cucurbitae	0.572	0.186	0.324	-0.107	-0.294
B. ruiliensis	0.551	0.243	0.511	-0.071	-0.369

Note: * = Significant at 5% level of significance

** = significant at 1% level of significance

The correlation studies between different species of fruit fly and abiotic factors in three different cucurbits revealed that only some species (*B. dorsalis*, *B. tuberculata* and *Z. cucurbitae*) was significantly correlated (positive).

The findings are in partial conformity with the findings of Wazir *et al*. (2019) who reported that the population of *B. cucurbitae* was highly significant and positively correlated with morning humidity, whereas the studies failed to establish any significant correlation with other abiotic factors, except the species *B. ruiliensis* which showed a significant positive correlation with maximum temperature during 2021 on pumpkin. Similar results were reported by Syed (1971), Ye (2021) and Vargas *et al*. (2003) who stated that maximum number of *B. cucurbitae* was recorded in the warm months of each year where relative humidity was observed as a crucial factor which impact the pest incidence.

On the other hand, Abhilash *et al*. (2017) had found that the population of melon fruit fly was significantly and positively correlated with maximum and minimum temperature but negatively correlated with relative humidity and rainfall. Similarly, Khan and Naveed (2017) who found a positive correlation between the fruit fly population and temperature and a negative correlation with relative humidity.

The non-significant correlation of different species of fruit flies in cucurbits with most of the abiotic factors in the present investigation might be due to some other factors like availability of tender fruits with soft skin. In the present investigation during the last week of May upto middle of June, the population of fruit flies species was observed highest in different cucurbits and during that period maximum number of tender fruits with soft skin was available in the fields. Therefore, the population might be increasing with the increase of tender fruits in the field during that period. Liu and Yeh (1982) and

Tariq *et al*. (2002) also correlated the population of fruit flies with the ripening of fruit crops which facilated easy oviposition inside the soft skin of fruits by the female fruit fly.

4.3 Species diversity and relative abundance of natural enemies of fruit flies

4.3.1 Species identification

Natural enemies of fruit flies recorded during the present study are presented in Table 4.19. The five natural enemies that have been reported during the year 2020 and 2021 are *Oecophylla smaragdina* (Hymenoptera: Formicidae), *Tapinoma* sp. (Hymenoptera: Formicidae), *Polyrhachis* sp. (Hymenoptera: Formicidae), *Crematogaster* sp. (Hymenoptera: Formicidae) and *Paederus fuscipes* (Coleoptera: Staphylinidae).

4.3.2 Diversity indices of natural enemies of fruit flies on important cucurbits of Nagaland

Diversity is a measure of species diversity in a community. It provides essential information about reality and commonness of species in a community. The present findings followed Simpson Diversity index which measures the probability that two individuals randomly selected from a sample will belong to the same species (Simpson, 1949). The value of this index ranges between 0 and 1 which indicates greater the value, greater the sample diversity.

Shannon-Weiner index accounts for both abundance and evenness of species in a community. The value of Shannon-Weiner diversity index usually falls in the range of 1.0 and 3.5 and rarely surpasses 4.5, whereas species evenness ranges between 0 and 1 with 1 being complete evenness.

In the year 2020 the values obtained for Shannon-Weiner index (H'), Simpson diversity index (SDI) and evenness (E_H) of natural enemies were 1.38, 0.294 and 0.860 (cucumber field), 1.37, 0.295 and 0.852 (pumpkin field) and 1.39, 0.288 and 0.861 (ash gourd field), respectively (Table 4.19).

In 2021, the values obtained for H', SDI and E_H were 1.36, 0.299 and 0.843 (cucumber field), 1.35, 0.301 and 0.838 (pumpkin field) and 1.34, 0.305 and 0.832 (ash gourd field), respectively (Table 4.19).

Pooled data as presented in Table 4.19 revealed that the value of H' obtained from cucumber, pumpkin and ash gourd field was 1.37, 1.36 and 1.36, respectively. SDI value in cucumber, pumpkin and ash gourd field was 0.296, 0.299 and 0.297, respectively and E_H value was 0.853, 0.845 and 0.847 from cucumber, pumpkin and ash gourd, respectively.

4.3.3 Relative abundance of natural enemies of fruit flies in different cucurbit

4.3.3.1 Cucumber

Data (Table 4.20 and Fig 4.12) from two years experimental trials revealed that *Oecophylla smaragdina* was the most abundant species as compared to *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. *and Paederus fuscipes*. The percent relative abundance of *O. smaragdina* in the year 2020 was observed to be 44.47 followed by *Tapinoma* sp. with 23.06, *Polyrhachis* sp. with 18.07, *Crematogaster* sp. with 8.53 and *Paederus fuscipes* with 5.87. Similarly in the year 2021, highest relative abundance percentage was observed for *O. smaragdina* with 43.58 followed by *Tapinoma* sp. with 26.85,

Table 4.19 Diversity of fruit fly natural enemies in different cucurbit crops recorded during 2020 and 2021

Species	**First year (2020)**			**Second year (2021)**			**Pooled**		
	Cucumber	**Pumpkin**	**Ash gourd**	**Cucumber**	**Pumpkin**	**Ash gourd**	**Cucumber**	**Pumpkin**	**Ash gourd**
O. smaragdina	1371.00	1279.00	1178.00	1279.00	1255.00	1243.00	1325.00	1267.00	1210.50
***Tapinoma* sp.**	711.00	755.00	790.00	788.00	815.00	812.00	749.50	785.00	801.00
***Polyrhachis* sp.**	557.00	522.00	483.00	510.00	498.00	485.00	533.50	510.00	484.00
***Crematogaster* sp.**	263.00	239.00	219.00	220.00	209.00	197.00	241.50	224.00	208.00
Paderus fuscipes	181.00	146.00	165.00	138.00	129.00	122.00	159.50	137.50	143.50
Total number of species	3083.00	2941.00	2835.00	2935.00	2906.00	2859.00	3009.00	2923.50	2847.00
Shannon-wiener diversity index (H')	1.38	1.37	1.39	1.36	1.35	1.34	1.37	1.36	1.36
Simpson diversity index (SDI)	0.294	0.295	0.288	0.299	0.301	0.305	0.296	0.299	0.297
Evenness of species (E_H)	0.860	0.852	0.861	0.843	0.838	0.832	0.853	0.845	0.847

* Population from 5 vines of ecological plots

Polyrhachis sp. with 17.38, *Cremtogaster* sp. with 7.50 and lowest was found for *Paederus fuscipes* with 4.70.

Pooled data (Table 4.20 and Fig 4.12) revealed that the relative abundance percentage was highest for *O. smaragdina* with 44.03 followed by *Tapinoma* sp. with 24.91, *Polyrhachis* sp. with 17.73, *Cremtogaster* sp. with 8.02 and least was found for *Paederus fuscipes* with 5.30.

4.3.3.2 Pumpkin

The data (4.20 and Fig 4.12) from two years experimental trials revealed that *Oecophylla smaragdina* was the most abundant species as compared to *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. *and Paederus fuscipes*. The relative abundance percentage of *O. smaragdina* in the year 2020 was observed to be 43.49 followed by *Tapinoma* sp. with 25.67, *Polyrhachis* sp. with 17.75, *Crematogaster* sp. with 8.13 and *Paederus fuscipes* with 4.96. In the year 2021, also similar trend was observed with highest relative abundance percentage for *O. smaragdina* (43.19) followed by *Tapinoma* sp. (28.04), *P*olyrhachis sp. (17.14), *Crematogaster* sp. (7.19) and lowest for *Paederus fuscipes* (4.44).

Pooled data (Table 4.20 and Fig 4.12) revealed that the relative abundance percentage of *O. smaragdina* was 43.34 followed by *Tapinoma* sp. with 26.85 *Polyrhachis* sp. with 17.44, *Crematogaster* sp. with 7.66 and *Paederus fuscipes* with 4.70.

4.3.3.3 Ash gourd

The data (4.20 and Fig 4.12) from two years experimental trials revealed that *Oecophylla smaragdina* was the most abundant species as compared to *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. *and Paederus fuscipes*. The relative abundance percentage of *O. smaragdina* in the year 2020 was observed to be 41.55 followed by *Tapinoma* sp. with 27.87, *Polyrhachis*

Table 4.20 Relative abundance (%) of fruit flies' natural enemies recorded during 2020 and 2021

Species	Relative abundance (%)								
	2020			2021			Pooled		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
O. smaragdina	44.47	43.49	41.55	43.58	43.19	43.48	44.03	43.34	42.52
***Tapinoma* sp.**	23.06	25.67	27.87	26.85	28.04	28.40	24.91	26.85	28.13
***Polyrhachis* sp.**	18.07	17.75	17.04	17.38	17.14	16.96	17.73	17.44	17.00
***Crematogaster* sp.**	8.53	8.13	7.72	7.50	7.19	6.89	8.02	7.66	7.31
Paederus fuscipes	5.87	4.96	5.32	4.70	4.44	4.27	5.30	4.70	5.04

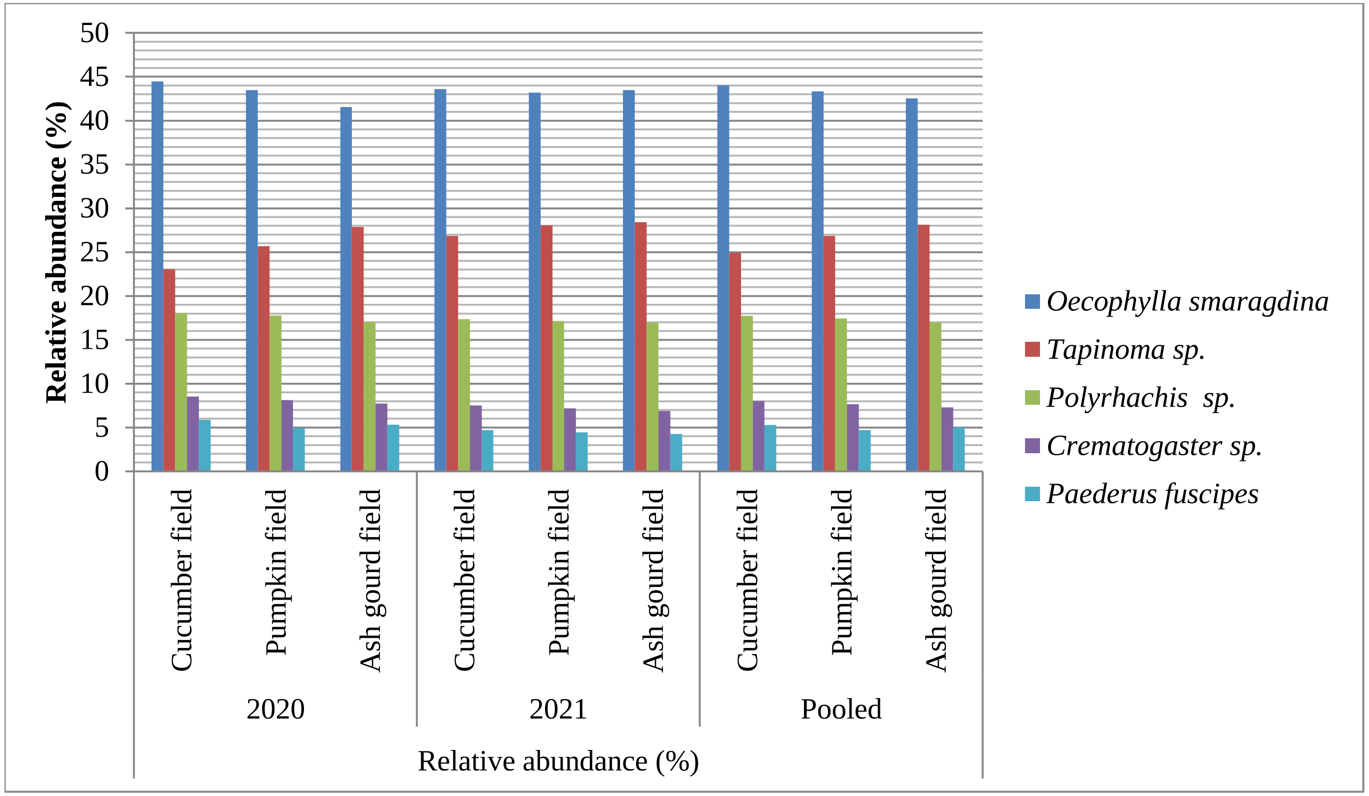


Fig 4.12 Relative abundance (%) of natural enemies of fruit flies recorded during 2020 and 2021

sp. with 17.04, *Crematogaster* sp. with 7.72 and *Paederus fuscipes* with 5.82. In the year 2021 the relative abundance percentage of *O. smaragdina* was observed to be 43.48 followed by *Tapinoma* sp. with 28.40, *Polyrhachis* sp. with 16.96, *Crematogaster* sp. with 6.89 and *Paederus fuscipes* with 4.27.

Pooled data (Table 4.20 and Fig 4.12) revealed that the highest relative abundance was observed for *O. smaragdina* with 42.52% followed by *Tapinoma* sp. with 28.13%, *Polyrhachis* sp. with 17.00%, *Crematogaster sp.* with 7.31% and *P. fuscipes* with 5.04%.

The result of the current study corroborates the finding of Fernandes *et al.* (2012) who reported that the ants were the most effective genus, removing 93 per cent of the fruit fly larvae. El Keroumi *et al*. (2012) who reported that ant predation behavior below Argan trees, highlights the promising use of dominant ant species as potential agents of Mediterranean fruit fly bio-control in the Argan forest and surrounding ecosystems. Hodgson *et al*. (1998) stated that fruit fly immature stages are naturally exposed to a variety of predators, among which the most important are ants (Formicidae) and rove beetles (Staphylinidae), which have been detected to cause significant mortality.

4.4 Seasonal abundance of natural enemies of fruit flies in different cucurbits and their correlation with abiotic factors

4.4.1 Seasonal abundance of natural enemies of fruit flies in different cucurbits

Seasonal abundance of different species of fruit flie’s natural enemies was monitored from the ecological plots (5 nos.) which were maintained 5 m away from the main field. The population was counted at weekly interval from 5 ramdomly selected vines per plot.

It was evident from table 4.19 during 2020-2021, five species of natural enemy *viz*., *Oecophylla smaragdina, Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. and *Paederus fuscipes* were observed at the experimental field. Their abundance in different cucurbits field were discussed in the following subheadings.

4.4.1.1 Seasonal abundance of *O. smaragdina* in different cucurbits

Experimental results of the present investigation revealed that the incidence of *O. smaragdina* appeared initially at 15 SMW in cucumber and ash gourd field whereas in pumpkin field they appeared for the first time on 16 SMW (Table 4.21 and Fig 4.13). The peak incidence of *O. smaragdina* was recorded on 5th July (27 SMW) in all the cucurbits representing mean population of 34.60, 33.60 and 32.00 numbers/vine, respectively on cucumber, pumpkin and ash gourd and the lowest mean population was observed on 12th April (15 SMW) in cucumber and ash gourd field with mean population of 0.40 and 1.00 numbers/vine, respectively, while in pumpkin field lowest population was recorded on 19th April (16 SMW) with mean population of 2.40 numbers/vine.

In the year 2021 the abundance of *O. smaragdina* started from 17 SMW and reached its peak on 11th July (28 SMW) with mean population of 33.80 and 33.20 numbers/vine in cucumber and ash gourd, respectively. However, in pumpkin field highest population was recorded on 4th July (27 SMW) with mean population of 33.20 numbers/vine (Table 4.22 and Fig 4.14).

4.4.1.2 Seasonal abundance of *Tapinoma* sp. in different cucurbits

During 2020 *Tapinoma* sp. was found to be active from 17 SMW i.e., on 26th April till the harvest time with mean population of 3.80, 1.80 and 2.40 numbers/vine in cucumber, pumpkin and ash gourd field, respectively and thereafter their population increases gradually reaching its peak on 28 SMW i.e., on 12th July 2020 with 25.40, 25.00 and 26.00 numbers/vine in cucumber, pumpkin and ash gourd field, respectively (Table 4.23 and Fig 4.15). In 2021 from Table 4.24 and Fig 4.16 it can be expressed that their population was recorded for the first time on 25th April (17 SMW) and the highest mean population was recorded on 11th July (28 SMW) with mean population of 26.20, 26.40 and 26.60 numbers/vine respectively from cucumber, pumpkin and ash gourd. The lowest population was recorded on 25th April (17 SMW) with 2.80, 3.00 and 3.20 numbers/vine from cucumber, pumpkin and ash gourd, respectively.

4.4.1.3 Seasonal abundance of *Polyrhachis* sp. in different cucurbits

In 2020 the weekly observation of *Polyrhachis* sp. indicated that the population fluctuated over the growing season of crop, ranging from zero population on 15th March to 29th March 2020 (11 SMW to 13 SMW) in cucumber field while in pumpkin and ash gourd fields zero population was recorded on 15th March to 19th April 2020 to a maximum on 28th June (26 SMW) with 15.80, 16.00 and 15.40 numbers/vine in cucumber, pumpkin and ash gourd field, respectively. The first incidence was recorded on 5th April (14 SMW) in cucumber field with 0.6 numbers/vine but in pumpkin and ash gourd field it was first recorded on 26th April (17 SMW) with 5.20 and 4.60 numbers/vine (Table 4.25 and Fig 4.17).

Table 4.21 Seasonal abundance of *O. smaragdina* recorded during March-July 2020 on different cucurbit crops

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.09	14.7	96	41	0.0	0	0.0	0.0
12	29.9	13.3	95	38	6.7	0	0.0	0.0
13	33.5	15.2	88	29	0.0	0	0.0	0.0
14	33.7	14.5	89	35	9.6	0	0.0	0.0
15	32.5	16.4	88	39	4.6	0.4	0.0	1.0
16	29.9	18.4	91	60	53.1	2.0	2.4	2.6
17	27.3	18.0	93	72	78.1	8.2	7.0	5.4
18	30.7	20.3	90	60	20.5	13.2	11.40	6.6
19	32.2	19.9	87	56	22.6	15	13.60	11.8
20	32.0	21.5	92	61	4.1	16.8	15.0	14.0
21	30.5	22.9	92	79	38.6	20.2	20.2	19.20
22	30.1	21.2	92	63	74.0	22.0	21.20	20.0
23	31.9	22.7	94	68	16.5	24.4	23.20	21.2
24	33.2	24.7	92	80	67.1	26.0	23.60	22.6
25	33.1	24.3	92	71	111.9	28.4	25.20	24.6
26	31.8	23.9	92	73	53.6	33.6	32.20	28.6
27	32.9	24.6	94	78	79.5	34.6	33.60	32.0
28	32.9	24.8	93	71	29.7	29.4	27.20	26.0

*Mean population from 5 vines

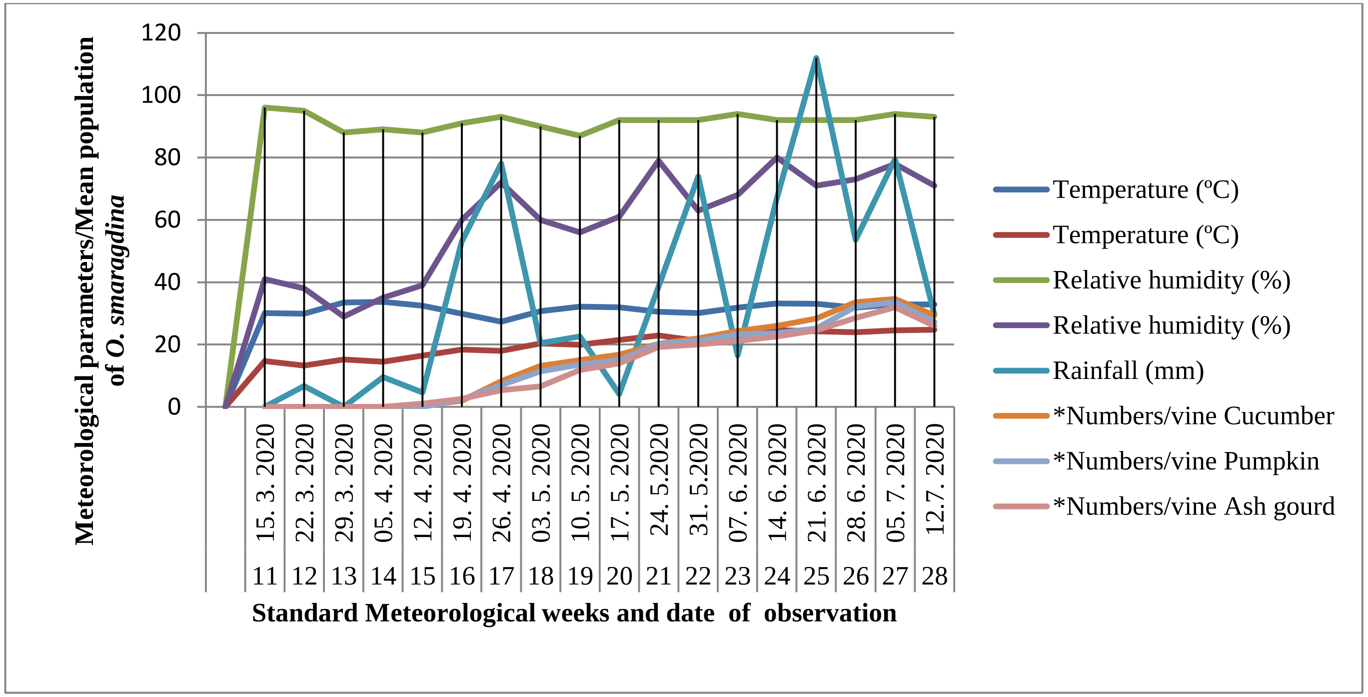


Fig 4.13 Seasonal abundance of *O. smaragdina* recorded during March-July 2020 on different cucurbit crops

a. ***Oecophylla smaragdina***

b. ***Tapinoma* sp.**

c. ***Crematogaster* sp.**

d. ***Polyrhachis* sp.**

e. ***Paederus fuscipes***

Plate 4: Different species of natural enemy for fruit flies

Table 4.22 Seasonal abundance of *O. smaragdina* recorded during March-July 2021 on different cucurbit crops

SMW	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.8	15.4	95	41	2.9	0.00	0.00	0.00
12	33.0	15.8	90	29	0.0	0.00	0.00	0.00
13	32.5	14.6	90	36	32.8	0.00	0.00	0.00
14	32.6	15.8	88	34	14.6	0.00	0.00	0.00
15	34.6	17.7	90	34	15.1	0.00	0.00	0.00
16	32.6	18.8	87	41	17.9	0.00	0.00	0.00
17	34.4	18.8	83	27	0.0	9.60	9.40	9.00
18	32.2	20.5	85	49	31.1	12.20	11.80	11.60
19	30.3	20.6	89	62	19.4	13.80	13.40	13.20
20	31.7	21.6	91	58	3.2	16.60	16.20	16.00
21	35.6	23.9	92	60	31.1	16.60	16.20	16.00
22	33.1	22.9	91	61	17.4	18.00	17.60	17.40
23	33.6	23.6	92	63	39.1	22.40	22.00	21.80
24	33.0	24.8	93	75	19.5	24.20	23.80	23.60
25	33.0	24.5	93	67	43.4	26.80	26.40	26.20
26	33.0	25.0	93	69	37.6	29.80	29.40	29.20
27	33.2	24.7	89	73	19.2	32.00	33.20	31.40
28	32.4	24.7	93	70	105.7	33.80	31.60	33.20

*Mean population from 5 vines

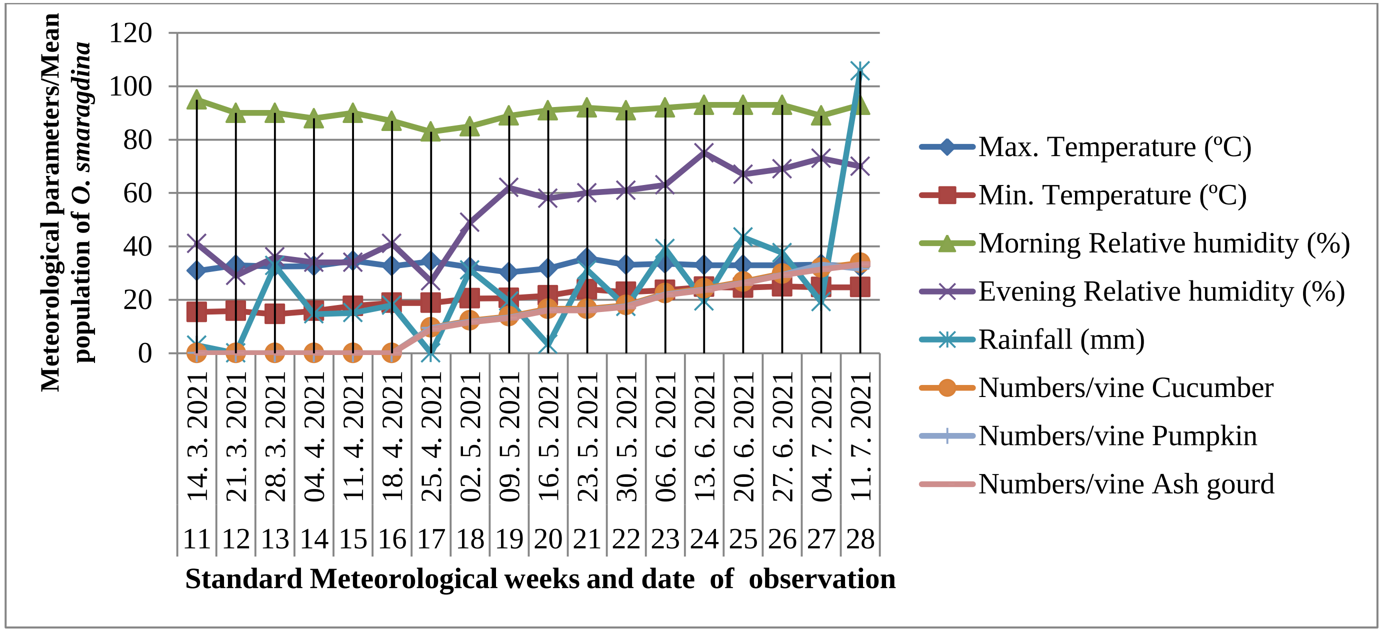


Fig 4.14 Seasonal abundance of *O. smaragdina* recorded during March-July 2021 on different cucurbit crops

Table 4.23 Seasonal abundance of *Tapinoma* sp. recorded during March-July 2020 on different cucurbit crops

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.09	14.7	96	41	0.0	0.00	0.00	0.00
12	29.9	13.3	95	38	6.7	0.00	0.00	0.00
13	33.5	15.2	88	29	0.0	0.00	0.00	0.00
14	33.7	14.5	89	35	9.6	0.00	0.00	0.00
15	32.5	16.4	88	39	4.6	0.00	0.00	0.00
16	29.9	18.4	91	60	53.1	0.00	0.00	0.00
17	27.3	18.0	93	72	78.1	3.80	1.80	2.40
18	30.7	20.3	90	60	20.5	4.60	5.00	5.40
19	32.2	19.9	87	56	22.6	6.60	6.00	6.40
20	32.0	21.5	92	61	4.1	8.80	8.80	8.40
21	30.5	22.9	92	79	38.6	8.40	8.00	8.40
22	30.1	21.2	92	63	74.0	9.20	11.40	12.00
23	31.9	22.7	94	68	16.5	11.20	13.40	14.00
24	33.2	24.7	92	80	67.1	13.60	16.0	16.40
25	33.1	24.3	92	71	111.9	14.40	14.40	15.20
26	31.8	23.9	92	73	53.6	16.60	17.80	18.40
27	32.9	24.6	94	78	79.5	19.60	23.40	25.0
28	32.9	24.8	93	71	29.7	25.40	25.00	26.0

*Mean population from 5 vines

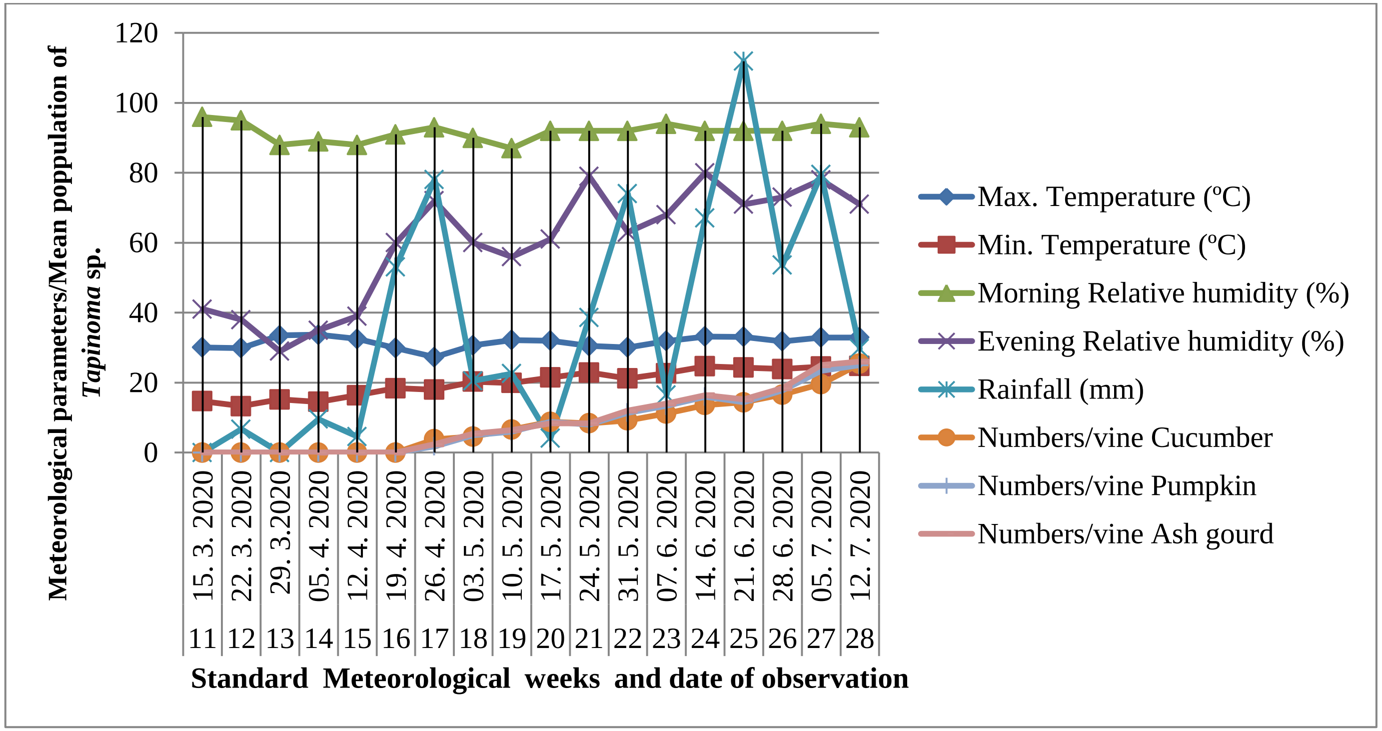


Fig 4.15 Seasonal abundance of *Tapinoma* sp. recorded during March-July 2020 on different cucurbit crops

Table 4.24 Seasonal abundance of *Tapinoma* sp. recorded during March-July 2021 on different cucurbit crop

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.8	15.4	95	41	2.9	0.00	0.00	0.0
12	33.0	15.8	90	29	0.0	0.00	0.00	0.0
13	32.5	14.6	90	36	32.8	0.00	0.00	0.0
14	32.6	15.8	88	34	14.6	0.00	0.00	0.0
15	34.6	17.7	90	34	15.1	0.00	0.00	0.0
16	32.6	18.8	87	41	17.9	0.00	0.00	0.0
17	34.4	18.8	83	27	0.0	2.80	3.00	3.20
18	32.2	20.5	85	49	31.1	5.60	5.80	6.00
19	30.3	20.6	89	62	19.4	5.60	6.80	6.00
20	31.7	21.6	91	58	3.2	6.60	8.80	7.00
21	35.6	23.9	92	60	31.1	8.60	8.80	9.00
22	33.1	22.9	91	61	17.4	12.20	12.40	12.60
23	33.6	23.6	92	63	39.1	14.20	14.40	14.60
24	33.0	24.8	93	75	19.5	16.60	16.80	17.00
25	33.0	24.5	93	67	43.4	15.40	15.60	15.80
26	33/0	25.0	93	69	37.6	18.60	18.80	19.00
27	33.2	24.7	89	73	19.2	25.20	25.40	25.60
28	32.4	24.7	93	70	105.7	26.20	26.40	26.60

*Mean population from 5 vines

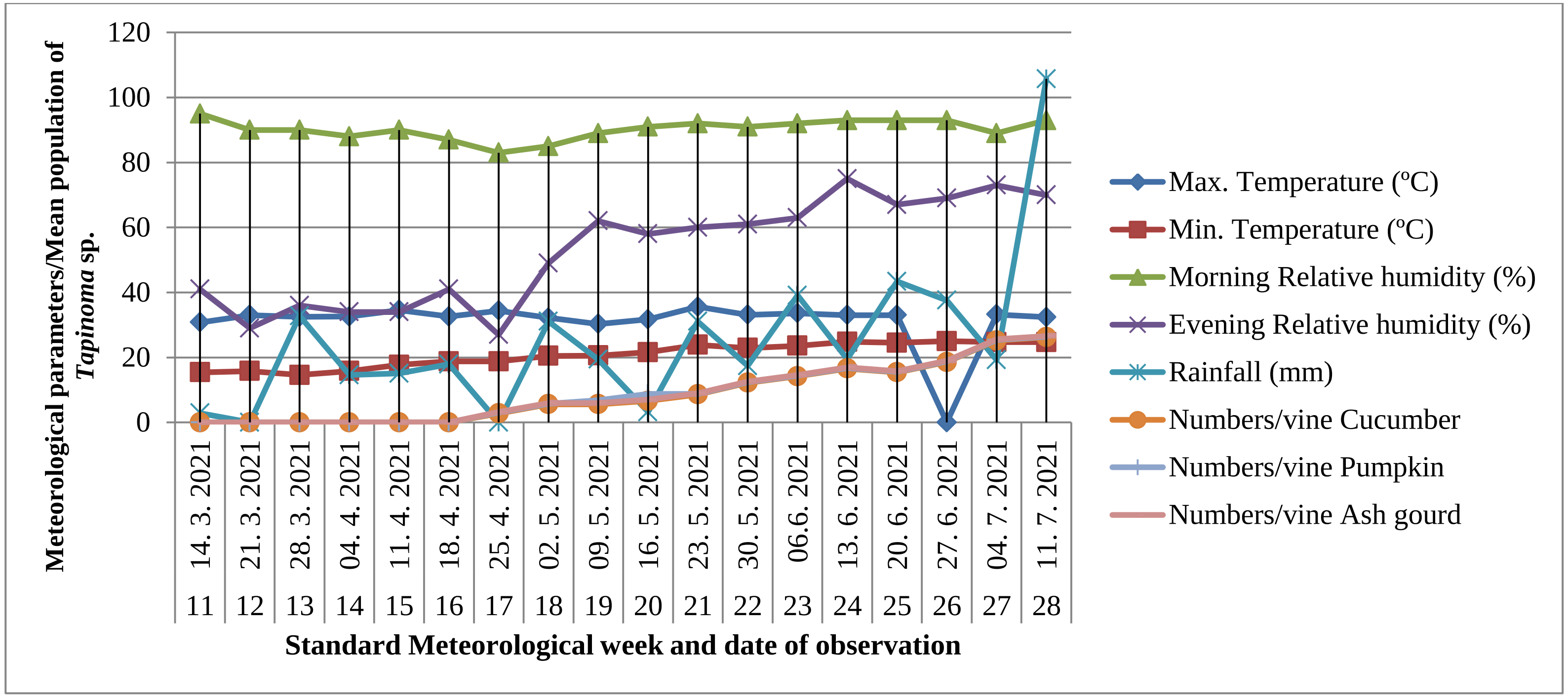


Fig 4.16 Seasonal abundance of *Tapinoma* sp. recorded during March-July 2021 on different cucurbit crops

During 2021 (Table 4.26 and Fig 4.18) zero population of *Polyrhachis* sp. was recorded on 14th March to 18th April (11SMW to 16 SMW) in cucumber, pumpkin and ash gourd field to a maximum on 27th June (26 SMW) with 15.80, 15.60 and 15.40 numbers/vine, respectively. The first incidence was recorded on 25th April (17 SMW) in cucumber, pumpkin and ash gourd field with 5.00, 4.80 and 4.60 numbers/vine, respectively.

4.4.1.4 Seasonal abundance of *Crematogaster* sp. in different cucurbits

The weekly observation of *Crematogaster* sp. indicated that the population fluctuated over the growing season of crops with zero population was recorded during the first two months of observation i.e., from 15th March to 26th April (11 SMW to 17 SMW) 2020 (Table 4.27 and Fig 4.19). It was first appeared on 3rd May (18 SMW) with 2.20, 1.80 and 1.60 numbers/vine, respectively in cucumber, pumpkin and ash gourd. The highest mean population was recorded on 28th June (26 SMW) in cucumber with 8.00 numbers/vine, while in pumpkin and ash gourd field highest mean population was recorded on 5th July (27 SMW) with 7.60 and 7.20 numbers/vine respectively.

The incidence of *Crematogaster* sp. in the year 2021 appeared initially at 17 SMW on 25th April with 0.60, 0.60 and 0.40 numbers/vine respectively in cucumber, pumpkin and ash gourd reaching its peak on 4th July (27 SMW) with mean population of 7.20, 7.00 and 6.80 numbers/vine (Table 4.28 and Fig 4.20).

Table 4.25 Seasonal abundance of *Polyrhachis* sp. recorded during March-July 2020 on different cucurbit crops

SMW	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.09	14.7	96	41	0.0	0	0.0	0.0
12	29.9	13.3	95	38	6.7	0	0.0	0.0
13	33.5	15.2	88	29	0.0	0	0.0	0.0
14	33.7	14.5	89	35	9.6	0.6	0.0	0.0
15	32.5	16.4	88	39	4.6	1.6	0.0	0.0
16	29.9	18.4	91	60	53.1	2.2	0.0	0.0
17	27.3	18.0	93	72	78.1	5.00	5.20	4.60
18	30.7	20.3	90	60	20.5	6.20	5.60	4.80
19	32.2	19.9	87	56	22.6	6.40	5.60	4.60
20	32.0	21.5	92	61	4.1	6.80	6.80	6.60
21	30.5	22.9	92	79	38.6	6.60	6.40	6.00
22	30.1	21.2	92	63	74.0	6.80	6.20	5.80
23	31.9	22.7	94	68	16.5	7.60	7.00	6.40
24	33.2	24.7	92	80	67.1	9.80	9.20	8.40
25	33.1	24.3	92	71	111.9	12.40	13.20	12.40
26	31.8	23.9	92	73	53.6	15.80	16.00	15.40
27	32.9	24.6	94	78	79.5	12.00	12.60	11.60
28	32.9	24.8	93	71	29.7	11.60	10.60	10.0

*Mean population from 5 vines

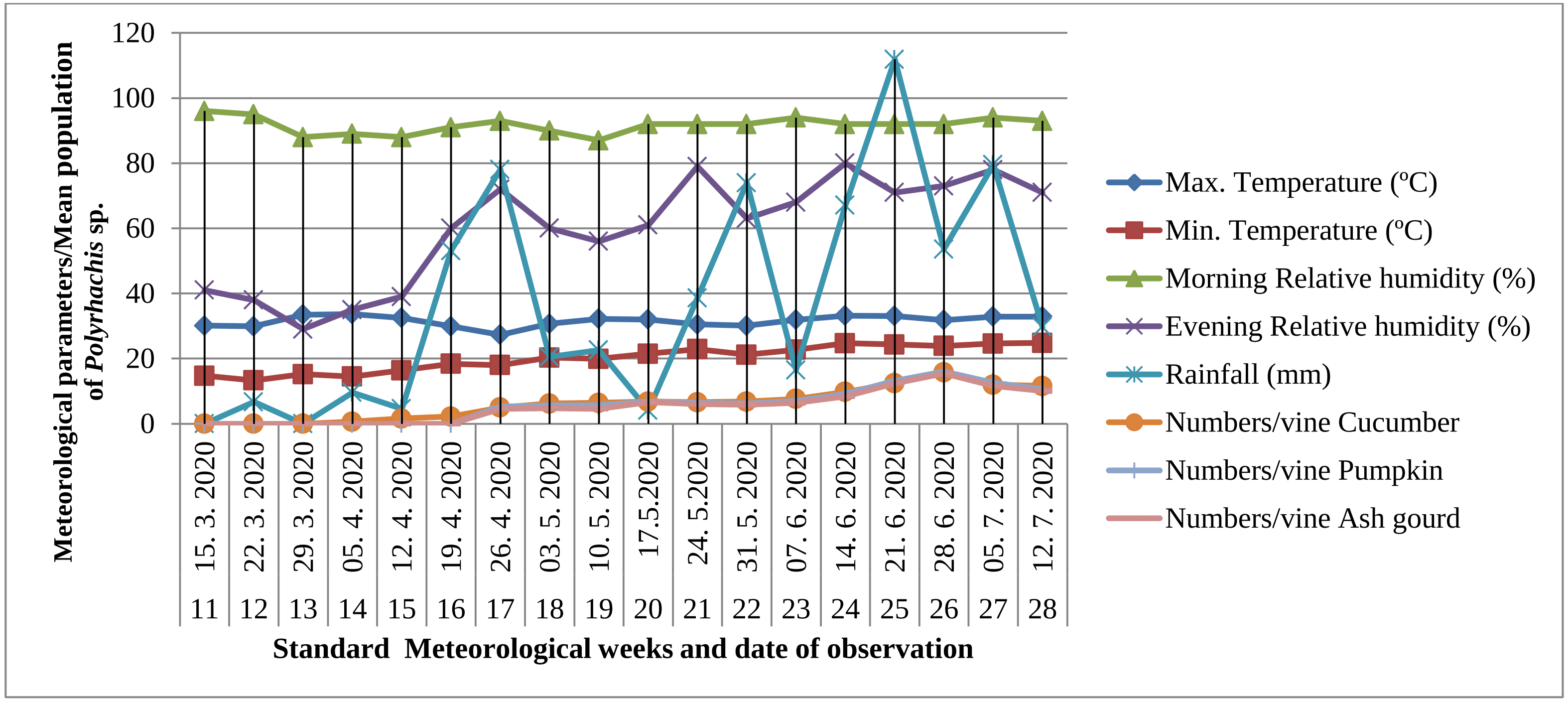


Fig 4.17 Seasonal abundance of *Polyrhachis* sp. recorded during March-July 2020 on different cucurbit crops

Table 4.26 Seasonal abundance of *Polyrhachis* sp. recorded during March-July 2021 on different cucurbit crops

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.8	15.4	95	41	2.9	0.00	0.00	0.0
12	33.0	15.8	90	29	0.0	0.00	0.00	0.0
13	32.5	14.6	90	36	32.8	0.00	0.00	0.0
14	32.6	15.8	88	34	14.6	0.00	0.00	0.0
15	34.6	17.7	90	34	15.1	0.00	0.00	0.0
16	32.6	18.8	87	41	17.9	0.00	0.00	0.0
17	34.4	18.8	83	27	0.0	5.00	4.80	4.60
18	32.2	20.5	85	49	31.1	5.40	5.20	5.00
19	30.3	20.6	89	62	19.4	5.40	5.20	5.00
20	31.7	21.6	91	58	3.2	6.60	6.40	6.20
21	35.6	23.9	92	60	31.1	6.20	6.00	5.80
22	33.1	22.9	91	61	17.4	6.00	5.80	5.60
23	33.6	23.6	92	63	39.1	6.80	6.60	6.40
24	33.0	24.8	93	75	19.5	9.00	8.80	8.60
25	33.0	24.5	93	67	43.4	13.00	12.80	12.60
26	33.0	25.0	93	69	37.6	15.80	15.60	15.40
27	33.2	24.7	89	73	19.2	12.40	12.20	11.80
28	32.4	24.7	93	70	105.7	10.40	10.20	10.00

*Mean population from 5 vines

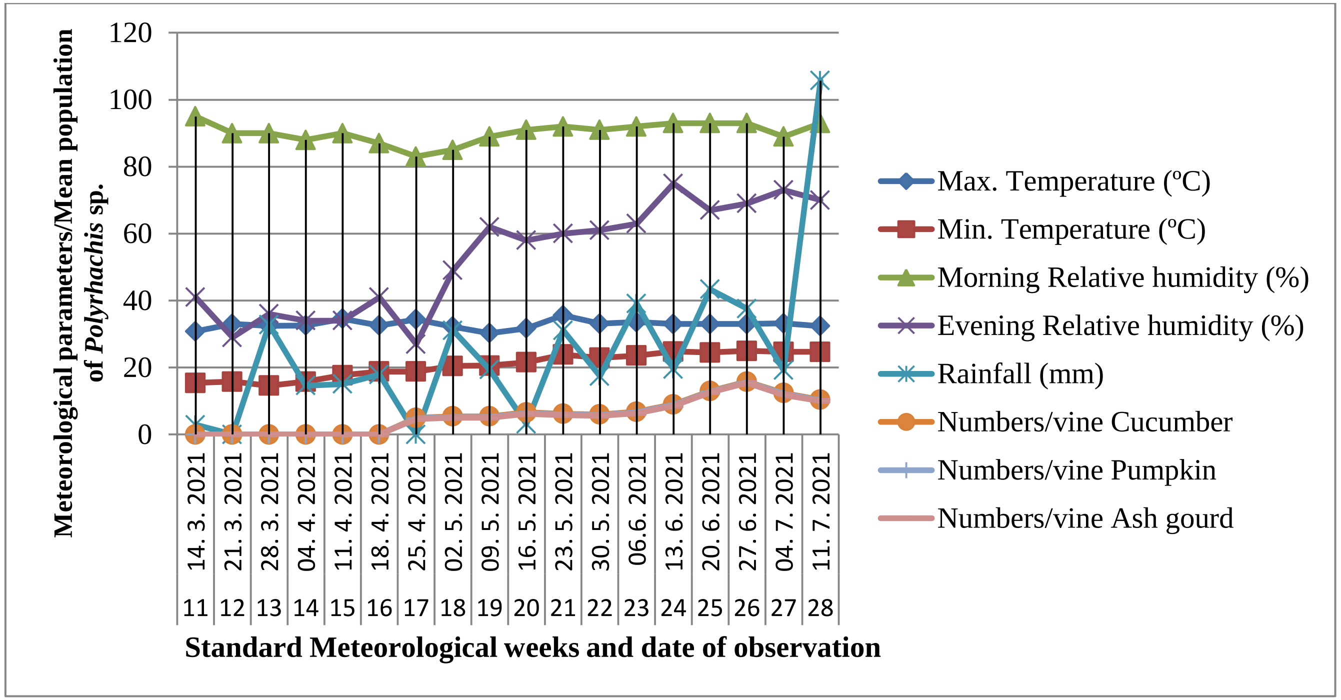


Fig 4.18 Seasonal abundance of *Polyrhachis* sp. recorded during March-July 2021 on different cucurbit crops

4.4.1.5 Seasonal abundance of *Paederus fuscipes* in different cucurbits

Similarly, for *P. fuscipes* their population fluctuated during the study period i.e., 2020 and 2021. In 2020 as depicted in Table 4.29 and Fig 4.21, zero population was recorded on 15th March to 26th April in cucumber field, whereas in pumpkin and ash gourd field till 3rd May. Their population was recorded initially on 3rd May (18 SMW) in cucumber with 0.60 numbers/vine but in pumpkin and ash gourd field first population was recorded on 10th May (19 SMW) with 0.20 and 0.60 numbers/vine respectively, reaching its peak on 12th July (28 SMW) in cucumber and ash gourd having same mean population of 7.20 numbers/vine, while in pumpkin highest population was recorded on 5th July with 6.60 numbers/vine.

In 2021 (Table 4.30 and Fig 4.22) *P. fuscipes* was first observed on 9th May (19 SMW) in cucumber and ash gourd but in pumpkin it was first observed on 16th May (20 SMW). After that it increases gradually and reached its peak on 20th June 2021 (25 SMW) with mean population of 6.40, 6.20 and 6.00 numbers/vine in cucumber, pumpkin and ash gourd, respectively. Lowest mean population was recorded on 9th May (19 SMW) in cucumber and ash gourd with same mean population of 0.20 numbers/vine but in pumpkin it was observed on 16th May (20 SMW) with mean count of 0.40 numbers/vine.

From the present findings it can be illustrated that the population of all the five species of natural enemies *viz*., *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. and *P. fuscipes* were nil in the month of March till 3rd week of April in both the year. The abundance of these five species was highest in the month of June and July. The present findings are in agreement with Francinaldo *et al*. (2017) who reported that a total of 255 specimens of *Paederus* species were collected with highest mean population

Table 4.27 Seasonal abundance of *Crematogaster* sp. recorded during March-July 2020 on different cucurbit crops

SMW	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.09	14.70	96	41	0.00	0.00	0.00	0.00
12	29.9	13.30	95	38	6.70	0.00	0.00	0.00
13	33.5	15.20	88	29	0.00	0.00	0.00	0.00
14	33.7	14.50	89	35	9.60	0.00	0.00	0.00
15	32.5	16.40	88	39	4.60	0.00	0.00	0.00
16	29.9	18.400	91	60	53.10	0.00	0.00	0.00
17	27.3	18.00	93	72	78.10	0.00	0.00	0.00
18	30.7	20.30	90	60	20.50	2.20	1.80	1.60
19	32.2	19.90	87	56	22.60	2.20	1.80	1.80
20	32.0	21.50	92	61	4.10	2.40	2.20	1.80
21	30.5	22.90	92	79	38.60	3.00	2.60	2.20
22	30.1	21.20	92	63	74.00	4.80	4.20	3.80
23	31.9	22.70	94	68	16.50	5.00	4.60	4.20
24	33.2	24.70	92	80	67.10	5.20	5.00	4.60
25	33.1	24.30	92	71	111.90	6.00	5.40	5.00
26	31.8	23.90	92	73	53.60	8.00	7.20	6.80
27	32.9	24.60	94	78	79.50	7.80	7.60	7.20
28	32.9	24.80	93	71	29.70	6.00	5.40	4.80

*Mean population from 5 vines

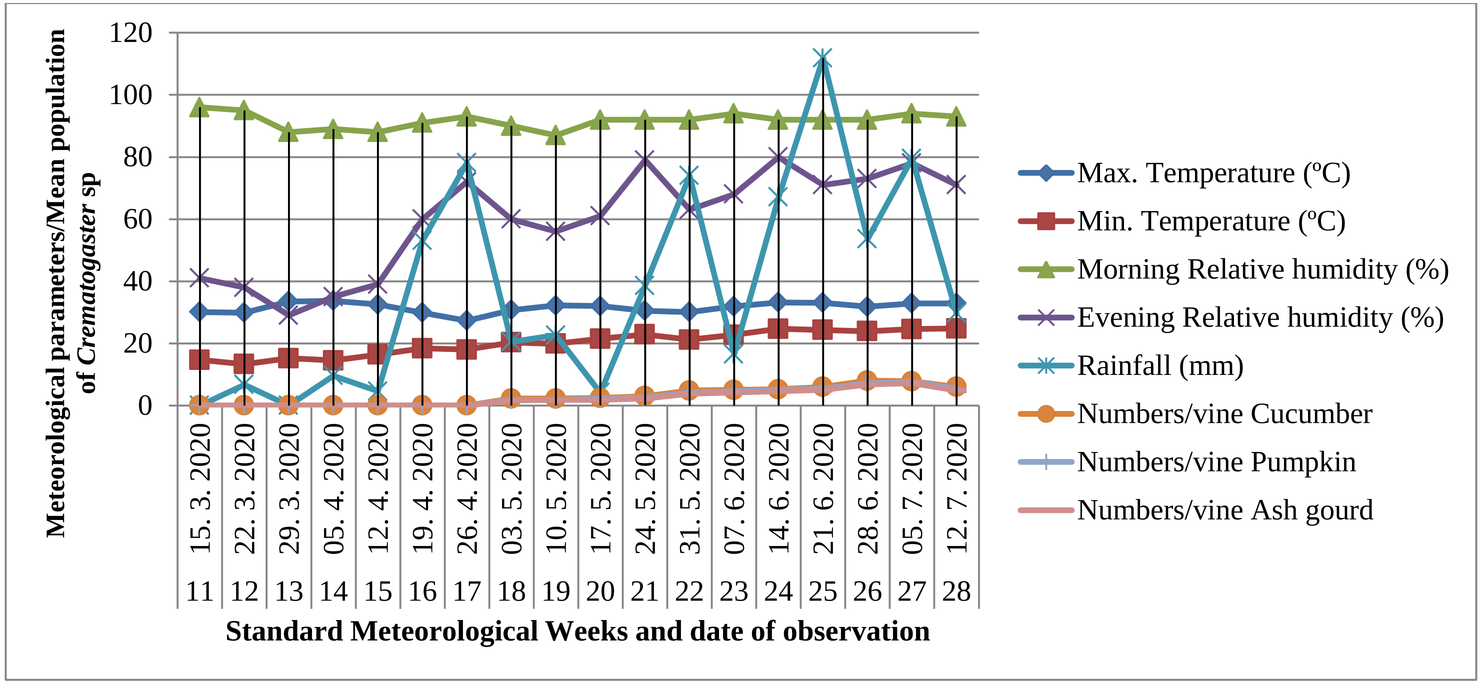


Fig 4.19 Seasonal abundance of *Crematogaster* sp. recorded during March-July 2020 on different cucurbit crops

Table 4.28 Seasonal abundance of *Crematogaster* sp. recorded during March-July 2021 on different cucurbit crops

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/Vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.8	15.4	95	41	2.9	0.00	0.00	0.0
12	33.0	15.8	90	29	0.0	0.00	0.00	0.0
13	32.5	14.6	90	36	32.8	0.00	0.00	0.0
14	32.6	15.8	88	34	14.6	0.00	0.00	0.0
15	34.6	17.7	90	34	15.1	0.00	0.00	0.0
16	32.6	18.8	87	41	17.9	0.00	0.00	0.0
17	34.4	18.8	83	27	0.0	0.60	0.60	0.40
18	32.2	20.5	85	49	31.1	1.40	1.20	1.00
19	30.3	20.6	89	62	19.4	1.40	1.20	1.00
20	31.7	21.6	91	58	3.2	1.80	1.60	1.40
21	35.6	23.9	92	60	31.1	2.20	2.00	1.80
22	33.1	22.9	91	61	17.4	3.80	3.60	3.40
23	33.6	23.6	92	63	39.1	4.20	4.00	3.80
24	33.0	24.8	93	75	19.5	4.60	4.40	4.20
25	33.0	24.5	93	67	43.4	5.00	4.80	4.60
26	33/0	25.0	93	69	37.6	6.80	6.60	6.40
27	33.2	24.7	89	73	19.2	7.20	7.00	6.80
28	32.4	24.7	93	70	105.7	5.00	4.80	4.60

*Mean population from 5 vines

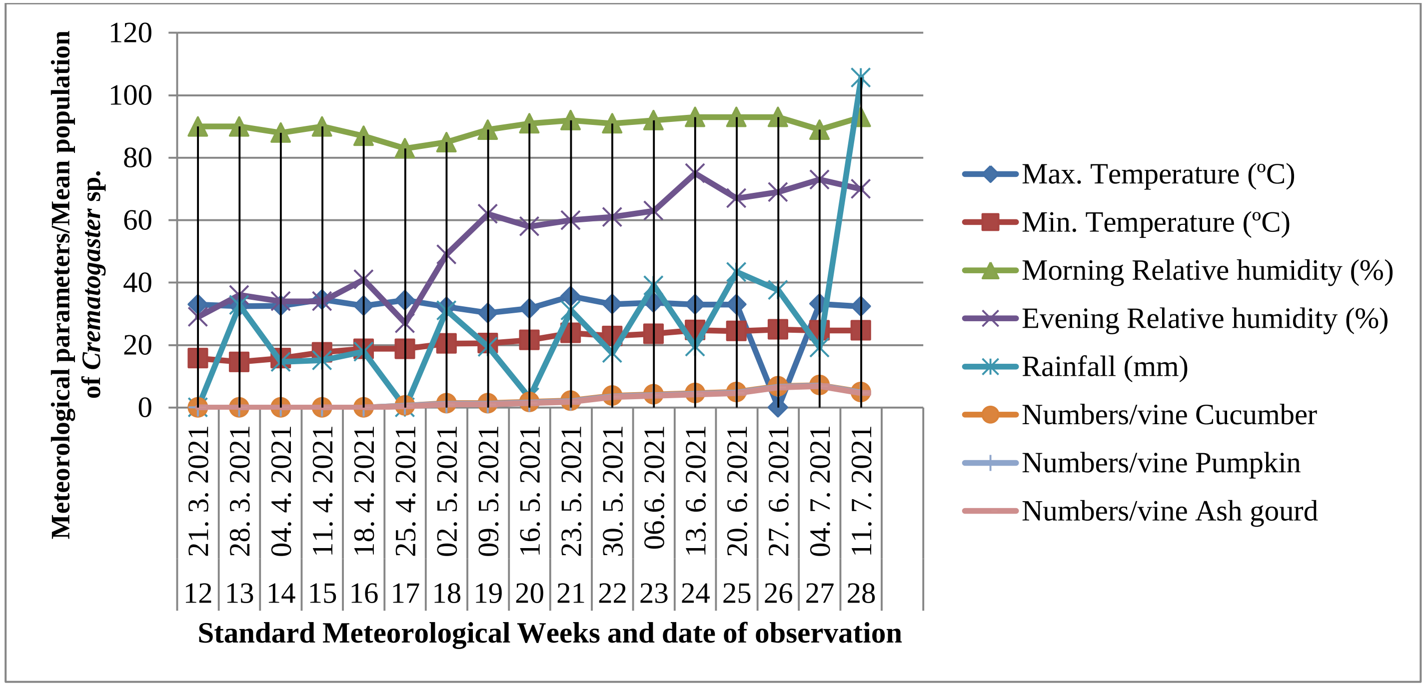


Fig 4.20 Seasonal abundance of *Crematogaster* sp. recorded during March-July 2021 on different cucurbit crops

Table 4.29 Seasonal abundance of *P. fuscipes* recorded during March-July 2020 on different cucurbit crops

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
	Max.	Min.	Morning	Evening		Cucumber	Pumpkin	Ash gourd
11	30.09	14.7	96	41	0.0	0	0.0	0.0
12	29.9	13.3	95	38	6.7	0	0.0	0.0
13	33.5	15.2	88	29	0.0	0	0.0	0.0
14	33.7	14.5	89	35	9.6	0.00	0.0	0.0
15	32.5	16.4	88	39	4.6	0.00	0.0	0.0
16	29.9	18.4	91	60	53.1	0.00	0.0	0.0
17	27.3	18.0	93	72	78.1	0.00	0.00	0.00
18	30.7	20.3	90	60	20.5	0.60	0.00	0.00
19	32.2	19.9	87	56	22.6	0.80	0.20	0.60
20	32.0	21.5	92	61	4.1	2.40	0.40	0.60
21	30.5	22.9	92	79	38.6	2.80	2.20	2.40
22	30.1	21.2	92	63	74.0	2.60	2.20	2.40
23	31.9	22.7	94	68	16.5	3.60	3.00	3.40
24	33.2	24.7	92	80	67.1	4.40	3.80	4.20
25	33.1	24.3	92	71	111.9	3.00	2.60	3.20
26	31.8	23.9	92	73	53.6	4.40	4.20	4.60
27	32.9	24.6	94	78	79.5	4.40	6.60	4.40
28	32.9	24.8	93	71	29.7	7.20	4.00	7.20

*Mean population from 5 vines

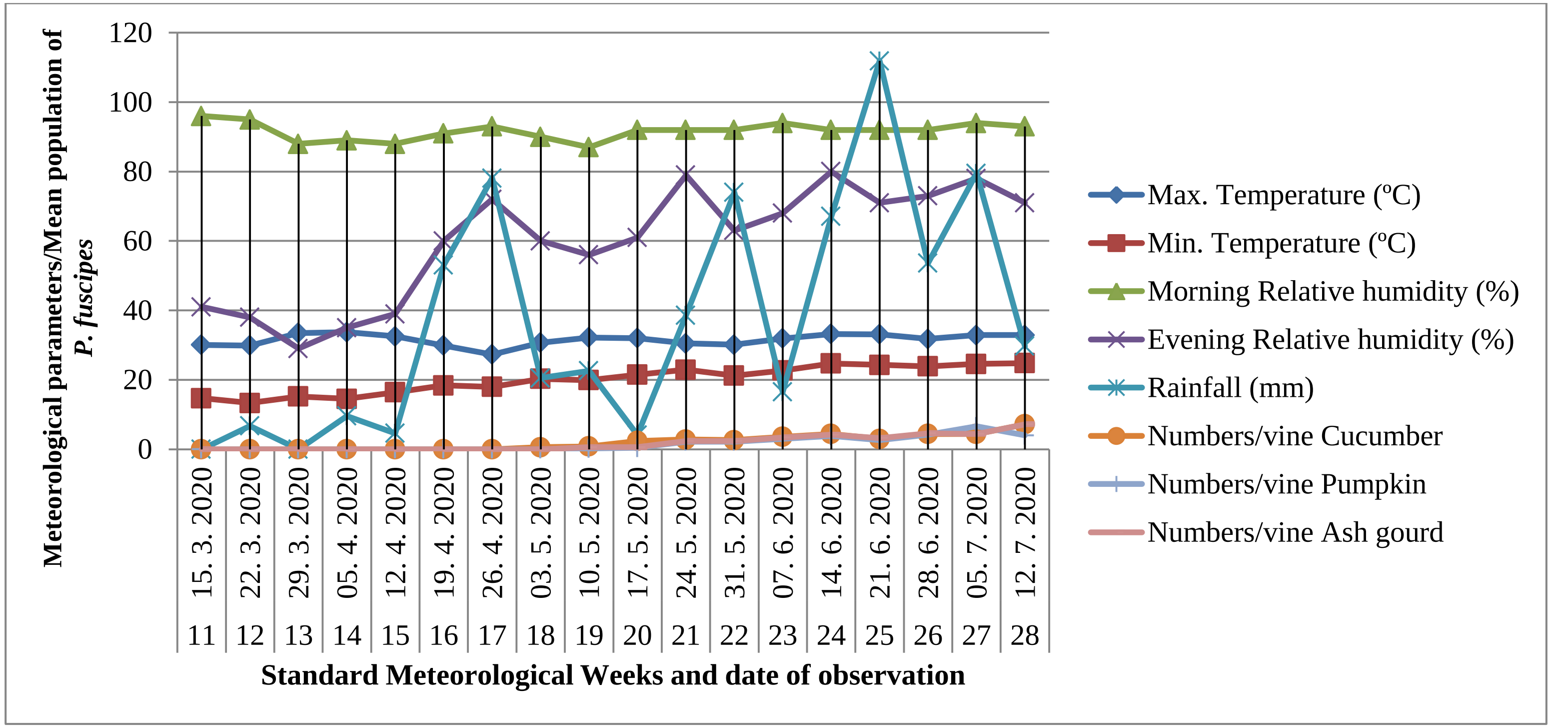


Fig 4.21 Seasonal abundance of *P. fuscipes* recorded during March-July 2020 on different cucurbit crops

Table 4.30 Seasonal abundance of *P. fuscipes* recorded during March-July 2021 on different cucurbit crops

SMW	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)	*Numbers/vine		
						Cucumber	Pumpkin	Ash gourd
	Max.	Min.	Morning	Evening				
11	30.8	15.4	95	41	2.9	0.00	0.00	0.0
12	33.0	15.8	90	29	0.0	0.00	0.00	0.0
13	32.5	14.6	90	36	32.8	0.00	0.00	0.0
14	32.6	15.8	88	34	14.6	0.00	0.00	0.0
15	34.6	17.7	90	34	15.1	0.00	0.00	0.0
16	32.6	18.8	87	41	17.9	0.00	0.00	0.0
17	34.4	18.8	83	27	0.0	0.00	0.00	0.00
18	32.2	20.5	85	49	31.1	0.00	0.00	0.00
19	30.3	20.6	89	62	19.4	0.20	0.00	0.20
20	31.7	21.6	91	58	3.2	0.40	0.40	0.40
21	35.6	23.9	92	60	31.1	2.00	1.80	1.60
22	33.1	22.9	91	61	17.4	2.00	1.80	1.60
23	33.6	23.6	92	63	39.1	2.80	2.60	2.40
24	33.0	24.8	93	75	19.5	3.60	3.40	3.20
25	33.0	24.5	93	67	43.4	6.40	6.20	6.00
26	33/0	25.0	93	69	37.6	4.00	3.80	3.60
27	33.2	24.7	89	73	19.2	3.80	3.60	3.40
28	32.4	24.7	93	70	105.7	2.40	2.20	2.00

*Mean population from 5 vines

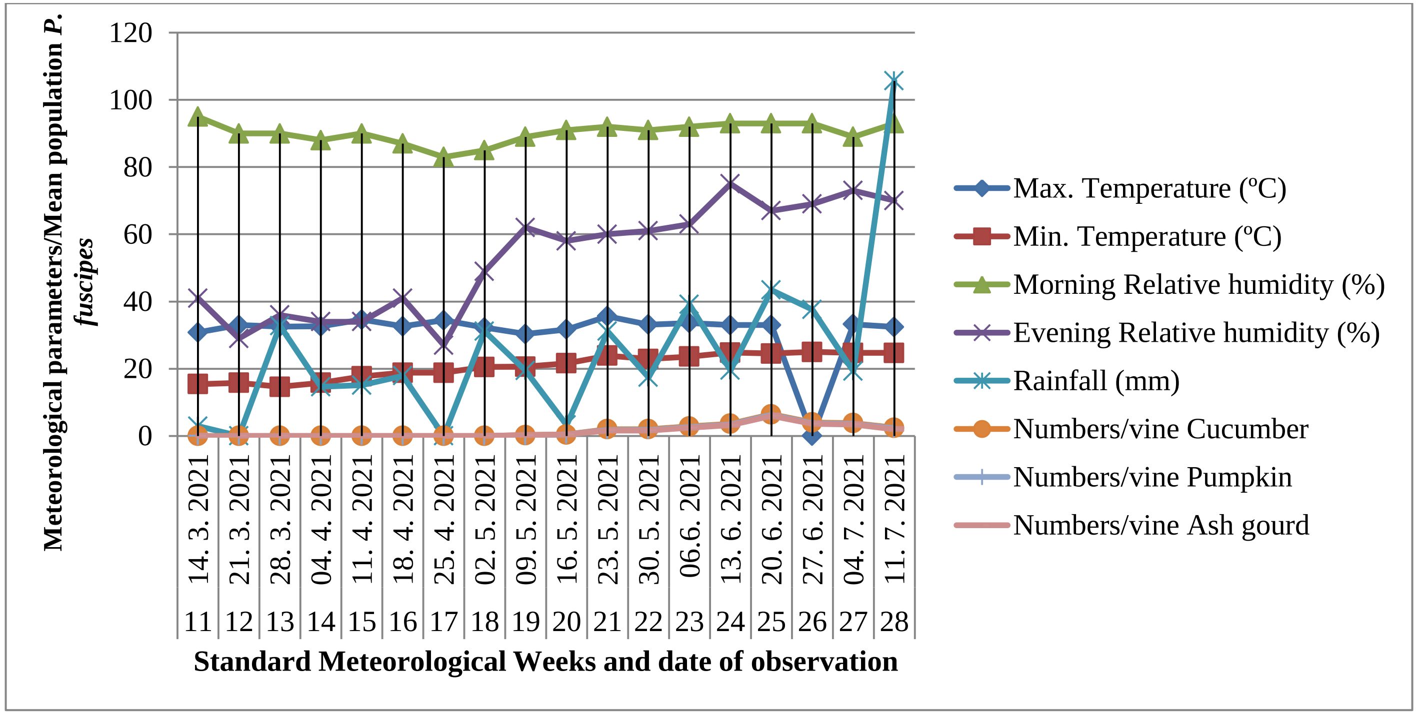


Fig 4.22 Seasonal abundance of *P. fuscipes* recorded during March-July 2021 on different cucurbit crops

were recorded mostly during June followed by July. Balog *et al*. (2008) also reported that the abundance of rove beetle was highest in June, July and August, while it was lowest in spring and autumn.

Though some workers had carried out their work on the seasonal abundance of *P. fuscipes*, no such information was available on the other four species i.e. *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp. and *Crematogaster* sp. Hence, the present findings on the above mentioned other four species of natural enemy could not be discussed.

4.4.2 Correlation between different species of natural enemies of fruit flies with abiotic factors

The following subheadings discussed the correlation study conducted in two seasons, 2020 and 2021, in three different cucurbit crops, including cucumber, pumpkin and ash gourd between various species of natural enemies and abiotic factors like maximum and minimum temperatures, relative humidity and rainfall.

4.4.2.1 Correlation between different species of natural enemies of fruit flies with abiotic factors in cucumber

The relationship between *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp. and *Crematogaster* sp. with abiotic variables for the year 2020 (Table 4.31) had shown to have significant positive relation with minimum temperature, evening relative humidity and rainfall, while in case of *P. fuscipes*, their relation with abiotic factors were found to have significant positive association

Table 4.31 Correlation coefficient (r) of fruit fly natural enemies with abiotic factors during March- July 2020 on cucumber

Natural enemies	Pearson's correlation coefficient (r)				
	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
O. smaragdina	0.155	0.972**	0.198	0.904**	0.614**
***Tapinoma* sp.**	0.230	0.950**	0.251	0.854**	0.522*
***Polyrhachis* sp.**	0.174	0.952**	0.084	0.891**	0.643**
***Crematogaster* sp.**	0.342	0.936**	0.208	0.778**	0.514*
P. fuscipes	0.360	0.939**	0.271	0.782**	0.431

Note: * = Significant at 5% level of significance

** = Significant at 1% level of significance

Table 4.32 Correlation coefficient (r) of fruit fly natural enemies with abiotic factors during March- July 2021 on cucumber

Natural enemies	**Pearson's correlation coefficient(r)**				
	Temperature (ºC)		**Relative humidity (%)**		**Rainfall (mm)**
	Max.	**Min.**	**Morning**	**Evening**	
O. smaragdina	0.101	0.947**	0.244	0.880**	0.490*
***Tapinoma* sp.**	0.109	0.953**	0.320	0.912**	0.557*
***Polyrhachis* sp.**	0.105	0.930**	0.208	0.848**	0.435
***Crematogaster* sp.**	0.120	0.955**	0.341	0.917**	0.485*
P. fuscipes	0.221	0.899**	0.553*	0.884**	0.478*

Note: * = Significant at 5% level of significance

** = Significant at 1% level of significance

with minimum temperature (0.939**) and evening relative humidity (0.782**) only.

According to 2021 correlation analysis (Table 4.32) all the four species of natural enemy viz., *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp. and *Crematogaster* sp. exhibited a significant positive connection with minimum temperature, evening relative humidity and rainfall. However, *P. fuscipes* exhibited a significant positive relation with all the abiotic factors studied except maximum temperature.

4.4.2.2 Correlation between different species of natural enemies of fruit flies with abiotic factors in Pumpkin

The correlation study during 2020 between the different species of natural enemies and abiotic factors such as maximum and minimum temperature, relative humidity and rainfall are expressed in Table 4.33. Data thus recorded shows that the relationship between the population of *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. and *P. fuscipes* with abiotic factors revealed a significant positive correlation with minimum temperature, evening relative humidity and rainfall.

In 2021, the correlation study (Table 4.34) between the different species of natural enemies and abiotic factors revealed that the population of *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp. and *Crematogaster* sp. had significant positive relation with minimum temperature, evening relative humidity and rainfall. But for *P. fuscipes* their populations were found to have significant positive correlation with minimum temperature, morning relative humidity, evening relative humidity and rainfall.

Table 4.33 Correlation coefficient (r) of fruit fly natural enemies with abiotic factors during March- July 2020 on Pumpkin

Natural enemies	Pearson's correlation coefficient (r)				
	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
O. smaragdina	0.140	0.968**	0.223	0.909**	0.617**
***Tapinoma* sp.**	0.279	0.954**	0.258	0.835**	0.537*
***Polyrhachis* sp.**	0.140	0.927**	0.208	0.872**	0.618**
***Crematogaster* sp.**	0.350	0.938**	0.222	0.781**	0.519*
P. fuscipes	0.358	0.887**	0.346	0.769**	0.553*

Note: * = Significant at 5% level of significance

** = Significant at 1% level of significance

Table 4.34 Correlation coefficient (r) of fruit fly natural enemies with abiotic factors during March- July 2021 on Pumpkin

Natural enemies	Pearson's correlation coefficient(r)				
	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
O. smaragdina	0.104	0.947**	0.241	0.882**	0.478*
***Tapinoma* sp.**	0.087	0.952**	0.314	0.915**	0.542**
***Polyrhachis* sp.**	0.105	0.930**	0.212	0.849**	0.437*
***Crematogaster* sp.**	0.128	0.882**	0.407	0.871**	0.488*
P. fuscipes	0.269	0.881**	0.552*	0.850**	0.471*

Note: * = Significant at 5% level of significance

** = Significant at 1% level of significance

4.4.2.3 Correlation between different species of natural enemies of fruit flies with abiotic factors in Ash gourd

In 2020 (Table 4.35) the population of *O. smaragdina* was shown to have a significant positive correlation with minimum temperature (0.978**), evening relative humidity (0.902**) and rainfall (0.623**). Similarly the other four species of natural enemy i.e *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. and *P. fuscipes* also exhibited a significant positive relationship with minimum temperature, evening relative humidity and rainfall. According to the 2021 correlation analysis (Table 4.36), *O. smaragdina* and other three species of natural enemy *viz*., *Tapinoma* sp., *Crematogaster* sp. and *P. fuscipes* had a significant positive relationship with minimum temperature, evening relative humidity and rainfall. Whereas, the population of *Polyrhachis* sp. association with minimum temperature, morning relative humidity and evening relative humidity were found significantly positive.

As per the present investigation, abiotic factors such as minimum temperature, evening relative humidity influences the abundance of all the above five mentioned different species of natural enemies. Seasons also affect their population, e.g. by rain fall. Months receiving more rainfall (June- July) normally show maximum abundances. Months with less rainfall or with no rainfall indicated lower abundances, i.e. in March, April and May. There was normally a maximum abundance and diversity during June-July. This finding is an agreement with the findings of Nasir *et al*. (2012) who reported that the

Table 4.35 Correlation coefficient (r) of fruit fly natural enemies with abiotic factors during March- July 2020 on Ash gourd

Natural enemies	Pearson's correlation coefficient (r)				
	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
O. smaragdina	0.187	0.978**	0.205	0.902**	0.623**
***Tapinoma* sp.**	0.264	0.953**	0.260	0.841**	0.551*
***Polyrhachis* sp.**	0.145	0.930**	0.219	0.871**	0.618**
***Crematogaster* sp.**	0.354	0.934**	0.216	0.777**	0.527*
P. fuscipes	0.371	0.899**	0.301	0.756**	0.499*

Note: * = Significant at 5% level of significance

** = Significant at 1% level of significance

Table 4.36 Correlation coefficient (r) of fruit fly natural enemies with abiotic factors during March- July 2021 on Ash gourd

Natural enemies	Pearson's correlation coefficient (r)				
	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
O. smaragdina	0.100	0.948**	0.250	0.883**	0.494*
***Tapinoma* sp.**	0.110	0.953**	0.310	0.909**	0.552**
***Polyrhachis* sp.**	0.105	0.931**	0.216	0.850**	0.440
***Crematogaster* sp.**	0.127	0.951**	0.367	0.917**	0.494*
P. fuscipes	0.205	0.894**	0.550*	0.882**	0.468*

Note: * = Significant at 5% level of significance

** = Significant at 1% level of significance

population is also impacted by the seasons, for example by rainfall. The highest abundances often occur in July and August, the months with the most rainfall.

Lower abundance was recorded in those months where there was less rain or no rain at all, i.e., during May, September and October. The highest population was recorded between July and August month. These findings are in agreement with Koller *et al*., 2002 and Nikbakhtzadeh and Tirgari, 2008.

4.5 Extent of damage caused by fruit fly on different cucurbits

In the year 2020, fruit damage (Table 4.37 and Fig 4.23) was noticed throughout the study period i.e., May to July 2020. The maximum fruit damage in cucumber crop was observed on 24 SMW with 72.62%, followed by 23 SMW with 66.50% and lowest (37.76%) damage was found on 26 SMW. Similarly in pumpkin, maximum fruit damage was observed on 24 SMW with 60.37% followed with 54.47% on 23SMW and minimum damage was recorded on 26 SMW with 29.83%. In case of ash gourd maximum damage of 51.29% was observed on 23 SMW followed with 39.09% on 22 SMW and lowest per cent damage was recorded on 20 SMW with 16.63.

In the year 2021 (Table 4.38 and Fig 4.23) the maximum fruit damage in cucumber crop was observed on 24 SMW with 76.81%, followed by 23 SMW with 63.50% and lowest (37.03%) damage was found on 28 SMW. In pumpkin also, maximum fruit damage was observed on 24 SMW with 57.87% followed with 52.00% on 23 SMW and minimum damage was recorded on 26 SMW with 22.57%. In case of ash gourd maximum damage of 56.89% was observed on 23 SMW followed with 34.60% in 22 SMW and lowest per cent damage was recorded on 20 SMW with 18.52.

The average damage percentage from Table 4.37 and Table 4.38, revealed that among the three different cucurbits highest damage per cent was recorded in cucumber with 42.42 and 43.42 followed by pumpkin with 30.76 and 29.24 and the lowest damage percentage was observed in ash gourd with 16.29 and 16.82 in 2020 and 2021, respectively.

From the results, it is evident that out of the three different cucurbits, cucumber was mostly preffered by fruit flies than the other two crops as the highest damaged percentage was recorded from cucumber. In addition to this it was also observed that all the cucurbit crops (cucumber, pumpkin and ash gourd) exhibited highest damaged percentage in the month of June where maximum number of fruit flies abundance occurred.

Results of this finding are in agreement with the findings of Kumar *et al.* (2006) who reported 73.83% damage due to melon fruit fly infestation from cucumber crop. Losses of 100% of cucurbit crop harvests have been frequently observed from fruit fly infestation (Philippe *et al*., 2010), Gupta and Verma (1992) reported that about 80% damage due to fruit fly infestation on cucumber and bottle gourd, 60% on bitter gourd and 50% on sponge gourd in Himachal Pradesh. The present finding are in partial agreement with Pradhan (1976) who reported that the yield losses caused by cucurbit fruit fly in different cucurbits were 28.7- 59.2, 24.7-40.0, 27.3-49.3, 19.4-22.1 and 0-26.2% in pumpkin, bitter gourd, bottle gourd, cucumber and sponge gourd, respectively in Nepal. In contrary with the present finding Kabir *et al*. (1991) reported that minimum yield losses due to fruit fly infestation was observed in cucumber (19.19%) and maximum in sweet gourd (69.96%).

Table 4.37 Extent of damage (%) caused by fruit flies on different cucurbits during 2020

SMW	Extent of damage (%)		
	Cucumber	**Pumpkin**	**Ash gourd**
18	44.31	0.00	0.00
19	37.99	30.57	0.00
20	40.55	31.57	16.63
21	50.32	42.59	28.76
22	57.68	49.12	39.09
23	66.50	54.47	51.29
24	72.62	60.37	25.16
25	58.85	39.81	18.33
26	37.76	29.83	0.00
27	0.00	0.00	0.00
28	0.00	0.00	0.00
Average damage (%)	42.42	30.76	16.29

Table 4.38 Extent of damage (%) caused by fruit flies on different cucurbits during 2021

SMW	Extent of damage (%)		
	Cucumber	Pumpkin	Ash gourd
18	0.00	0.00	0.00
19	37.03	27.07	0.00
20	43.92	33.24	18.52
21	52.47	36.95	29.17
22	61.53	48.99	34.60
23	63.50	52.00	56.89
24	76.81	57.87	25.88
25	58.14	42.92	19.99
26	43.25	22.57	0.00
27	41.06	0.00	0.00
28	0.00	0.00	0.00
Average damage (%)	43.43	29.24	16.82

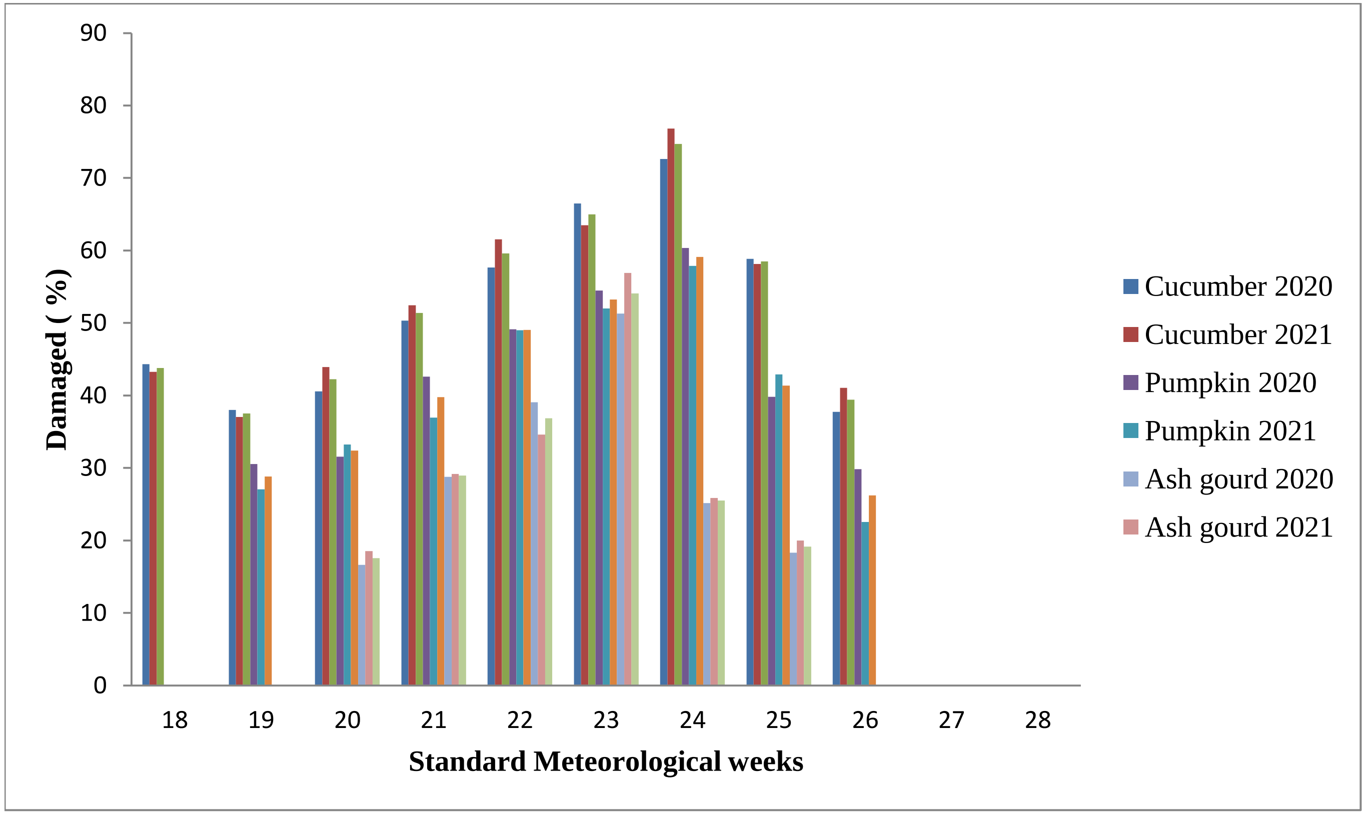


Fig 4.23 Extent of damage (%) caused by fruit flies on different cucurbits during 2020 and 2021

4.6 Evaluation of the effectiveness of different attractants against major fruit fly complex

4.6.1 Effect of different attractants on *B. dorsalis* adult in different cucurbits

The data pertaining to trapping of *B. dorsalis* during May to July 2020 and 2021 with respect to effect of treatments are depicted in Table 4.39 and Fig 4.24.

Significant differences among different treatments for trapping of *B. dorsalis* adult were observed.

During 2020, methyl eugenol (T_1) attracted mean population of 184.00, 150.00 and 130.40 flies/trap from cucumber, pumpkin and ash gourd, respectively which was found significantly superior over all other treatments. The second best treatment was found in cue lure (T_2) which attracted 107.20, 96.40 and 91.00 flies/trap from cucumber, pumpkin and ash gourd, respectively. The least number of flies trapped was found in Banana poison bait (T_3) which attracted 25.00, 24.60 and 22.00 flies/trap from cucumber, pumpkin and ash gourd, respectively.

Similarly, in 2021, methyl eugenol (T_1) had attracted 177.80, 125.60 and 129.80 flies/trap from cucumber, pumpkin and ash gourd, respectively which was found significantly superior over all the other treatments followed by cue lure (T_2) which attracted 66.80, 93.40 and 81.60 flies/trap from cucumber, pumpkin and ash gourd, respectively. The minimum number of flies trapped was observed in banana poison bait (T_3) which attracted 26.60, 30.20 and 22.60 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

Pooled data also revealed that T_1 (methyl eugenol) was observed to attract maximum mean population of 180.90, 137.80 and 130.10 flies/trap from cucumber, pumpkin and ash gourd, respectively. It was followed by T_2 i.e. cule lure which attracted 87.00, 94.90 and 86.30 flies/trap from cucumber, pumpkin and ash gourd field, respectively. The Lowest mean population was attracted in T_3 (banana poison bait) with 25.80, 27.40 and 22.30 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

4.6.2 Effect of different attractants on *Z. tau* adult in different cucurbits

The observations on mean population of *Z. tau* flies attracted in different attractants were recorded from starting of flowering till harvesting during 2020 and 2021. The details are presented in Table 4.40 and Fig 4.25.

In 2020, *Z. tau* was found to be significantly attracted more towards cue lure (T_2) with mean population of 52.20, 51.20 and 60.40 flies/trap from cucumber, pumpkin and ash gourd field, respectively followed by banana poison bait (T_3) which attracted 32.60, 28.40 and 28.60 flies/trap from cucumber, pumpkin and ash gourd field, respectively. The Lowest mean population

Table 4.39 Effect of different attractants against *B. dorsalis* during 2020 and 2021

Attractants	2020 (Mean population count/trap)			2021 (Mean population count/trap)			Pooled (Mean population count/ trap)		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
Methyl eugenol: (T_1)	184.00 (13.60)	150 (12.28)	130.4 (11.46)	177.8 (13.37)	125.60 (11.25)	129.80 (11.43)	180.90 (13.49)	137.80 (11.78)	130.10 (11.41)
Cue lure: (T_2)	107.20 (10.40)	96.40 (9.87)	91.00 (9.59)	66.80 (8.23)	93.40 (9.71)	81.60 (9.08)	87.00 (9.38)	94.90 (9.79)	86.30 (9.28)
BPB: (T_3)	25.00 (5.09)	24.6 (5.05)	22.00 (4.79)	26.60 (5.25)	30.20 (5.62)	22.60 (4.85)	25.80 (5.17)	27.40 (5.35)	22.30 (4.72)
Untreated (Control): (T_0)	4.60 (2.32)	4.00 (2.16)	3.20 (1.95)	1.20 (1.44)	5.00 (2.44)	3.00 (1.94)	2.90 (1.97)	4.50 (2.31)	3.10 (1.65)
SEm±	0.145	0.192	0.17	0.111	0.103	0.134	0.082	0.128	0.149
CD (P= 0.05)	0.453	0.599	0.595	0.347	0.321	0.416	0.255	0.398	0.465

***Figures in parenthesis are square root transformation value**

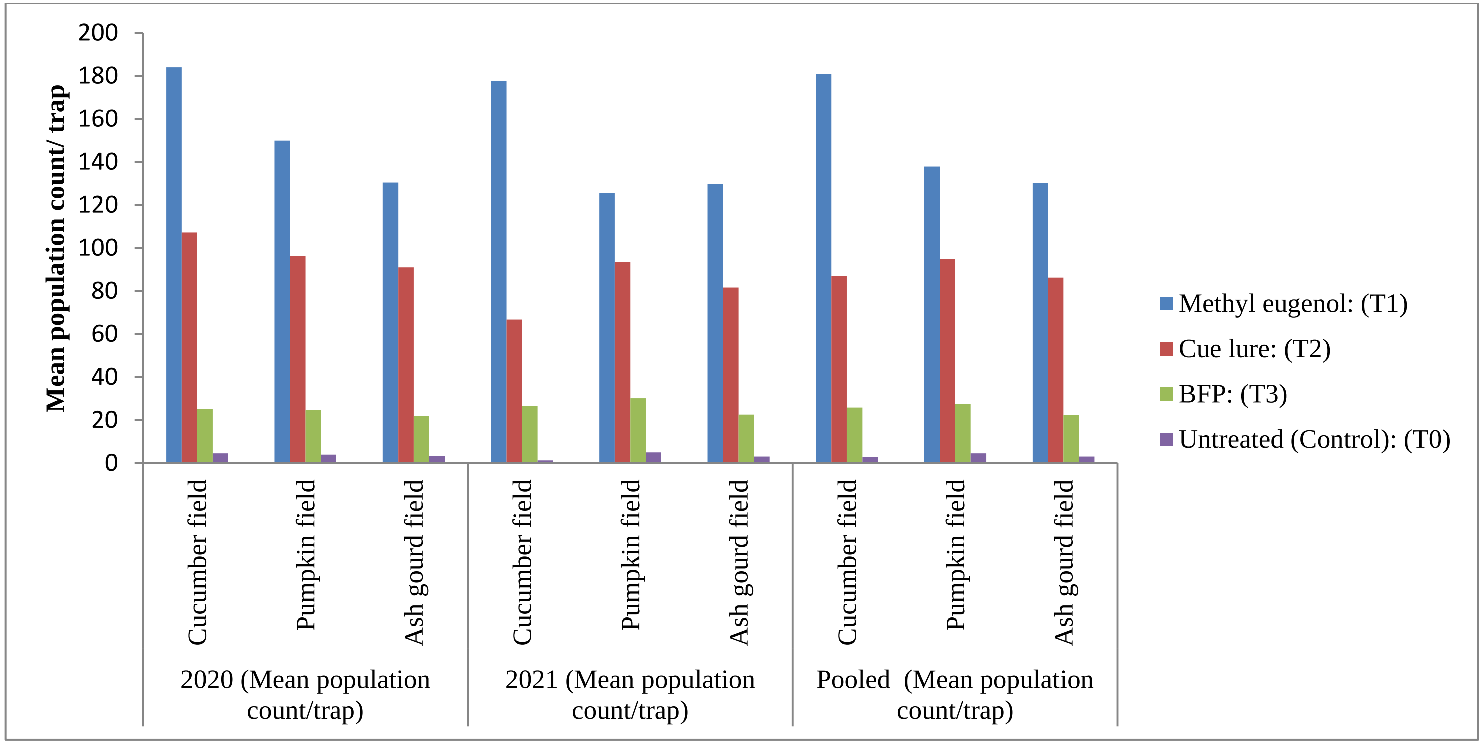


Fig 4.24 Effect of different attractants on the population of *B. dorsalis* during 2020 and 2021

was recorded on methyl eugenol (T_1) with 18.80, 13.40 and 22.60 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

In 2021, similar trend was observed where treatment T_2 (cue lure) have attracted 47.20, 47.20 and 57.00 flies/trap from cucumber, pumpkin and ash gourd, respectively shows significantly superior over all the treatments followed by T_3 (banana poison bait) which attracted 29.00, 25.60 and 27.20 flies/trap from cucumber, pumpkin and ash gourd, respectively. The minimum number of flies trapped was observed in T_1 (methyl eugenol) which attracted 13.80, 17.00 and 21.20 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

Pooled data showed that cue lure (T_2) had attracted the maximum number of *Z. tau* with mean population of 49.70, 49.20 and 58.70 flies/trap from cucumber, pumpkin and ash gourd, respectively. The second best treatment was banana poison bait (T_3) which attracted 30.80, 27.00 and 27.90 flies/trap from cucumber, pumpkin and ash gourd, respectively and lowest population was recorded from methyl eugenol (T_1) with 16.30, 15.20 and 21.90 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

4.6.3 Effect of different attractants on *B. tuberculata* adult in different cucurbits

The information about *B. tuberculata* trapping from May to July 2020 and 2021 with regards to the impact of treatments is shown in Table 4.41 and Fig 4.26.

In 2020, a significant difference among the various treatments used to capture *B. tuberculata* adult was observed. Methyl eugenol (T_1) was much more effective than all other treatments in 2020, attracting a mean population of 36.00, 40.20 and 44.60 flies/trap from cucumber, pumpkin and ash gourd,

Table 4.40 Effect of different attractants against *Z. tau* during 2020 and 2021

Attractants	2020 (Mean population count/trap)			2021 (Mean population count/trap)			Pooled (Mean population count/ trap)		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
Methyl eugenol: (T_1)	18.80 (4.435)	13.40 (3.79)	22.60 (4.76)	13.80 (3.84)	17.00 (4.23)	21.20 (4.59)	16.30 (4.15)	15.20 (4.02)	21.90 (4.68)
Cue lure: (T_2)	52.20 (7.29)	51.20 (7.22)	60.40 (7.77)	47.20 (6.94)	47.20 (6.94)	57.00 (7.55)	49.70 (7.12)	49.20 (7.08)	58.70 (7.66)
BPB: (T_3)	32.60 (5.79)	28.40 (5.42)	28.60 (5.34)	29.00 (5.48)	25.60 (5.14)	27.20 (5.20)	30.80 (5.64)	27.00 (5.29)	27.90 (5.27)
Untreated (Control): (T_0)	2.00 (1.70)	1.20 (1.46)	2.00 (1.38)	1.40 (1.48)	1.40 (1.51)	1.80 (1.31)	1.70 (1.60)	1.30 (1.49)	1.90 (1.34)
SEm±	0.157	0.090	0.103	0.159	0.137	0.115	0.129	0.112	0.100
CD (P= 0.05)	0.490	0.280	0.351	0.496	0.427	0.393	0.401	0.350	0.311

***Figures in parenthesis are square root transformation value**

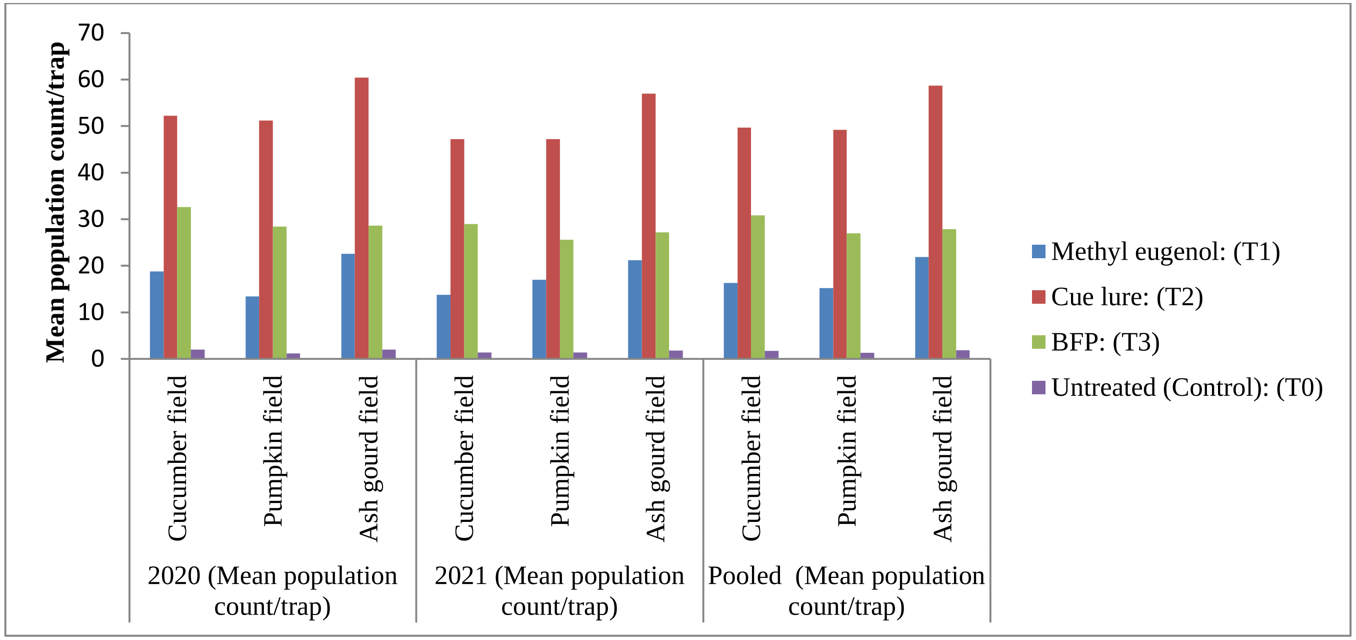


Fig 4.25 Effect of different attractants on the population of *Z. tau* during 2020 and 2021

respectively. Cue lure (T_2), which trapped 15.40, 11.00 and 17.80 flies/trap from cucumber, pumpkin and ash gourd, respectively, was the second-best treatment. Banana poison bait (T_3), which attracted 7.20, 7.60 and 12.00 flies/trap from cucumber, pumpkin and ash gourd, respectively had the lowest number of flies captured.

In 2021, methyl eugenol (T_1) was found to be significantly superior to all other treatments which attracted 35.20, 42.60 and 42.00 flies/trap from cucumber, pumpkin and ash gourd, respectively. Cue lure (T_2) attracted 16.00, 16.00 and 15.60 flies/trap from cucumber, pumpkin and ash gourd, respectively. The banana poison bait (T_3), which attracted 6.80, 9.20 and 10.20 flies/trap from cucumber, pumpkin and ash gourd fields, respectively, was found to trap the least flies.

The combined data showed that T_1, or methyl eugenol, had attracted the highest number of flies from cucumber, pumpkin and ash gourd with 35.60, 41.40 and 43.30 flies/trap, respectively. T_2 (cue lure), which trapped 15.70, 13.50 and 16.70 flies/trap from the fields of cucumber, pumpkin and ash gourd, respectively, comes next. The lowest mean population was trapped to T_3 (banana poison bait) from cucumber, pumpkin and ash gourd field, with 7.00, 8.40 and 11.10 flies/trap, respectively.

4.6.4 Effect of different attractants on *Z. cucurbitae* adult in different cucurbits

The observations on mean population of *Z. cucurbitae* flies attracted in different attractants were recorded from starting of flowering till harvesting during 2020 and 2021. The details are presented in Table 4.42 and Fig 4.27.

In 2020, *Z. cucurbitae* was found to be significantly attracted more towards cue lure (T_2) with mean population of 19.80, 23.00 and 25.80 flies/trap from cucumber, pumpkin and ash gourd field, respectively followed by banana poison bait (T_3) which attracted 9.40, 8.60 and 12.60 flies/trap from cucumber, pumpkin and ash gourd field, respectively. Lowest mean population was recorded on methyl eugenol (T_1) with 6.20, 3.60 and 5.40 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

In 2021, similar trend was recorded where treatment T_2 (cue lure) had attracted 25.80, 20.40 and 24.20 flies/trap from cucumber, pumpkin and ash gourd, respectively which was significantly superior over all the treatments followed by T_3 (banana poison bait) which attracted 10.40, 8.00 and 10.00 flies/trap from cucumber, pumpkin and ash gourd, respectively. The minimum number of flies trapped was observed in T_1 (methyl eugenol) which attracted 3.20, 4.80 and 3.80 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

Pooled data shows that cue lure (T_2) had attracted maximum number of *Z. cucurbitae* with mean population of 22.80, 21.70 and 25.00 flies/trap from cucumber, pumpkin and ash gourd, respectively. The second best treatment was banana poison bait (T_3) which attracted 9.90, 8.30 and 11.30 flies/trap from cucumber, pumpkin and ash gourd, respectively and lowest population was recorded from methyl eugenol (T_1) with 4.70, 4.20 and 4.60 flies/trap from cucumber, pumpkin and ash gourd field, respectively.

Table 4.41 Effect of different attractants against *B. tuberculata* during 2020 and 2021

Attractants	2020 (Mean population count/trap)			2021 (Mean population count/trap)			Pooled (Mean population count/ trap)		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
Methyl eugenol: (T_1)	36.00 (6.08)	40.20 (6.41)	44.60 (6.75)	35.20 (6.01)	42.60 (6.60)	42.00 (6.55)	35.60 (6.05)	41.40 (6.51)	43.30 (6.65)
Cue lure: (T_2)	15.40 (4.04)	11.00 (3.46)	17.80 (4.32)	16.00 (4.10)	16.00 (4.10)	15.60 (4.07)	15.70 (4.07)	13.50 (3.80)	16.70 (4.20)
BPB: (T_3)	7.20 (2.84)	7.60 (2.91)	12.00 (3.60)	6.80 (2.67)	9.20 (3.19)	10.20 (3.33)	7.00 (2.80)	8.40 (3.06)	11.10 (3.46)
Untreated (Control): (T_0)	1.60 (1.61)	1.40 (1.52)	2.60 (1.89)	2.20 (1.72)	1.40 (1.52)	2.00 (1.68)	1.90 (1.70)	1.40 (1.52)	2.30 (1.80)
SEm±	0.151	0.121	0.107	0.160	0.158	0.155	0.097	0.130	0.114
CD (P= 0.05)	0.471	0.376	0.334	0.498	0.493	0.510	0.303	0.406	0.355

***Figures in parenthesis are square root transformation value**

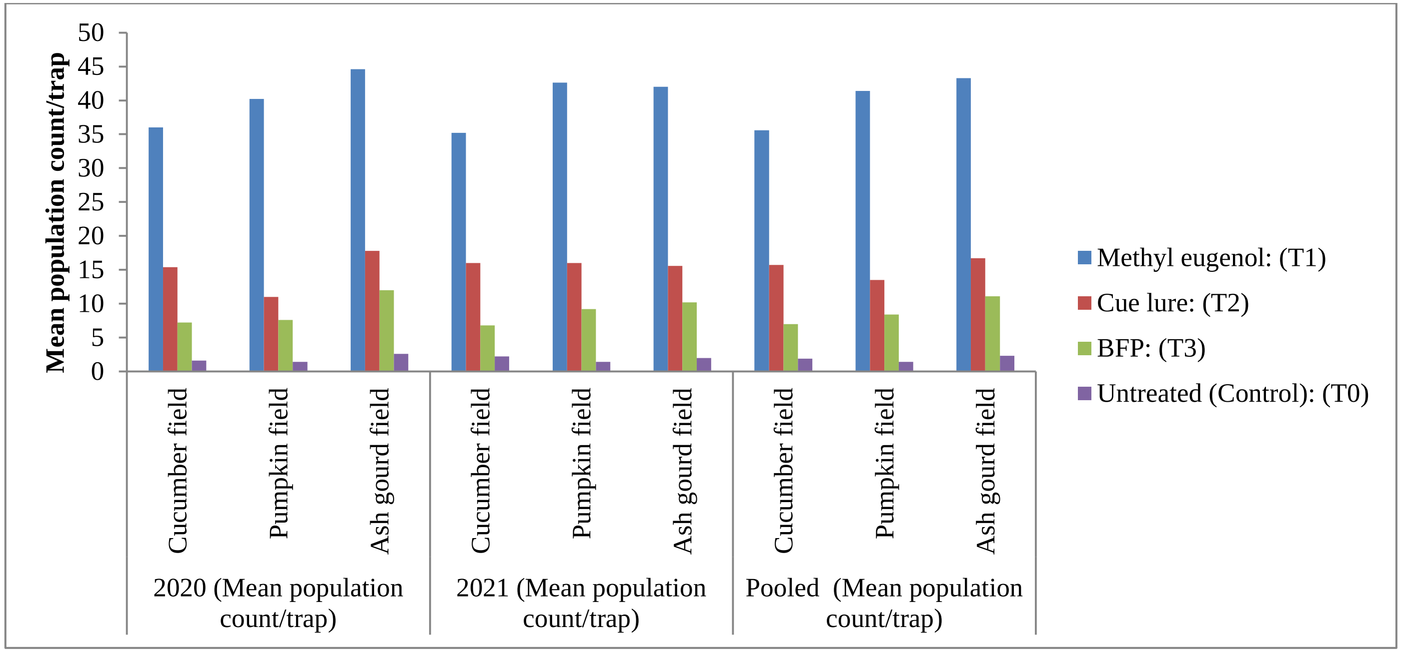


Fig 4.26 Effect of different attractants on the population of *B. tuberculata* during 2020 and 2021

Table 4.42 Effect of different attractants against *Z. cucurbitae* during 2020 and 2021

Attractants	2020 (Mean population count/trap)			2021 (Mean population count/trap)			Pooled (Mean population count/ trap)		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
Methyl eugenol: (T_1)	6.20 (2.62)	3.60 (2.05)	5.40 (2.50)	3.20 (1.99)	4.80 (2.38)	3.80 (2.17)	4.70 (4.88)	4.20 (2.24)	4.60 (2.35)
Cue lure: (T_2)	19.80 (4.55)	23.00 (4.89)	25.80 (5.17)	25.80 (5.17)	20.4 (4.62)	24.20 (5.01)	22.80 (3.30)	21.70 (4.76)	25.00 (5.09)
BPB: (T_3)	9.40 (3.21)	8.60 (3.10)	12.60 (3.68)	10.40 (3.35)	8.00 (2.99)	10.00 (3.32)	9.90 (2.33)	8.30 (3.04)	11.30 (3.50)
Untreated (Control): (T_0)	1.20 (1.43)	1.20 (1.44)	1.40 (1.51)	0.60 (1.25)	1.60 (1.58)	1.40 (1.51)	0.90 (1.36)	1.40 (1.51)	1.40 (1.51)
SEm±	0.174	0.190	0.150	0.174	0.155	0.102	0.149	0.149	0.099
CD (P= 0.05)	0.541	0.591	0.467	0.543	0.483	0.317	0.463	0.464	0.309

***Figures in parenthesis are square root transformation value**

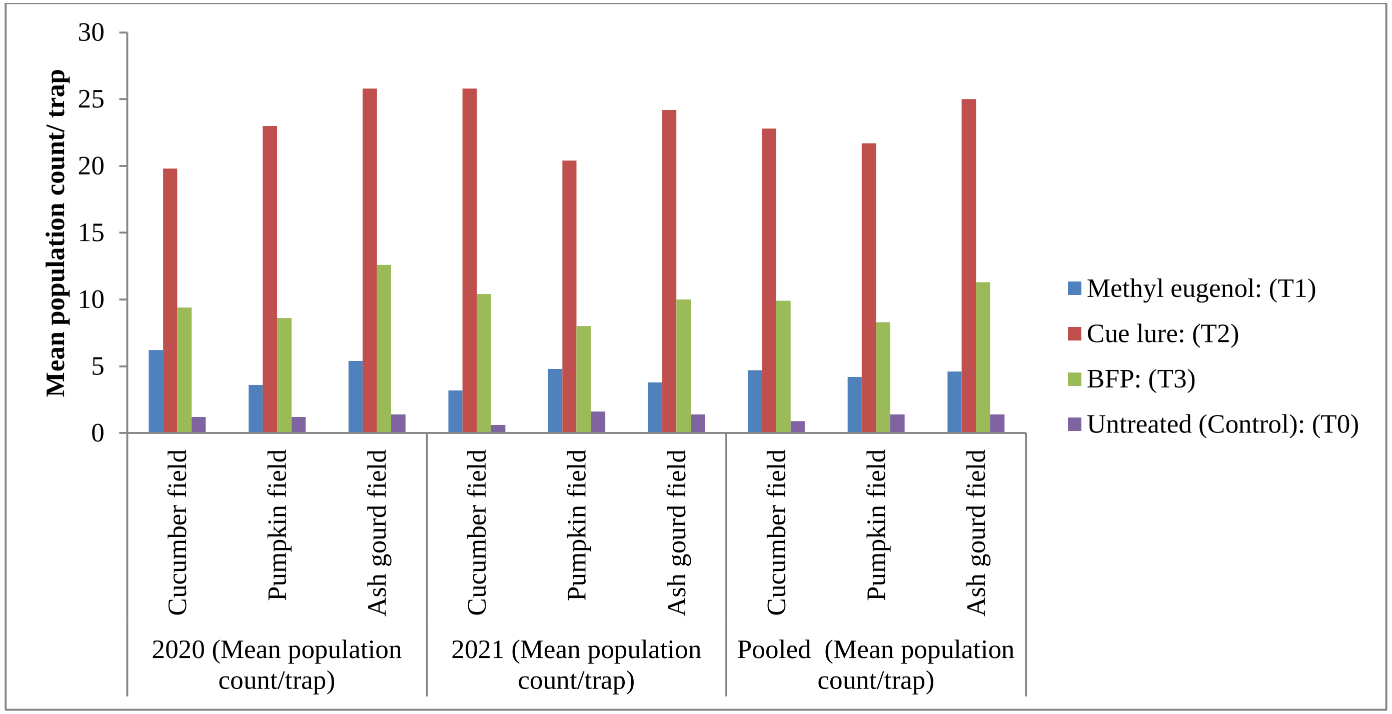


Fig 4.27 Effect of different attractants on the population of *Z. cucurbitae* during 2020 and 2021

4.6.5 Effect of different attractants on *B. ruiliensis* adult in different cucurbits

The information about *B. ruiliensis* trapping from May to July 2020 and 2021 with regards to the effect of treatments is shown in Table 4.43 and Fig. 4.28.

In 2020, a significant difference among the various treatments used to capture mature *B. ruiliensis* adult was observed. Methyl eugenol (T_1) was much more effective than all other treatments in 2020, attracting a mean population of 9.00, 9.20 and 10.20 flies/trap from cucumber, pumpkin and ash gourd, respectively. Cue lure (T_2), which catched 2.60, 3.00 and 3.60 flies/trap from cucumber, pumpkin and ash gourd, respectively, was the second best treatment. Banana poison bait (T_3), which attracted 1.00, 1.20 and 1.00 flies/trap from cucumber, pumpkin and ash gourd, respectively had the lowest number of flies captured.

In 2021, methyl eugenol (T_1) was found to be significantly superior to all other treatments which attracted 11.00, 6.80 and 8.80 flies/trap from cucumber, pumpkin and ash gourd, respectively. Cue lure (T_2) attracted 4.20, 3.00 and 3.00 flies/trap from cucumber, pumpkin and ash gourd, respectively. The banana poison bait (T_3), which attracted 2.20, 0.60 and 0.80 flies/trap from cucumber, pumpkin and ash gourd fields, respectively was found to trap the least flies.

The combined data showed that T_1, or methyl eugenol, had attracted the highest number of flies from cucumber, pumpkin and ash gourd with 10.00, 8.00 and 9.50 flies/trap, respectively. T_2 (cule lure), which drawn 3.40, 3.00 and 3.30 flies/trap from the fields of cucumber, pumpkin and ash gourd, respectively comes next. The lowest mean population was drawn to T_3 (banana poison bait) from cucumber, pumpkin and ash gourd field, with 1.60, 0.90 and 0.90 flies/trap, respectively.

From the present findings, it can be illustrated that methyl eugenol was the most effective attractant for *B. dorsalis*, *B. tuberculata* and *B. ruiliensis*, while cue lure was found to be effective attractant for *Z. tau* and *Z. cucurbitae*. The current findings are consistent with Mahammad *et al*. (2004), who reported that methyl eugenol traps were the most appealing and effective for fruit fly monitoring of *B. zonata* and *B. dorsalis*. Roomi *et al*. (1993) also stated that methyl eugenol traps are the most outstanding option for controlling fruit flies because they have both olfactory and phagostimulatory action. The current findings are also in agreement with the results of Cunningham (1989), who reported that methyl eugenol, when applied in combination with an insecticide impregnated into a suitable substrate, forms the foundation of the male annihilation technique and has been successfully used for the eradication and management of several *Bactrocera* species.

The current findings are also in line with the findings of Ferrar (2010) who reported that methyl eugenol attracts males of many *Bactrocera* species, but not members of the *Bactrocera* subgenus (*Zeugodacus*), which includes the melon fruit fly. Khan *et al*. (2010) also discovered that cue-lure was more effective for *B. cucurbitae* with higher male catches of fruit flies (171.83 flies/trap/week) than methyl eugenol (81.69 flies/trap/week).

Table 4.43 Effect of different attractants against *B. ruiliensis* during 2020 and 2021

Attractants	2020 (Mean population count/trap)			2021 (Mean population count/trap)			Pooled (Mean population count/ trap)		
	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field	Cucumber field	Pumpkin field	Ash gourd field
Methyl eugenol: (T_1)	9.00 (3.26)	9.20 (3.10)	10.20 (2.98)	11.00 (3.05)	6.80 (2.68)	8.80 (3.38)	10.00 (3.16)	8.00 (2.90)	9.50 (3.20)
Cue lure: (T_2)	2.60 (1.95)	3.00 (1.82)	3.60 (1.59)	4.20 (1.81)	3.00 (1.83)	3.00 (2.08)	3.4 (1.89)	3.00 (1.83)	3.30 (1.92)
BPB: (T_3)	1.00 (1.14)	1.20 (1.22)	1.00 (1.09)	2.20 (1.04)	0.60 (1.02)	0.80 (1.62)	1.60 (1.10)	0.90 (1.15)	0.90 (1.41)
Untreated (Control): (T_0)	0.00 (0.71)	0.00 (0.71)	0.40 (0.71)	0.40 (0.81)	0.00 (0.70)	0.20 (0.91)	0.20 (0.88)	0.00 (0.71)	0.30 (0.83)
SEm±	0.193	0.156	0.272	0.171	0.122	0.165	0.168	0.110	0.134
CD (P= 0.05)	0.603	0.486	0.847	0.533	0.380	0.514	0.524	0.341	0.417

***Figures in parenthesis are square root transformation value**

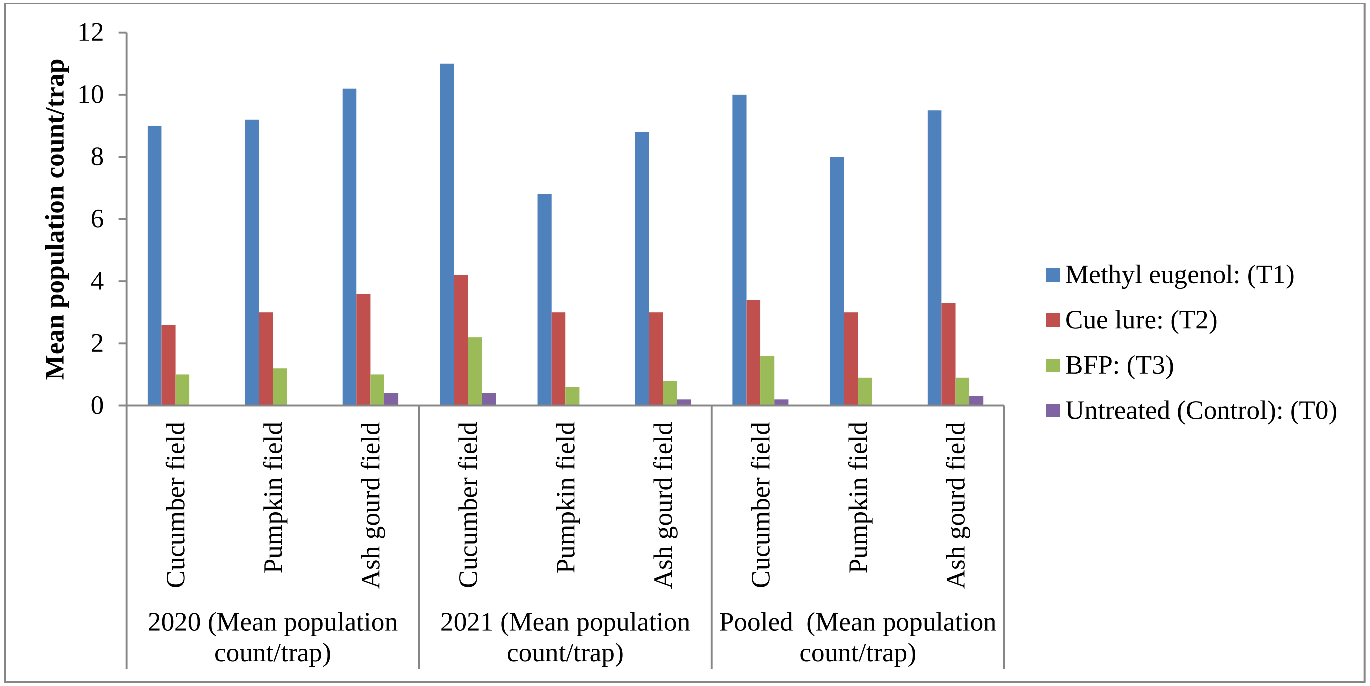


Fig 4.28 Effect of different attractants on the population of *B. ruiliensis* during 2020 and 2021

4.7 Development of DNA barcodes for different species of fruit flies and their natural enemies of cucurbitaceous crops ecosystem

4.7.1 Qualitative and Quantitative estimation of DNA

The quality and quantity of extracted DNA was determined by using Nanodrop (Digital spectrophotometer). Blank control of 1X TE buffer was used for all the batches of DNA estimations. The quality and quantity of DNA varied for all the specimens. Extraction of DNA was done following Qiagen DNeasy Blood and Tissue Kit. The details of the estimation are presented in (Table 4.44). The highest concentration of DNA was recorded in *Oecophylla smaragdina* (35.60ng/µl) and the lowest in *Crematogaster* sp. (7.91ng/µl). From the 260/280 ratios of wavelength, phenol/Chloroform and protein contamination were detected in some samples.

4.7.2 PCR amplification of COI gene

Multiple specimens of all ten identified species were successfully amplified using standard DNA barcoding primers LCO & HCO and LepF1 & LepR1. The barcoding primers were designed to amplify a 709bp PCR fragment from the partial COI gene. All of the specimens were successfully amplified with the targeted PCR fragment (709bp) of the partial COI gene.

Table 4.44 Qualitative and quantitative estimation of DNA from fruit flies and their natural enemies

Sl. No.	DNA code	Name of sample	Order	DNA Concentration (ng/µl)	260/280 ratio
1	S1	*Bactrocera dorsalis*	Diptera	27.38	2.04
2	S32	*Bactrocera tuberculata*		15.24	2.10
3	S3	*Zeugodacus cucurbitae*		23.27	1.83
4	BT	*Zeugodacus tau*		15.69	1.50
5	S31	*Bactrocera ruiliensis*		15.24	2.10
6	P3	*Polyrhachis* sp.	Hymenoptera	14.79	2.15
7	WA	*Oecophylla smaragdina*		35.60	1.62
8	SA	*Tapinoma* sp.		10.43	1.73
9	BA	*Crematogaster* sp.		7.91	2.79
10	RB	*Paederus fuscipes*	Coleoptera	9.47	2.52

DNA extraction and PCR amplification were performed in batches on different dates for multiple specimens of the same species. All specimens yielded acceptable clear bands, and amplified bands were detected using gel electrophoresis on 1.5% agarose gel. The insect species' amplified gel image is shown in (Plate 5).

4.7.3 Sequencing of partial PCR fragment of COI gene

Two specimens were sequenced for each species using the standard barcoding primers (i.e. LCO & HCO and LepF1 & LepR1). The Sanger sequencing of all the samples was done commercially by sending 40µl of post PCR product in frozen condition to M/S Eurofins Genomics India Pvt. Ltd, Bangalore and sequencing was carried out bi-directionally (from both the ends 5’ and 3’) for all the samples. Re-sequencing was done for samples which gave poor quality sequence due to degradation of post PCR product or excess quantity of DNA. From the total 10 species, good quality sequence were obtained from 10 species but 1 species *viz*., *Polyrhachis* sp. resulted in poor sequence.

4.7.4 Sequencing analysis

Utilizing the Pregap and Gap programme in the Staden Package software, sequencing analysis was carried out after the DNA samples were successfully sequenced (Staden *et al*., 2000). Each species' sequencing analysis was done separately, and the software's sequences were manually checked for errors to ensure error-free results. To create a high-quality sequence, the sequences' messy/ambiguous 5' and 3' ends were cut out. A series of bar graphs displaying the sequence quality were produced after the confidence level was also examined; the majority of the sequences displayed a high confidence level. The resulting sequence's overall length ranged from 380 to 647bp depending on the species (Table 4.44 and 4.45).

The shortest sequence length was found in *Polyrhachis* sp., which had 380 base pairs. *Zeugodacus cucurbitae*, *Zeugodacus tau* and *Bactrocera dorsalis* all displayed good quality partial COI sequence lengths of 649, 638 and 647bp, respectively. Using the NCBI web-based program's invertebrate genetic code option, all sequences were converted into proteins. The lengths of the different proteins varied from 126 to 215 amino acids. In addition to their nucleotide sequence length and protein translation, each detected species' specifics are provided in Table 4.45.

4.7.5 Submission of sequences to NCBI

The final analysed sequences were submitted to National Center for Biotechnology Information (NCBI) for accession numbers. The accession numbers were obtained for the representative partial COI gene sequence of 10 identified species *viz*., ON725008, ON725009, ON725110, ON725111, ON725112, ON725013, ON724399, ON724947, ON738447 and ON738454. The nucleotide length, protein length along with NCBI accession numbers are presented in Table 4.45.

4.7.6 Blast analysis

The analysed sequences were subjected to BLASTN search on online portal of National Centre for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/). The correct identity and homologous species were established by comparing with a library or database of sequences that resembles the sequence. All the sequences were analysed individually by nucleotide Blastn search at NCBI portal and the first three hits were recorded. The blast results with 99-100% homology to NCBI database were considered as similar species and molecular identity of the test species was confirmed.

Table 4.45 List of identified species along with nucleotide length, protein length and NCBI accession number

Sl. No.	Name of insect	Order	Nucleotide length (bp)	Protein length	Accession number
1	*Zeugodacus cucurbitae*	Diptera	649	215	ON724399
2	*Zeugodacus tau*		638	212	ON724947
3	*Bactrocera dorsalis*		647	215	ON725008
4	*Bactrocera ruiliensis*		591	196	ON725009
5	*Bactrocera tuberculata*		577	191	ON725010
6	*Tapinoma* sp.	Hymenoptera	582	190	ON738447
7	*Oecophylla smaragdina*		585	195	ON725011
8	*Crematogaster* sp.		567	195	ON738454
9	*Polyrhachis* sp.		380	126	ON725012
10	*Paederus fuscipes*	Coleoptera	583	194	ON725013

However for those species with blast result below 99%, the identity was established till genus level in this research. Accordingly, the molecular identities of all the insect species collected from different cucurbitaceous crops ecosystem of Nagaland were established. The details of the NCBI BLASTN search results for all the insects are presented in Table 4.46 – Table 4.47.

4.7.7 Development of DNA barcodes

All nucleotide sequences submitted to NCBI had their DNA barcode pictures created using web-based software (http://biorad-ads.com/.DNABarcodeWeb/) (Plate 6 and 7).

It is crucial to appropriately identify any insect pests before taking any control measures because wrong identification could lead to ineffective treatment and may even aggravate the impact caused by a particular pest species (Rivera and Currie, 2009). The cytochrome oxidase I (COI) based technique has been developed in response to the declining number of taxonomists and other identification experts and allows for the identification of all developmental stages of insects, their food webs and biotypes, which may not be possible with morphology-based taxonomy (Jinbo *et al.*, 2011, Srinivasan *et al.*, 2013, Jalali *et al.*, 2015).

Agriculturally significant insect pests' DNA barcoding has assisted in finding perplexing and maybe new species (Burns *et al.*, 2008). This is congruent with our research findings that the insect species *Bactrocera ruiliensis,* which has not previously been documented from Nagaland, is found in the environment of cucurbits.

The identification of insect pest species has been made easier because to the numerous new invasive insect pest species that have been reported from India and North East India.

Table 4.46 NCBI BLASTN search results for different fruit flies species as on 30.06.22

Sample name code/Common name	Molecular identity	Voucher code	NCBI results	First three NCBI matches	Seq Length
S1_*Bactrocera dorsalis* S1_*Bactrocra dorsalis*	*Bactrocera dorsalis*	SASRD-HDDS1	99.85% similarity with *Bactrocera dorsalis*	MG689904 (99.85%) MG689087 (99.85%) MG688191 (99.85%)	647 bp
BT_*Bactrocera tau* BT_*Bactrocera tau*	*Zeugodacus tau*	SASRD-HDDBT	99.69% similarity with *Zeugodacus tau*	MH97372 (99.69%) MH900081 (99.53%) KP711431 (99.53%)	638 bp
32_*Bactrocera tuberculata* 32_*Bactrocera tuberculata*	*Bactrocera tuberculata*	SASRD-HDDF32	99.82% similarity with *Bactrocera tuberculata*	MW600175 (99.82%) KT151120 (99.62%) MK411590 (99.48%)	577 bp
S3_*Bactrocera cucurbitae* S3_*Bactrocera cucurbitae*	*Zeugodacus cucurbitae*	SASRD-HDDS3	100% similarity with *Zeugodacus cucurbitae*	MN016981.1 (100%) KY113187.1 (99.54%) KU096057.1 (99.38%)	649 bp
S31_*Bactrocera ruiliensis* S31_*Bactrocera ruiliensis*	*Bactrocera ruiliensis*	SASRD-HDDS31	100% similarity with *Bactrocera ruiliensis*	NC046952 (100%) KT151117 (100%)	591 bp

Table 4.47 NCBI BLASTN search results for different fruit flie's natural enemies as on 30.06.22

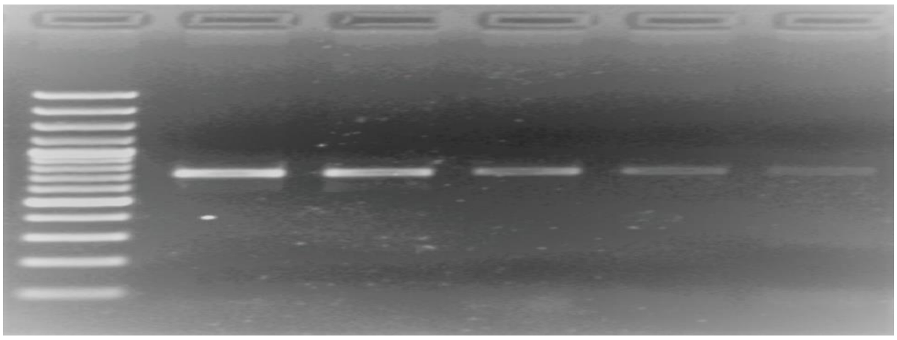
Sample name code/Common name	Molecular identity	Voucher code	NCBI results	First three NCBI matches	Seq Length
WA_LepF1 Ant WA_LepR1 Ant	*Oecophylla samaragdina*	SASRD-HDDW AF1	99.83% similarity with *Oecophylla samaragdina*	MF278656 (99.83%) JQ681064 (99.66%) JQ344565 (99.65%)	585 bp
SA_LepF1 Ant SA_LepR1 Ant	*Tapinoma* sp.	SASRD-HDDSA F1	98.80% similarity with *Tapinoma* sp.	MK950207 (98.80%) KY837778 (97.81%) KY838112 (97.77%)	582 bp
BA1_LepF1 Ant BA1_LepR1 Ant	*Crematogaster* sp.	SASRD-HDDBA F1	98.64% similarity with *Crematogaster* sp.	MN010606 (85.64%) OL664470 (85.64%) HM418763 (85.07%)	567 bp
P3_LepF1 Ant P3_LepR1 Ant	*Polyrhachis* sp.	SASRD-HDDPPP 3F1	97.63% similarity with *Polyrhachis paracamponota*	JQ681070 (97.63%) MW056455.1 (91.32%) KY831806.1 (89.92%)	380 bp
RB_LepF1 Rove beetle RB_LepR1 Rove beetle	*Paederus fuscipes*	SASRD-HDDRB F1	99.14% similarity with *Paederus fuscipes*	MH916764 (99.14%) MG581161 (98.80%) KM441774. (98.45%)	583 bp

The South American tomato pinworm (*Tuta absoluta*), which has successfully invaded India, was discovered and identified via DNA barcoding in Meghalaya in 2017 after being reported for the first time in Maharashtra in 2014 (Sankarganesh *et al*., 2017).

In North East India in 2017, the invasive tomato leaf miner *Liriomyza sativae* was also found and identified by DNA barcoding (Firake *et al*., 2017). Additionally, prior experiments with DNA barcoding had shown that it was particularly effective at identifying invasive and other taxonomically challenging insect species. For instance, Behere *et al*. (2007) used DNA barcoding to study the genetic diversity of *H. armigera* on a global scale, the diversity of fruit flies (Manger, 2015), pests of cole crops (Lalrinfeli, 2015), pests of cereal crops (Kuotsu, 2016), pests of solanaceous crops (Sankarganesh *et al*., 2017) and pests of cucurbitaceous crops (Pongen, 2018). In contrast to the approximately 59,000 recognised insect species, comprehensive DNA information on insect species is still quite scarce in India, having produced only 3,694 barcodes of known species. However, there are only roughly 1.63,617 barcodes of described species in the equivalent world wide scenario; therefore much effort is needed to catch up with the global scenario (IBIn, 2022).

The DNA barcoding technique utilized in this study has shown to be extremely helpful in accurately identifying insect pests and natural enemies in the cucurbit ecosystem.This method has demonstrated to be a reliable and effective tool over the past ten years, reaching species level resolution in 95 to 97% of cases (Hebert *et al*., 2004). The thorough information on DNA barcodes produced by this work will undoubtedly be useful as a diagnostic tool for identifying and providing more efficient management of cucurbit insect pests.

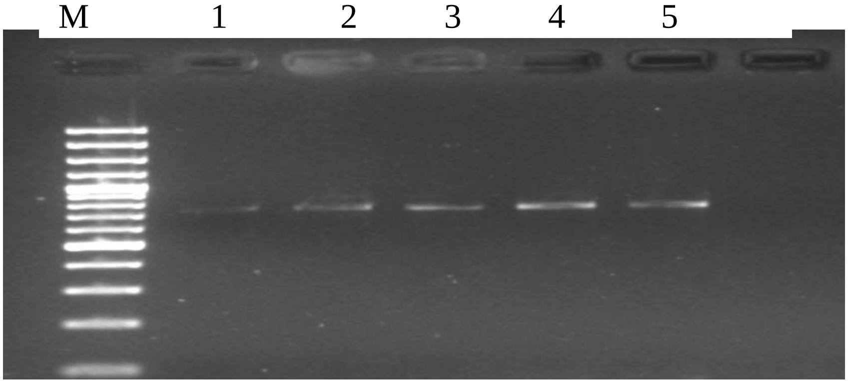
M 1 2 3 4 5



NB: M=100bp ladder

Lane 1 to 5**:** Lane 1- *B. dorsalis*, 2- *Z. cucurbitae*, 3- *B. ruiliensis,* 4- *B. tuberculata*, 5- *Z. tau*

M 1 2 3 4 5

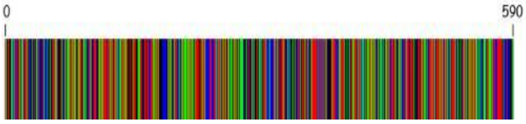


NB: M=100bp ladder

Lane 1 to 5**:** Lane 1- *Polyrhachis* sp., 2- *Crematogaster* sp., 3- *O. smaragdina*, 4- *Tapinoma* sp., 5- *P. fuscipes*

Plate 5: PCR amplification of different species of fruit flies and their natural enemy species using LepF1 and LepR1 primers on 1.5% agarose gel

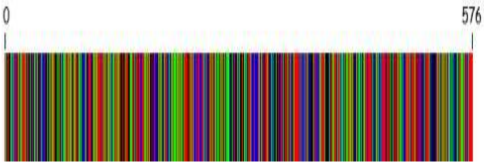
1) *Bactrocera dorsalis*



2) *Bactrocera ruiliensis*

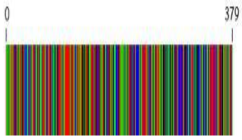
3) *Zeugodacus tau*

4) *Zeugodacus cucurbitae*



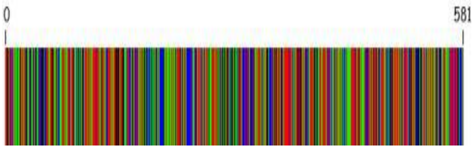
5) *Bactrocera tuberculata*

Plate 6: Translated image of nucleotide sequences of different species of fruit flies on important cucurbits of Nagaland



1) *Polyrhachis* sp.

2) *Crematogaster* sp**.**



3) *Tapinoma* sp.

4) *Oecophylla smaragdina*

5) *Paederus fuscipes*

Plate 7: Translated image of nucleotide sequences of different species of fruit flie's natural enemy on important cucurbits of Nagaland

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present study entitled "**Diversity of fruit flies and their natural enemies on important cucurbits of Nagaland**" was carried out in different experimental farms of SAS, Nagaland University, Medziphema Campus Nagaland during summer season of 2020- 2021.

The significant findings from the different experiments were summarized below:

1. The fruit flies adults were collected at standard week throughout the study period by employing different attractants such as methyl eugenol, cue lure and banana poison bait traps, while their natural enemy data were recorded by direct counting which were identified to species level with the help of molecular identification.
2. Five species of fruit fly *viz*., *B. dorsalis*, *Z. tau*, *B. tuberculata*, *Z. cucurbitae* and *B. ruiliensis* along with five species of natural enemy *viz*., *O. smaragdina*, *Tapinoma* sp., *Polyrhachis* sp., *Crematogaster* sp. and *P. fuscipes* were recorded in cucurbit crops ecosystem in Nagaland. The fruit fly species *B. ruiliensis* have been reported for the first time from Nagaland.
3. A total of 2544.00, 2347.50 and 2409.50 numbers of fruit flies adult were collected from cucumber, pumpkin and ash gourd field, respectively during the entire experimental period of May to July (pooled of 2020 and 2021). Among the species, *B. dorsalis* was found to be the most abundant with 1483.00, 1323.00 and 1209.00 (pooled of 2020 and 2021) number of individuals from cucumber, pumpkin and ash gourd field, respectively, whereas, *B. ruiliensis* was least abundant with only 76.00, 59.50 and 70.00 (pooled of 2020 and 2021) number of individuals from cucumber, pumpkin and ash gourd filed, respectively.

4. The relative abundance of *B. dorsalis* in cucumber, pumpkin and ash gourd field was represented with 58.29, 56.36 and 50.19% followed by *Z. tau* with 19.35, 19.74 and 22.90%, *B. tuberculata* with 11.83, 13.78 and 15.23% and *Z. cucurbitae* with 7.53%, 7.58% and 8.78% . Whereas, *B. ruiliensis* was the least abundant species in all cucurbit fields with 2.99, 2.54 and 2.90% in cucumber, pumpkin and ash gourd field, respectively (pooled of 2020 and 2021).

5. In the year 2020, the peak incidence of *B. dorsalis* was recorded on 14th June (24 SMW) on all the cucurbits representing mean population of 18.65, 16.50 and 15.40 flies/trap on cucumber, pumpkin and ash gourd, respectively. Highest population of *Z. tau* was recorded on last week of May (22 SMW) with a mean catch of 4.60, 4.40 and 6.25 flies/trap on cucumber, pumpkin and ash gourd, respectively. For *B. tuberculata* highest population was recorded on 7th June (23 SMW) with mean population of 6.45, 6.40 and 7.00 flies/trap in cucumber, pumpkin and ash gourd, respectively. For *Z. cucurbitae,* highest incidence was on 31st May (i.e., 22 SMW) with mean population 2.30, 2.30 and 2.65 flies/trap in cucumber, pumpkin and ash gourd, respectively. The peak incidence of *B. ruiliensis* was recorded on 22 SMW with 0.70 and 1.10 flies/trap in cucumber and pumpkin; however in ash gourd highest population was recorded on 23 SMW (7th June) with 1.20 flies/trap.

6. In 2021, the peak incidence of *B. dorsalis* was recorded on 20th June (25 SMW) in cucumber and pumpkin with mean population of 13.45 and 15.50 flies/trap. However, in ash gourd the highest population was recorded on 13th June (24 SMW) with mean population of 15.40 flies/trap. The highest population of *Z. tau* was recorded on 6th June (23 SMW) with mean population of 3.85 flies/trap in cucumber, while in pumpkin and ash gourd peak incidence was observed on 30th May (22 SMW) with mean population of 5.45 and 7.30 flies/trap. For *B. tuberculata*, the highest population was recorded on 13th June (24 SMW) with 3.20 flies/trap in cucumber, while in pumpkin and ash gourd

on 6th June (23 SMW) with 5.40 and 6.80 flies/trap. The highest incidence of *Z. cucurbitae* was observed on 6th June (23 SMW) in cucumber with 1.85 flies/trap, while in pumpkin and ash gourd on 30th May (i.e., 22 SMW) with mean population 2.15 and 2.50 flies/trap, respectively. The peak incidence of *B. ruiliensis* was recorded on 23 SMW with 1.05 and 0.85 flies/trap in cucumber and pumpkin; however, in ash gourd highest population was recorded on 21 SMW (23rd May) with 0.75 flies/trap.

7. In 2020, all the five species of fruit flies were found to have non-significant correlation with the abiotic factors in the three cucurbit fields. However, in 2021, on cucumber, the population of *B. dorsalis*, *B. tuberculata* and *Z. tau* had shown significant positive correlation with morning relative humidity. Similarly on pumpkin and ash gourd also the population of *B. dorsalis* had shown significant positive correlation with morning relative humidity. On the other hand, the population of *B. ruiliensis* was found to have significant positive correlation with maximum temperature only on pumpkin during 2021.

8. A total of 3009, 2923.50 and 2847.00 numbers of natural enemies were collected from cucumber, pumpkin and ash gourd field, respectively during the entire experimental period of March to July (pooled of 2020 and 2021). Among these species *O. smaragdina* was found to be the most abundant with 1325, 1267 and 1210.50 number of individuals from cucumber, pumpkin and ash gourd field, respectively. Whereas *P. fuscipes* was least abundant with only 159.5, 137.5 and 143.5 numbers of individuals from cucumber, pumpkin and ash gourd field, respectively.

9. The relative abundance of *O. smaragdina* in cucumber, pumpkin and ash gourd field was represented with 44.03, 43.34 and 42.52%, followed by *Tapinoma* sp. with 24.91, 26.85 and 28.13%, *Polyrhachis* sp. with 17.73, 17.44 and 17.00% and *Crematogaster* sp. with 8.02, 7.66 and 7.31%, respectively. Whereas, *P. fuscipes* was the least abundant species in all cucurbit fields with

5.30, 4.70 and 5.04% in cucumber, pumpkin and ash gourd field, respectively (pooled of 2020 and 2021).

10. In 2020, the peak incidence of *O. Smaragdina* was recorded on 5th July (27 SMW) on all the cucurbits with 34.60, 33.60 and 32.00 numbers/vine in cucumber, pumpkin and ash gourd, respectively). The highest abundance of *Tapinoma* sp. was observed on 12th July (28 SMW) with a mean catch of 25.40, 25.00 and 26.00 numbers/vine on cucumber, pumpkin and ash gourd, respectively. The highest population of *Polyrhachis* sp. was recorded on 28th June (26 SMW) with mean population of 15.80, 16.00 and 15.40 numbers/vine in cucumber, pumpkin and ash gourd, respectively. The abundance of *Crematogaster* sp. was highest on 28th June (26 SMW) in cucumber with 8.00 numbers/vine, while in pumpkin and ash gourd highest population was recorded on 5th July (27 SMW) with 7.60 and 7.20 numbers/vine, respectively. The peak abundance of *P. fuscipes* was recorded on 28 SMW with 7.20 and 7.20 numbers/vine in cucumber and ash gourd, while in pumpkin the highest population was recorded on 5th July (27 SMW) with 6.60 numbers/vine.

11. In 2021, the abundance of *O. smaragdina* reached its peak on 11th July (28 SMW) with mean population of 33.80 and 33.20 numbers/vine in cucumber and ash gourd, however in pumpkin highest population was recorded on 4th July (27 SMW) with mean population of 33.20 numbers/vine. The highest population of *Tapinoma* sp. was recorded on 11th July (28 SMW) with mean population of 26.20, 26.40 and 26.60 numbers/vine from cucumber, pumpkin and ash gourd, respectively. The weekly mean population of *Polyrhachis* sp. was highest on 27th June (26 SMW) with mean population of 15.80, 15.60 and 15.40 numbers/vine in cucumber, pumpkin and ash gourd, respectively. The highest population of *Crematogaster* sp. was observed on 4th July (27 SMW) with mean population of 7.20, 7.00 and 6.80 numbers/vine in cucumber, pumpkin and ash gourd field, respectively. The population of *P. fuscipes*

reached its peak on 20th June (25 SMW) with mean population of 6.40, 6.20 and 6.00 numbers/vine in cucumber, pumpkin and ash gourd, respectively.

12. The correlation studies revealed that the natural enemies of fruit flies had a significant positive relation with minimum temperature, evening relative humidity and rainfall on three different cucurbits.

13. The Shannon-Weiner diversiy index (H'), Simpson diversity index (SDI) and evenness of species (E_H) index were computed from all the three cucurbit crops for fruit flies and their natural enemies. For fruit flies H' value was in the range of 1.18 to 1.29, SDI value was in the range of 0.336 to 0.397 and E_H value was in the range of 0.736 to 0.799. For natural enemies the H' value was in the range of 1.36 to 1.37, SDI in the range of 0.296 to 0.299 and E_H value was in the range of 0.845 to 0.853 (pooled of 2020 and 2021).

14. In 2020, the maximum fruit damage throughout the study period was found on cucumber with total of 72.62% on 24 SMW, followed by pumpkin with 60.37% on 24 SMW, whereas minimum fruit damage was recorded on ash gourd with 51.29% during 23 SMW. Similarly, in 2021, the highest damage percentage was recorded in cucumber with 76.81 on 24 SMW followed by pumpkin with 57.87 on 24 SMW and lowest infestation was recorded in ash gourd with 56.89% on 23 SMW.

15. Methyl eugenol was found the most effective attractant for *B. dorsalis* (130.10 to 180.90 flies/trap), *B. tuberculata* (35.60 to 43.30 flies/trap) and *B. ruiliensis* (8.0 to 10.0 flies/trap). Whereas cue lure was found to be effective attractant for *Z. tau* (48.60 to 58.70 flies/trap) and *Z. cucurbitae* (21.70 to 25.00 flies/trap).

16. DNA was successfully extracted from multiple specimens of 10 insect species (5 fruit flies and 5 natural enemies) and the quality and quantity of DNA was good for all the specimens.

17. The final good quality nucleotide sequence length of partial COI gene varied from 577 to 649 bp for fruit fly species and 380 to 585 bp for natural enemy species.

18. The species level identity was developed for 7 species (*B. dorsalis*, *Z. tau*, *B. tuberculata*, *Z. cucurbitae*, *B. ruiliensis*, *O. smaragdina* and *P. fuscipes*) and remaining 3 (*Tapinoma* sp., *Polyrhachis* sp. and *Crematogaster* sp.) was identified upto genus level due to absence of matching molecular data at NCBI. All the analyzed sequences have been deposited to International GeneBank (NCBI) with accession numbers ON725008 to ON725013, ON724399, ON724947, ON738447 and ON738454.

19. This study had generated DNA barcodes for 10 insect species (5species of fruit fly and 5 species of natural enemy).

20. The fruit fly, *Bactrocera ruiliensis* have been reported for the first time from Nagaland.

CONCLUSION

The following conclusions are drawn based on the above findings:

1. *B. dorsalis* was the most dominant species with the highest relative abundance percentage and for their natural enemy *O. smaragdina* was found to have the highest relative abundance percentage in all the cucurbit crops of Nagaland.

2. The diversity of fruit flies and their natural enemies was relatively low in all the cucurbit fields. However, they were distributed evenly as the value of evenness of species (E_H) was found high in all the cucurbit fields.

3. The abundance of fruit flies was the highest during last week of May upto middle of June, while for natural enemies the highest population was recorded in the month of June and July.

4. The most preffered host for fruit flies among the three cucurbit crops (cucumber, pumpkin and ash gourd) was cucumber recording the highest percentage of infestation.

5. Methyl eugenol was found to be the most effective attractant for *B. dorsalis*, *B. tuberculata* and *B. ruiliensis*, while cue lure was found to be the most effective attractant for *Z. tau* and *Z. cucurbitae*.

6. The research study also concludes the presence of 10 insect species (5 fruit fly species and 5 natural enemies) in cucurbitaceous crops ecosystem in Nagaland.

7. The fruit fly, *B. ruiliensis* have been reported for the first time from Nagaland.

FUTURE LINE OF WORK

According to the current preliminary investigation, numerous kinds of fruit flies are attacking various cucurbits and causing serious damage. The outcome of the current experiment offers some intriguing questions for future research, including the relationship between abiotic and biotic variables and seasonal abundance throughout the year in various cropping seasons.

Application of chemical insecticide is dangerous as they harmed both target and non- target beneficial insects and unnecessary when managing fruit flies. Therefore, in order to make vegetables profitable to cultivate, it is essential that future effortsshouldbe made fully on the aforementioned line of work with fruit flies using cost-effective and sound pest management techniques.

The experiment findings showed that the ecology of cucurbitaceous crops in Nagaland has 10 insect species, including 5 different kinds of fruit flies and 5 natural enemies. The thorough molecular information obtained in this study for a total of 10 species seen in Nagaland's cucurbitaceous crops environment might be used as a diagnostic tool at both morphological and molecular levels. Furthermore, detailed knowledge of the diversification of fruit flies and their related natural enemies in the area would be beneficial for creating better pest management plans.

Furthermore, the diversity of fruit flies and their natural enemies studies can also be conducted in different districts of Nagaland as in the current study it was only conducted in Medziphema area of Nagaland, Dimapur district.

REFERENCES

REFERENCES

Abhilash, J., Naveen, N, E., Patil, S, U., Sharanabasappa and Mohankumar K.S. 2017. Monitoring of melon fruit fly (*Bactrocera cucurbitae)* Col. (Diptera: Tephritidae) in relation to weather parameters. *Journal of Entomology and Zoology Studies*. **5**(5): 1930-1935.

Abro, Z., Waseem, A., Naheed, B. and Noor, A. S. 2017. Population Densities of Melon Fruit Fly *Bactrocera cucurbitae* (Coquillett) in Vegetables Agro-Ecosystem in District Hyderabad, Sindh, Pakistan. *Sarhad Journal of Agriculture*. **33**(2): 331-337.

Ajuru, M. and Nmom, F. 2017. Review on the economic uses of species of cucurbitaceae and their sustainability in Nigeria. *Americal Journal of Plant Biology*. **2**(1):17-24.

Amin, M.R. 1995. Effect of some indigenous materials and pesticides incontrolling fruit fly *Bactrocera cucurbitae* (Coquillett) on cucumber. M.Sc. Thesis, Institute of PostgraduateStudies in Agriculture, Gazipur, Bangladesh.

Amin, M. R., Sarkar, T. and Chun, I. J. 2011. Comparison of host plants infestation level and life history of fruit fly (*Bactrocera cucurbitae* Coquillett) on cucurbitaceous crops. *Horticulture, Environment and Biotechnology*. **52**: 541.

Anonymous. 1988. Comparative efficacy of bagging and trapping in controlling fruit fly, *Bactrocera cucurbitae* (Coquillett). Attacking kakrol (*Momordica diocia* Roxb). Research Report in fruit fly in cucurbits. Paper presented at the International Review Workshop held on Aug. 28-30, 1988, Division of Entomology; BARI, Gazipur, 17 pp.

Anonymous. 2019. Economic survey of Nagaland- 2016-17, Area and production published by Ministry of Agriculture. 2019.

Apriyadi, R., Saputra, H. M., Sintia, S. and Andini, D. E. 2021. The diversity of fruit fly (Diptera: Tephritidae) on combination of attractant and different trap height in cucumber field (*Cucumis sativus* L.). **In**: Proceedings of the Earth and Environmental Science. 926: 1- 7.

Badii, K.B., Billah, M.K., Afreh-Nuamah, K. and Obeng-Ofori, D. 2015. Species Composition and Host Range of Fruit-Infesting Flies (Diptera: Tephritidae) in Northern Ghana. *International Journal of Tropical Insect Science*. **35**(3): 137-151.

Balog, A., Viktor, M. and Laszlo, F. 2008. Patterns in distribution, abundance and prey preferences of parasitoid rove beetles *Aleochara bipustulata* (L.)

(Coleoptera: Staphylinidae, Aleocharinae) in Hungarian agroecosystems. *North-Western Journal of Zoology*. **4**(1): 6-15.

Bateman, M. A. 1972. The ecology of fruit flies. *Annual Review of Entmology*. **17**: 493-518.

Behere, G. T., Tay, W. T., Russell, D. A., Heckel, D. G., Appleton, B. R., Kranthi, K. R. and Batterham, P. 2007. Mitochondrial DNA analysis of field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and of its relationship to *H. zea*. *BMC Evolutionary Biology*. **7**(1): 1-17.

Behere, G.T., Firake, D.M., Tay, W.T., Thakur, N.S.A., and Ngachan, S.V. 2016. Complete mitochondrial genome sequence of a phytophagous ladybird beetle, *Henosepilachna pusillanima* (Mulsant) (Coleoptera: Coccinellidae). *Mitochondrial DNA.* **A27**(1): 291-292.

Beroza, M., Alexander, B.H., Steiner, L.F., Mitchell, W.C. and Miyashita, D. H. 1960. New synthetic lure for the male melon fly. *Science*. **131**: 1044-1045.

Bharati, T. E., Sathiyanandam, V. K. R. and David, P. M. M. 2004. Attractiveness of some food baits to the melon fruit fly, *Bactrocera cucurbitae* (Coq.) (Diptera: Tephritidae). *International Journal of Tropical Insect Science.* **24**(2): 125- 134.

Bhowmik, P., Mandal, D. and Chatterjee, M. L. 2014. Studies on the infestation levels by melon fruit fly, *Bactrocera cucurbitae* Coquillett, on pointed gourd (*Trichosanthes dioica* Roxb.) and bitter gourd (*Momordica charantia* Linn.) in new alluvial zone of West Bengal. *Journal of Entomological Research.* **38**(3): 195-200.

Bisognin, D. A. 2002. Origin and evolution of cultivated cucurbits. *Ciencia Rural*. **32**(5): 715- 723.

Blacket, M. J., Semeraro, L. and Malipatil, M. B. 2012. Barcoding Queensland Fruit Flies (*Bactrocera Tryoni*): Impediments and Improvements. *Molecular Ecology Resources*. **12**(3):428–436.

Budiyanti, S., Hidrayani, and Hamid, H. 2019. Species diversity of fruit flies (Diptera: Tepthritidae) and their damage on vegetables in Padang, West Sumatera, Indonesia. *Asian Journal of Agriculture and Biology*. Special issue: 224-230.

Burns, J. M., Janzen, D. H., Hajibabaei, M., Hallwachs, W. and Hebert, P. D. N. 2008. DNA barcodes and cryptic species of skipper butterflies in the genus Perichares in Area de Conservacion Guanacaste, Costa Rica. Proc. Natl. Acad. Sci. USA. **105**(17): 6350-6355.

Chaudhary, F. K. and Patel, G. M. 2012. Dose of cue-lure for suppression of melon fly population in pumpkin. *AGRES*. **1**(2): 181-185.

Clarke, K. R. and Warwick, R. M. 2001. Changes in marine communities: an approach to statistical analysis and interpretation, 2nd edition, PRIMERE: Plymouth. 172pp

Cunningham, R. T., 1989. Male annihilation. In: World Crop Pests, Eds. Robinson, A. S. and Hooper, the Netherlands.

David, K. J., Hancock, D. L., Singh, S. K., Ramani, S., Behere, G. T. and Salini, S., 2017. New species, new records and updated subgeneric key of *Bactrocera Macquart* (Diptera: Tephritidae: Dacinae: Dacini) from India. *Zootaxa*. **4272**(3): 386-400.

Decker, K. B. and Yeargan, K. V. 2008. Seasonal phenology and natural enemies of the squash bug (Hemiptera: Coreidae) in Kentucky. *Environmental Entomology*. **37**(3): 670–678.

Deguine, J. P., Atiama-nurbel, T., Douraguia, E., Chiroleu, F. and Quilici, S. 2012. Species diversity within a community of the curcurbit fruit flies *Bactrocera cucurbitae*, *Dacus ciliates* and *Dacus demmerezi* roosting in corn borders near cucurbit production areas of Reunion Island. *Journal of Insect Science*. 12: 105-110.

Delomen, M. L. C., Mendioro, M. S. and Diaz, M. G. Q. 2013. Morphometric Analysis and DNA barcoding of Fruit Flies *Bactrocera occipitalis* (Bezzi) and *B. philippinensis* Drew and Hancock (Diptera: Tephritidae) from Cavite and Davao del Norte. *Philippine Journal of Science*. **142**(1): 69-76.

Demilo, A. B., Cunningham, R. T., and Mcgovern, T. P. 1994. Structural variants ofmethyl eugenol and their attractiveness to the oriental fruit-fly (Diptera: Tephritidae). *Journal of Economic Entomology*. **87**(4): 957-964.

Dhamdhere, S. V. and Odak, S. C. 1975. How to combat fruit fly. *Agricultural Research Journal of Kerala.* **13**: 33-55.

Dhillon, M. K., Singh, R., Naresh, J. S. and Sharma, H. C. 2005. The melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. *Entomological Journal of Insect Sciences*. **5**(1): 1- 16.

El Keroumi, A., Naamani, K., Soummane, H. and Dahbi, A. 2012. Seasonal dynamics of ant community structure in the Moroccan Argan Forest. *Journal of Insect Science*. **12**(94): 1-17.

Ekesi, S. and Billah, M. K. (2007). A field guide to the management of economically important tephritid fruit fkies in Africa. ICIPE Sciences Press, Nairobi, Kenya.

EPPO. *Chrysodeixis eriosoma* (Lepidoptera: Noctuidae) - Green semi-looper. EPPO.https://www.eppo.int/QUARANTINE/Alert_List/insects/ *Chrysodeixi seriosoma*. doc. [Visited on 7 July, 2022].

Fang, M. N. and Chang, C. P. 1984. The injury and seasonal occurrence of melon fly, *Dacus cucurbitae* Coq. (Diptera: Trypetidae) in Central Taiwan. *Plant Protection Bulletin*. Taiwan. **26**: 241–248.

Fernandes, W. D., Sant, M. V., Raizer, J. and Lange, D. 2012. Predation of Fruit Fly Larvae Anastrepha (Diptera: Tephritidae) by Ants in Grove. *Psyche*. **2012**: 1-7.

Ferrar, P. 2010. Fruit flies in Asia (especially Southeast Asia). Species, biology and management. Available online on www.Scribd.com/doc/…/Fruit-flies-in-Asia-Paperpaul-27-Aug-2010.

Firake, D. M., Sankarganesh, E., Sharma, B., Firake, P. D. and Behere, G. T. 2017. DNA barcoding confirmed the occurrence of invasive vegetables leaf miner, *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Northeast India. *Journal of Asia-Pacific Biodiversity*. **11**(1):56-60.

Francinaldo, S., Shelre, E. P. D. and Apoliana, A. S. 2017. Seasonal Abundance and the Effect of Light Trap Height on the Captures of the Vesicating Beetles of the Genus Paederus (Coleoptera: Staphylinidae) in Brazil. *Entomological News*. **127**(1): 21-27.

Ganie, S.A., Khan, Z.H., Ahangar, R.A., Bhat, H.A., Hussain, B. 2013. Population dynamics, distribution, and species diversity of fruit flies on cucurbits in Kashmir Valley. *Indian Journal of Insect Science*. **13:** 65.

Gariepy, T.D., Kuhlmann, U., Gillott, C., and Erlandson, M. 2007. Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of arthropods. *Journal of Applied Entomology*. **131**: 225-240.

Ghavami, S. 2008. The potential of predatory spiders as Biological control agents of cotton pests in Tehran provinces of Iran. *Asian Journal of Environmental Science.* **22**: 303 - 306.

Ghule, T.M., Uikey, B.L., Barma, P. and Jha, S. 2014. Incidence studies on some important insect pests of cucumber (*Cucumis sativus* L.). *The Ecoscan*. **8**(1&2): 177-180.

Gupta, J. N. and Verma, A. K. 1992. Population fluctuation of the maggots of fruit flies *Dacus cucurbitae* Coquillett and *Dacus tau* (Walker) infesting cucurbitaceous crops. *Advances of Plant Sciences*. **5**: 518-523.

Hancock, D. L., Hamacek, E., Llyoyd and Haris, M. M. 2000. The distribution and host plants of fruit flies (Diptera: Tepthritidae) in Australia. Queensland Department of Primary Industries, Australia.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and Jeremy, R. 2003. Biological Identifications through DNA Barcodes. *Proceeding of the Royal Society B*. **270**: 313–321.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings National Academy of Sciences*. **101**(41): 14812-14817.

Hodgson, P. J., Sivinski, J., Quintero, G. and Aluja, M. 1998. Depth of pupation and survival of fruit fly (*Anastrepha* spp.: Tephritidae) pupae in a range of agricultural habitats. *Environmental Entomology*. **27**: 1310–1314.

Hollingsworth, R., Vagalo, M. and Tsatsia, F. 1997. Biology of melon fly, with special reference to Solomon Island. **In**: Allwood, A.J. and Drew, R.A.I. editors. Management of fruit flies in the Pacific. Proceeding Australian Country Industrial Agricultural Research. **76**: 140-144.

Hossain M.A., Leblanc L., Momen M., Abdul, B. M. and Khan, S.A. 2019. Seasonal Abundance of Economically Important Fruit Flies (Diptera: Tephritidae: Dacinae) in Bangladesh, in Relation to Abiotic Factors and Host Plants. **In**: Proceedings of the Hawaiian Entomological Society. **51**(2): 25–37.

Hulcr, J., Miller, S. E., Setliff, G. P., Darrow, K., Mueller, M. D., Hebert, P. D. N. and Weiblen, G. D. 2007. DNA barcoding confirms polyphagy in a generalist moth, *Homona mermerodes* (Lepidoptera: Tortricidae). *Molecular Ecology*. **7**: 549-57.

http:/bioradads.com/.DNABarcodeWeb/. Accessed on 16th May, 2022.

https://ibin.iirs.gov.in/. Acceessed on 16th May, 2022.

http://www.ncbi.nlm.nih.gov/. Accessed on 15th May, 2022.

Hwang, Y. B., Kao, C. H. and Cheng, E. Y. 1997. The monitoring and control of the oriental fruit fly in Taiwan. *Bulletin of Plant Protection*, Taipei. **39**: pp 125-136.

Iqbal, A. 2018. Efficacy of different ecofriendly management practices in controlling cucurbit fruit fly on bitter gourd. M.Sc. (Ag) thesis, State Agricultural University, Sher-E-Bangla Agriculture University Dhaka, India. 29 p.

Ishtiaqe, A., Farman, U. and Shah, A. K. 1999. Efficacy of various insecticide and trap height in methyl eugenol baited trap against fruit flies. *Sarhad Journal of Agriculture*. **15** (6): 589-594.

Jalali, S. K., Ojha, R. and Venkatesan, T. 2015. New Horizon in Insect Science: Towards sustainable pest management. Springer India, Delhi. pp 13-23. DOI: 10.1007/978-81-322-2089-3_2.

Jenkins, C., Chapman, T.A., Micallef, J. L. and Reynolds, O. L. 2012. Molecular techniques for the detection and differentiation of host and parasitoid species and the implications for fruit fly management. *Insects.* 3: 763-788.

Jinbo, U., Kato, T. and Ito, M. 2011. Current progress in DNA barcoding and future implications for entomology. *Entomology*. **14**: 107-124.

Kabir, S. M. H., Rahman, R. and Molla, M. A. S. 1991. Host plants of Dacinaefruit flies (Diptera: Tephritidae) of Bangladesh. *Bangladesh Journal of Entomology*. **1**: 60- 75.

Kapoor, V. C. 1993. Indian fruit flies (Insecta: Diptera: Tephritidae) Oxford and IBH publishing. Co. Pvt. Ltd. New Delhi. 228.

Kapoor, V. C. 2005. Taxonomy and biology of economically important fruit flies of India. *Isreal Journal of Entomology*. **35-36**: 459-475.

Kate, A. O., Bharodia, R. K. Joshi, M. D. Pardeshi, A. M and Makadia, R. R. 2009. Seasonal incidence of fruit fly, *Bactrocera cucurbitae* (Coquillet) on cucumber. *Asian Science*. **4** (1 & 2): 83-84.

Kawashita, T., Rajapakase, G. B. J. P. and Tsuruta, K. 2004. Population survey of Bactrocera fruit flies by lure trap in Sri Lanka. *Research Bulletin of Plant Protection Service*. **40**: pp 83-87.

Khan, M. A., Gogi, D. A., Khaliq, A., Subhani, M. N. and Ali, A. 2010. Efficacy of methyl eugenol and cue-lure traps for monitoring melon fruit fly in relation to environmental conditions in bitter gourd. *Journal of Agriculture Research*. **48**: 523-530.

Khan, R. A. and Naveed, M. 2017. Occurrence and seasonal abundance offruit fly, *Bactrocera zonata* (Saunders), (Diptera: Tephritiadae) inrelation to meteorological factors. *Pakistan journal of Zoology*. **49**(3):999-1003.

Kishor, P., Kempraj, T., Elaiyabharathi, T. and Kavitha, C. 2018. Species Diversity and Relative Abundance of Fruit Flies (Tephritidae: Diptera) in Cucurbitaceous Vegetables. *Madras Agricultural Journal*. **105**(4-6): 196-200.

Koller, W. W., Alberto, G., Sergio, R. R. and Julio, M. 2002. Staphylinidae (Coleoptera) associated to cattle dung in Campo Grande, MS, Brazil. *Neotropical Entomology*. **31**(4):641-645.

Krishna, K. N. K., Abraham, V., Shivakumara, B., Krishnamoorthy, P.N. and Ranganath, H.R. 2006. Relative incidence of *Bactrocera cucurbitae* (Coquillett) and *Dacus ciliatus* on cucurbitaceous vegetables. Fruit Flies of Economic Importance: From Basic to Applied Knowledge. **In**: Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance, Salvador, Brazil. pp. 249-253.

Kumar, N. K., Verghese, A., Shivakumara, B., Krishnamoorthy, P. N. and Ranganath, H. R. 2006. Fruit flies of economic importance.From basic to applied knowledge. **In**: Proceedings of the 7th International symposium on fruit flies of economic importance, Salvador, Brazil, 249-253.

Kunprom, C. and Pramual, P. 2016. DNA barcode variability and host plant usage of fruit flies (Diptera: Tephritidae) in Thailand. *Genome*. **59**(10): 792-804.

Kuotsu, K. 2016. Development of DNA barcodes of Insect Pests and Natural Enemies of major Cereal crops in Mid Hills of Meghalaya. M. Sc. (Agri.) Thesis, submitted to Central Agriculture University Imphal, Umiam-793103, Meghalaya, India.

Lalrinfeli, R. 2015. Development of DNA barcodes for major insect pests and natural enemies of cole crops ecosystem in mid-altitude of Meghalaya. M. Sc. (Agri.) Thesis, submitted to Central Agriculture University Imphal, Umiam-793103, Meghalaya, India.

Laskar, N. and Chatterjee, H. 2010. The effect of meteorological factors on the population dynamics of melon fly, *Bactrocera cucurbitae* (Coq.) (Diptera: Tephritidae) in the foot hills of Himalaya. *Journal of Applied Sciences and Environmental Management*. **14**(3): 53-58.

Latha, S and Sathyanarayana, N, 2015. An illustrative guide for trapping, Monitoring & identification of economically important Fruit flies. National institute of plant health management, Hyderabad.

Lee, L.W.Y., Hwang, Y. B., Cheng, C.C and J.C. Chang. 1992. Population fluctuation of the melon fly, *Dacus cucurbitae*, in northeastern Taiwan. *Chinese Journal of Entomology*. **12**: 285-292.

Leinster, T. and Cobbold, C. A. 2012. Measuring diversity: the importance of species similarity. *Ecology*. **93**(3): 477–489.

Lengkong, M., Pelealu, J., Tulung, M., Mantiri, V. and Lengkong, E. F. 2017. Identification of Genetic Diversity in *Bactrocera*spp.from Minahasa Regency Based on COI Barcode. *International Journal of ChemTech Research*. **10**(9): 974- 982.

Liu, Y. C. and Yeh, C. C. 1982. Population fluctuation of oriental fruit fly *D. dorsalis* Hendel in sterile fly release and control area. *Chinese Journal of Entomology*. **2**:57–76.

Manger, A. 2015. Molecular characterization of fruit fly species of the genus *Bactrocera* in midaltitude of Meghalaya. M. Sc. (Agri.) Thesis, submitted to Central Agriculture University Imphal, Umiam-793103, Meghalaya, India.

Maharjan, R., Regmi, R. and Poudel, R. 2015. Monitoring and varietal screening cucurbit of fruit fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) on cucumber in Bhaktapur and Kathmandu, Nepal. *International Journal of Applied Science Biotechnology*. **3**(4): 714-720.

Mahmood, K. and Mishkatullah. 2007. Population dynamics of three species of *Bactrocera* (Diptera: Tephritidae: Dacinae) in BARI, Chakwal (Punjab). *Pakistan Journal of Zoology*. **39**(2): 123-126.

Majacunene, A., Cugala, D. and Ekesi, S. 2014. Assessing the efficacy ofintegrated pest management strategies for suppressing *Bactrocera invadens* in selected orchards in Manica Province, Mozambique. **In**: Proceeding of Fourth Biennial Conference of Maputo, Mozambique. 19-25: 501-502.

Makhmoor, H. D. and Singh, S. T. 1998. Effective concentration of methyleugenol for the control of guava fruit fly *Dacus dorsalis* (Hendel) inguava orchard. *Annals of Plant Protection Sciences*. **6**(2): 165-169.

Mohamood, E., Ekesi, S. and Kambal, M. 2009. Fruit flies their species composition, host range and field response to food-based and male lures attractants. Biophysical and Socio-economic Frame Conditions for the Sustainable Management of Natural Resources. Tropentag, October 6-8, 2009, Hamburg.

Muhammad, I., Muhammad, U., Muhammad, A. and Khan, I. A. 2004. Integrated pest management of mango against mealy bug and fruit fly. *International Journal of Agriculture and Biology*. **6**(3): 452-454.

Mwatawala, M.W., De Meyer, M., Makundi, R.H., and Maerere, A.P. 2006. Seasonality and host utilization of the invasive fruit fly, *Bactrocera invadens* (Diptera: Tephritidae) in central Tanzania. *Journal of Applied Entomology*. **130**(9–10): 530–537.

Nair, S. and Thomas, J. 1999. Effect of *Acorus calamus* L. Extracts on the longevity of *Bactrocera cucurbitae* (Coquillett). *Insect Environment*. **5**: 27.

Nair, N., Bhattacharjee, T., Thangjam, B., Giri, U. and Debnath, M. R. 2018. Species diversity of Dacine fruit flies (Diptera: Tephritidae: Dacinae: Dacini) in Tripura, N.E. *India Journal of Entomology and Zoology Studies.* **6**(1): 297-302.

Nasir, S., Akram. W. and Ahmed, F. 2012. The population dynamics, ecological and seasonal activity of *Paederus fuscipes* Curtis (Staphylinidae; Coleoptera) in the Punjab, Pakistan. *Environmental Science*. **4**: 36-41.

Nath, P. 2007. Cucurbits- Everyone’s Crop. **In**: Proceeding of the IIIrd International Symposium on Cucurbits, 485-491.

National Horticultural Board. 2019-20. Accessed on 20 July 2020.

Navendu, N., Prasenjit, P. and Dilip, N. 2020. Seasonal Incidence of Fruit Fly (*Zeugodacus tau*) in Cucurbit Ecosystem in Tripura. *International Journal of Current Microbiology and Applied Sciences*. **9**(11): 971-977

Nikbakhtzadeh, M. R. and Tirgari, S. 2008. Medically important beetles (Insecta: Coleoptera) of Iran. *Journal of Venomous Animals Toxins including Tropical Diseases*. **14**(4):597-618.

Pal, S., Singh, D. K. and Umrao, R. S. 2014. Population fluctuation and species diversity of Fruit fly, *Bactrocera* spp. *Annals of Plant Protection Sciences*. **22**(1): 31-33

Pandey, A. K., Nath, P. and Rai, A. B. 2008. Efficacy of some eco-friendly insecticides, poison baits and their combinations against bitter gourd fruit infestation by melon fruit fly (*Bactrocera cucurbitae* Coq.). *Vegetable Science*. **35**(2): 152-155.

Pandey, A. K., Nath, P., Rai, A. B., Kumar, A. and Gupta, S. 2010. Influence of poison baits and weather factors on fruit fly *Bactrocera cucurbitae* (Coq.) catches in cucurbits. *Vegetable Science*. **37**(1): 67-68.

Pawar, D. B., Moti, U. N. and Lawande, K. E. 1991. Monitoring of fruit fly population in bitter gourd crop with the help of lure trap. *Journal of Research Maharashtra Agricultural Universities*. **16**: 281.

Philippe, R., Jean-Philippe, D., Thierry, B. and Jean-François, V. 2010. Fruit flies (Diptera: Tephritidae) on vegetable crops in Reunion Island (Indian Ocean): State of knowledge, control methods and prospects for management. *Fruits.* **65**(2): 113-130.

Pongen, A. 2018. Development of DNA barcodes for major insect pests and natural enemies of Cucurbitaceous crops in Mid Hills of Meghalaya. M.Sc. (Ag) Thesis, Central Agricultural University, School of Crop Protection College of Post Graduate Studies, Umiam, Meghalaya, India

Preston, F. W. 1948. The commonness and rarity of species". *Ecology*. **29**(3): 254–283.

Prabhakar, C. S., Sood, P. and Mehta, P. K. 2012.Fruit fly (Diptera: Tephritidae) Diversity in Cucurbit Fields and Surrounding Forest Areas of Himachal Pradesh, a North-Western Himalayan State of India. *Archives of Phyto pathology and Plant Protection*. **45**(10): 1210-1217.

Pradhan, R.B. 1976. Relative susceptibilities of some vegetables grown in Kathmandu valley to *D. Cucurbitae* Coquillett. *Nepal Journal of Agriculture*. **12**: 67-75.

Pujar, K., Kempraj, K., Elaiyabharathi, T and Kavitha, C. 2018. Species diversity and relative abundance of fruit flies (Diptera: Tephritidae) in cucurbitaceous vegetables. *Madras Agricultural Journal*. **105**(4-6): 196-200.

Qureshi, M. Z., Hussain, S. A. H., Khan, L. and Kahttak, M. K. 2000. Varietalresistance and the effect of number of sprays of Dipterex 80sp againstfruit fly complex on cucumber (*cucumis sativus* L.). *Pakistan Journal of Biological sciences*. **3**: 1919-1920.

Rabindranath, K. and Pillai, K. S. 1986.Control of fruit fly of bitter gourd using synthetic pyrethroids. *Entomon*. **11**: 269–272.

Ramsamy M. P., Rawanansham T. and Joomaye. 1987. Studies on the control of *Dacus cucurbitae* Coq. and *Dacus demmerezi* Bezze (Diptera: Tephritidae) by male annihilation. *Revue Agricole et Sucriere de L'ile Maurice*. **66**(1–3): 105-114.

Ravikumar, C. H. 2005. Studies on fruit flies in guava and mango orchards with special reference to their management through mass trapping. M.Sc. (Agri.) Thesis, University of Agricultural Sciences. Dharwad. pp.90.

Rivera, J. and Currie, D. C. 2009. Identification of Nearctic black flies using DNA barcodes (Diptera: Simuliidae). *Molecular Ecology Resources*. **9**(1): 224-236.

Roomi, M. W., Abbas, T., Shah, A. H., Robina, S., Qureshi, A. A., Sain, S. S. and Nasir, K. A. 1993. Control of fruit flies by attractants of plant origin. *Pfianzeschutz*. **66**: 155-157.

Sankarganesh, E., Firake, D. M., Sharma, B., Verma, V. K.and Behere, G. T. 2017. Invasion of the South American Tomato Pinworm, *Tuta absolut*a, in northeastern India: A New Challenge and Biosecurity Concerns. *Entomologia Generalis*. **36**(4):335-345.

Santana, P. A., Gonring, A. H. R., Picanco, M. C., Ramos, R. S., Martins, J. C. and Ferreira, D. O. 2012. Natural biological control of *Diaphania* spp. (Lepidoptera: Crambidae) by social wasps. *Sociobiology*. **59**(2): 115-120.

Sapkota, R., Dahal, K. C. and Thapa, R. B. 2013.Damage assessment and management of cucurbit fruit flies in spring-summer squash. *International Journal of Nematology and Entomology*. **1**(4): 64-68.

Satarkar, V. R., Krishnamurthy, S. V., Faleiro, J. R. and Verghese, A. 2009. Spatial distribution of major *Bactrocera* fruit flies attracted to methyl eugenol in different ecological zones of Goa, India. *International Journal of Tropical Insect Science*. **29**(4): 195-201.

Simpson, E. H. 1949. Measurement of diversity. *Nature*. **163**: 688.

Singh, S. V., Mishra, A., Bisan, R. S., Malik, Y. P. and Mishra, A. 2000. Host preference of red pumpkin beetle, *Aulacophora foveicollis* and melon fruit fly, *Dacus cucurbitae*. *Indian Journal of Entomology*. **62**:242–246.

Singh, R. 2008. Studies on varietal susceptibility of oriental fruit fly, *Bactrocera dorsalis* (Hendel) on guava and its attraction to different poison baits. *Asian Journal of Biological Science*. **3**(2): 330-332.

Smit, J., Reijnen, B. and Stokvis, F. 2013. Half of the European fruit fly species barcoded (Diptera, Tephritidae); a feasibility test for molecular identification. *ZooKeys*. **365**: 279–305.

Srinivasan, R., Hsu, Y.C., Kadirvel, P. and Lin, M.Y. 2013. Analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex in Java Island, Indonesia based on mitochondrial cytochrome oxidase I sequences. *Philippine Agricultural Scientist.* **96**(3): 290-295.

Staden, R., Beal, K. F. and Bonfield, J. K. 2000. The Staden package. *Methods in Molecular Biology*. **132**: 115-300.

Sujit, K. B. 2005. Studies on the bionomics and management approaches of cucurbit Fruit Fly, *Bactrocera cucurbitae* on Bitter Gourd. M.S. Thesis. Department of Entomology. B.A.U. Mymensingh. pp 70.

Sultana, T., Uddin, Md. M. U., Rahman, Md. M. and Shahjahan, Md. (2017).Host preference and eco-friendly management of cucurbit fruit fly under field condition of Bangladesh. *Journal of Bioscience & Biotechnology*. **2**(1): 55-59.

Syed, R.A. 1971. Studies on tephritids and their natural enemies in Pakistan. *V. Dacus* (Strumete) Cucurbita Coq. Tech. Bull. Commonw. *Insect Biological Control*. **14**: 63-75.

Taira, T. L., Abot, A. R., Nicacio, J., Uchoa, M. A., Rodrigues, S. R. and Anderson, J. 2013. Fruit flies (Diptera, Tephritidae) and their parasitoids on cultivated and wild hosts in the Cerrado-Pantanalecotone in MatoGrosso do Sul, Brazil. *Guimaraes Revista Brasileira de Entomologia*. **57**(3): 300–308.

Tan, K. H. and Lee, S. L. 1982. The efficacy of different food attractants and their concentration for melon fly. *Bulletin of Entomological Research*. **72**: 709-716.

Tan, K., and Serit, M. 1994. Adult population dynamics of *Bactrocera dorsalis*(Diptera: Tephritidae) in relation to host phenology and weather in two villages of Penang Island, Malaysia. *Environment of Entomology*. **23**: 267–275.

Tariq, M., Hussain, S. I., Khokhar, K. M., Ahmad, M. and Hidayatullah, G. H. 2002. Studies on methyl eugenol as a sex attractant for fruit fly *D. zonatus* (Saund) in relation to abiotic factors in peach orchard. *Asian Journal of Plant Science*. **4**: 401–402.

Thomas, J., Faleiro, R., Vidya, C. V., Satarkar, V. R., Stonehouse, J. M., Verghese, A. and Mumford, J. D. 2005. Melon flies attraction and control by baits in Central Kerala. *Pest Management in Horticultural Ecosystems*. **11**(2): 110-112.

Uddin, M. J. 1996. Development of suitable package(s) of IPM components for the management of selected insect pests of cucumber. M.Sc. (Ag) Thesis. Institute of Post Graduate Studies in Agriculture. Gazipur, Bangladesh. 72p.

Ukey, M.S., Chandele, A.G., Jadhav, R. S and Wagh, S. S. 2014. Seasonalincidence of fruit flies, *Bactrocera* spp. (Diptera: Tephritidae) inrelation to abiotic factors. *Ecology, Environment and conservations*. **20**(1): 365-368.

Vargas, R.I., Miller, N. W. and Stark, J. D. 2003. Field trials of spinosad as a replacement fornaled ddvp, and Malathion in methyl eugenoland cue-lure bucket traps to attract and killmale oriental fruit flies and melon flies (Diptera: Tephritidae) in Hawaii. *Journal of Economic Entomology*. **96**: 1780-1785.

Vargas, R. I., Leblanc, L., Harris, E. J. and Nicholas, C. 2012. Regional Suppression of Bactrocera Fruit Flies (Diptera: Tephritidae) in the Pacific through Biological Control and Prospects for Future Introductions into Other Areas of the World. *Manoukis Insects*. **3**: 727-742.

Varun, A., Srinivasa, N., Saniya, T., Samantapudi, V. S. R., Chandra, P. S., Twinkle, S. and Pratap, D. 2022. DNA barcoding of fruit flies associated with cucurbit ecosystem and combination of Cue-Lure and Methyl Eugenol in trap is not effective for mass trapping of responsive fruit flies. *Phytoparasitica*. **50**: 683–695.

Vayssieres J. F., Carel, Y., Coubes, M. and Duyck, P. F. 2008. Development of immature stages and comparative demography of two cucurbit infesters in Reunion Island: *Bactrocera cucurbitae* and *Dacus ciliatus* (Diptera: Tephritidae). *Environmental Entomology*. **37**(2): 307–314.

Vayssieres, J. F., De Meyer, M., Ouagoussounon, I., Sinzogan, A., Adandonon, A., Korie, S. and De Bon, H. 2015. Seasonal abundance of mango fruit flies (Diptera: Tephritidae) and ecological implications for their management in mango and cashew orchards in Benin (Centre &North). *Journal of economic entomology*. **108**(5): 2213-2230.

Vignesh, R. and Viraktamath, S. 2015. Population dynamics of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) on Cucumber (*Cucumis sativus* L.). *Karnataka Journal of Agricultural Science*. **28**(4): 528-530.

Wang, Y., Chen, J., Jiang, L. Y. and Qiao, G. X. 2015. The complete mitochondrial genome of *Mindarus keteleerifoliae* (Insecta: Hemiptera: Aphididae) and comparison with other Aphididae insects. *International Journal of Molecular Science*. **16**: 30091-30102.

Wazir, Z. A., Singh, A. K. and Ramana, N. 2019. Seasonal incidence of fruit fly on Summer squash (*Cucurbita pepo* L.) and effect of weather parameters on population dynamics of fruit fly *Bactrocera cucurbitae* (Coquillett). *Journal of Entomology and Zoology Studies*. **7**(5): 167-170.

Weems, H.V.J and Heppner, J.B. 2001. Melon fly, *Bactrocera cucurbitae* (Coquillett). (Insecta: Diptera: Tephritidae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, and T.R. Fasulo, University of Floridae. University of Florida Publication. Pp 51105.

Wen, H. C. 1985. Field studies on melon fly (*Dacus cucurbitae*) and attractant experiment in southern Taiwan. *Journal of Agricultural Research of China***.** **34**: 228–235.

White, I. M. and Elson-Harris, M. M. 1992. Fruit flies of economic significance: their identification and bionomics. C.A.B., International. Walling ford, UK. 601.

Will, K.W. and Rubinoff, D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*. **20**(1): 47–55.

Wong, Tim, T. Y., Don, O. M. and Mohsen, M. R. 1991. Age related response of male melon flies *Dacuscucurbitae* (Diptera: Tephritidae) to cue-lure. *Journal of Chemical Ecology*. **17**: 2481-2487.

Ye, H. 2001. Distribution of the melon fly (Diptera:Tephritidae) in Yunnan province. *Entomologia Sinica*. **8**: 175-182.

Ye, H. and Liu, J. H. 2005. Population dynamics of the oriental fruit fly,*Bactrocera dorsalis* (Diptera: Tephritidae) in the Kunming area, southwestern China. *Insect Science*. **12**(5): 387-392.

Yong, H. S., Nag Y. F. and Lim, P. E. 2010. Diversity and abundance of Dacinae fruit flies (Insecta: Diptera: Tephritidae) in Chini 2, Runchang and Sungai Bebar, Pahang, Peninsular.*Malaysia Journal of Science and Technology in the Tropics*. **6**: 17-21.

Yong, H. S. 2016. Complete mitochondrial genome of three *Bactrocera* fruit flies of subgenus *Bactrocera* (Diptera: Tephritidae) and their phylogenetic Implications. *Journal of Entomology and Zoology Studies.* **5**(3): 1419-1421.

Zaman, M. 1995. Assessment of the male population of fruit flies through kairomone baited traps and the association of the abundance level with the environmental factors. *Sarhad Journal of Agriculture*. **11**: 657–670.

APPENDIX

APPENDIX-A

Nucleotide sequences of different species of fruit flies

1. *Zeugodacus cucurbitae*

TAGTGGGAACATCTCTTAGAATCTTAGTCCGGGCAGAACTGGGTCACC
CAGGAGCTTTAATCGGAGATGATCAAATCTATAATGTCATCGTAACAG
CTCATGCATTTGTTATGATTTTTTTCATAGTGATACCTATTATAATTGGA
GGATTTGGAAATTGACTAGTACCCCTAATACTAGGAGCGCCAGATATA
GCATTCCCTCGAATGAATAATATAAGATTTTGATTATTACCTCCCTCTC
TTACATTACTTTTAGTGAGCAGTATAGTAGAAAACGGAGCTGGTACAG
GTTGAACTGTTTATCCTCCCCTTTCATCAATTATCGCTCATGGTGGAGC
CTCAGTTGATTTAGCTATTTTTTCTCTACATTTAGCTGGTATTTCATCAA
TTTTAGGGGCCGTAAATTTCATTACTACAGTAATTAATATGCGATCAAC
AGGAATCACATTTGACCGGATACCTTTATTCGTTTGAGCTGTAGTATTG
ACAGCTCTTCTTTTACTTCTATCTCTACCTGTATTAGCCGGAGCTATTAC
TATACTTTTAACAGACCGAAATTTAAACACCTCTTTCTTCGACCCGGCT
GGTGGTGGAGACCCTATTTTATACCAACATTTATTTTGATTTTTTGGAC
ATCCAGAAGTTTA

2. *Zeugodacus tau*

GAGCTTGAGCAGGTATAGTAGGAACATCTCTTAGAATTTTAGTTCGAG
CAGAACTAGGACACCCAGGAGCTTTAATTGGAGATGACCAAATCTATA
ATGTGATCGTAACAGCTCATGCATTTGTTATAATTTTTTTCATGGTAAT
GCCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTCTAATATTA
GGAGCACCAGATATAGCGTTCCCTCGAATGAATAATATAAGATTTTGA
TTATTACCTCCCTCTCTTACATTACTTTTAGTGAGCAGTATAGTAGAAA
ACGGAGCTGGTACAGGTTGAACTGTTTACCCTCCCCTTTCATCAATTAT
CGCTCATGGTGGAGCCTCAGTTGATTTAGCTATTTTTTCTCTACATTTAG
CTGGTATTTCATCAATTTTAGGGGCTGTAAATTTCATTACTACAGTAAT
TAATATACGATCAACAGGGATTACATTTGACCGAATACCTTTATTCGTT
TGAGCTGTAGTATTAACAGCTCTTCTTTTACTTCTATCTCTCCCAGTATT
AGCTGGAGCTATTACTATACTTTTAACAGACCGAAACTTAAATACATCT

TTCTTCGACCCAGCTGGTGGTGGGGATCCTATTTTATACCAACACTTAT
TT

3. *Bactrocera dorsalis*
TGAGCAGGAATAGTAGGAACATCCCTTAGAATTTTAGTCCGAGCTGAA
CTCGGTCACCCAGGAGCTTTAATTGGTGACGATCAAATTTATAATGTAA
TTGTAACAGCTCATGCTTTCGTAATAATTTTCTTTATAGTTATACCAATT
ATAATTGGTGGATTTGGAAATTGACTTGTTCCTTTAATATTAGGAGCTC
CCGATATAGCATTTCCACGAATGAATAATATAAGATTTTGATTATTACC
TCCTTCCCTTACATTACTATTAGTAAGAAGTATAGTAGAAAACGGAGCT
GGTACAGGTTGAACAGTTTACCCACCCCTATCATCTGTTATTGCACACG
GAGGAGCTTCAGTTGACCTAGCTATTTTTTCACTTCACTTAGCGGGTAT
TTCCTCAATTTTAGGAGCAGTAAATTTCATTACAACAGTAATTAATATA
CGATCGACAGGAATCACCTTTGATCGAATACCTCTATTCGTTTGAGCAG
TTGTATTAACAGCTTTATTACTTTTATTATCATTACCAGTTTTAGCAGGG
GCTATTACTATATTACTAACAGACCGAAACTTAAATACTTCCTTTTTTG
ACCCTGCCGGAGGAGGAGATCCTATTCTTTACCAACATTTATTTTGATT
TTTTGGACA

4. *Bactrocera ruiliensis*
TAGTTGGAACATCTCTTAGAATCTTAGTCCGAGCTGAACTCGGTCACCC
AGGAGCTTTAATCGGTGACGATCAAATTTATAATGTAATTGTAACAGC
TCATGCTTTCGTAATAATTTTCTTTATAGTTATACCAATTATAATTGGTG
GATTTGGAAATTGGCTTGTTCCTTTAATATTAGGGGCCCCCGATATAGC
ATTTCCACGAATAAATAATATAAGATTTTGGTTATTACCCCCTTCCCTT
ACATTACTATTAGTAAGAAGTATAGTAGAAAACGGAGCTGGTACAGGT
TGAACAGTTTACCCACCCCTATCATCTGTTATTGCACACGGAGGAGCTT
CAGTTGATCTAGCTATTTTTTCACTTCACTTAGCGGGTATTTCCTCAATT
TTAGGAGCAGTTAATTTCATTACAACAGTAATTAATATACGATCAACA
GGAATTACCTTTGATCGAATACCTCTATTCGTTTGAGCGGTTGTATTAA
CAGCTTTATTACTTTTATTATCATTACCAGTTTTAGCAGGAGCTATTACT
ATATTATTAACAGACCGAAACTTAAATACTTCCTTTTTTGACCCTGCCG
GAG

5. *Bactrocera tuberculata*

TAGTTGGAACATCTCTTAGAATTTTAGTTCGAGCTGAACTAGGTCACCC
TGGAGCATTAATTGGAGATGACCAAATTTATAATGTAATTGTAACAGC
CCATGCTTTCGTAATAATTTTCTTTATAGTAATACCAATTATAATTGGT
GGATTTGGAAATTGACTTGTTCCTTTAATATTAGGTGCTCCCGATATAG
CATTTCCACGAATAAATAATATAAGATTTTGGTTATTACCTCCTTCCCT
TACACTGCTATTAGTAAGAAGTATAGTAGAAAACGGAGCTGGTACAGG
TTGAACAGTTTACCCTCCCCTATCATCTGTTATTGCACACGGAGGTGCT
TCAGTTGATCTAGCTATTTTTTCACTCCACTTAGCCGGGATCTCCTCAAT
TTTAGGGGCAGTTAATTTCATTACAACAGTAATTAATATGCGATCAACA
GGAATTTCTTTTGACCGAATACCACTTTTCGTTTGAGCAGTTGTATTAA
CAGCCTTATTACTTTTATTGTCTCTGCCAGTTTTAGCCGGGGCTATTACT
ATATTACTAACAGACCGGAACTTAAATACTTCATTTTT

APPENDIX-B

Nucleotide sequences of natural enemies of fruit flies

1. *Tapinoma* sp.

TTGGATCTTCAATAAGAATGATTATTCGAATCGAACTAGGAACCTGCG
GACCTTTAATTGGTAATGACCAAATTTATAATTCTATTGTAACAGGACA
TGCATTTATTATAATTTTCTTTATAGTTATACCTTTTATAATTGGTGGAT
TTGGAAACTTCCTAGTACCTCTAATATTAGGAGCACCAGACATAGCAT
ACCCCCGAATAAATAATATAAGATTTTGACTATTACCCCCCTCAATCCT
ATTACTTACTATTAGAAATTTTATTAGATCAGGAGTAGGTACAGGATG
AACAGTTTACCCTCCTCTAGCCTCTAATATTTATCATAATGGACCCTCA
GTTGATTTAGCTATTTTTTCTTTACATATTGCTGGTATATCCTCAATTTT
AGGAGCAATTAATTTTATCTCTACAATCATTAATATACACCATAAAAAT
TTTTCTATTGATAAAATTCCCTTATTAGTTTGATCCATTTTAATCACAGC
TGTATTATTATTACTATCTTTACCTGTATTAGCCGGAGCTATTACTATAT
TATTAACTGATCGCAATTTAAATACTTCTTTCTTCGACCCAT

2. *Oecophylla smaragadina*

ATAGGATCATCAATAAGAATAATTATTCGAATTGAATTAGGATCTCCT
AACTCAATTATTAATAATGATCAAATTTATAATACATTAGTAACTAGTC
ATGCATTCGTTATAATTTTTTTTATAGTTATACCTTTTATAATTGGAGGA
TTTGGAAATTTTTTAGTACCCTTAATATTAGGATCGCCTGATATAGCAT
ATCCTCGTATAAATAATATAAGATTTTGATTATTACCTCCATCAATTTTT
TTATTAATTTTAAGAAATTTTATTGATAATGGTAGGGGTACAGGATGAA
CAGTTTATCCTCCTTTAGCATCTAATATTTTTCATAGAGGTTCTTCTGTT
GATTTAACAATTTTTTCACTTCATATTGCAGGTATTTCATCAATTATAG
GAGCAATTAACTTTATTTCTACTATTCTAAATATACATCATAAAAATTT
TTCAATTGATAAAGTTCCTTTGCTTGTATGATCTATTCTAATTACAGCA
ATTTTATTATTATTATCATTACCTGTATTAGCAGGAGCAATTACTATAC
TACTTACAGATCGAAATTTAAATACCTCATTCTTTGATCCATCA

3. *Crematogaster* sp.

ATTGGGTCTTCTATAAGAATAATTATTCGTCTTGAACTCGGTTCATGTG
ACTCCCTAATTTATAATGATCAAATTTATAATGTCCTTGTCACTGGACA
TGCTTTTGTTATAATTTTTTTTATAGTTATACCATTTATAATTGGGGGAT
TTGGAAATTTCCTAGTACCACTCATACTTGGATCTCCTGATATAGCATA
TCCCCGAATGAACAATATAAGATTTTGACTCCTTCCCCCATCAATTCTT
CTTCTAATTCTTAGTAGATTTCTTAATACCGGCGTAGGTACTGGATGAA
CTATTTACCCACCCTTAGCCTCTAATATCTTTCATAGTGGCCCCTCAGTT
GATCTTTCAATCTTTTCCCTTCACATTGCAGGTATATCCTCAATTTTAGG
AGCTATTAATTTCATTGCTACCATTTTAAATATACACCACAAATCTCTC
TCACTTGATAAAATTACCCTACTAACTTGATCAATCTTAATTACAGCTG
TTCTCCTACTCTTATCATTACCTGTCCTTGCCGGTGCTATTACTATGCTT
TTAACTGATCGTAATCTAAATACC

4. *Paederus fuscipes*

AGTAGGAACATCATTAAGTTTACTAATTCGAGCTGAATTAGCAACCCC
AGGTTCATTAATTGGGGATGACCAAATTTATAATGTTATTGTTACAGCT
CATGCATTCATTATGATTTTTTTCATAGTTATACCTATTATAATTGGGGG
ATTTGGTAATTGATTAGTCCCTTTAATACTTGGAGCCCCTGATATAGCT
TTCCCTCGAATAAACAACATAAGATTTTGATTGTTGCCCCCAGCTTTAA
CACTTTTATTGATAAGAAGAATAGTAGAAAATGGTGCTGGAACAGGAT
GAACAGTGTACCCTCCTCTGTCATCAAATGCATTCCATAATGGATCTTC
TGTTGATCTTGCTATTTTTAGACTTCATTTAGCTGGTATTTCATCAATTT
TAGGTGCAATTAATTTTATTACTACAGCTTTAAATATACGAGCAAGAA
ACATATCTTACGAACAAATACCATTATTTGTTTGATCAGTTGCAATTAC
TGCTTTATTGTTACTTCTTTCATTACCAGTTTTAGCTGGAGCAATTACAA
TATTATTAACAGATCGAAATTTAAATACTTCATTCTTCGACCCT

5. *Polyrhachis* sp.

TAAGAATAATTATTCGCTTAGAATTAGGTTCACCCAACTCACTAATTCT
CAATGATCAAACTTTTAACTCTATTGTCACAAGACATGCTTTTATCATA

ATTTTTTTTATAGTTATACCATTTATAATTGGAGGATTTGGAAATTTCTT
AGTGCCTCTGATAATTGGAACTCCTGATATAGCAAACCCTCGTATAAAT
AACATAAGATTCTGACTCCTACCCCCATCAATTTCTTTATTACTCCTAA
GTAACTTTATTAATGAAGGGTCAGGAACAGGATGAACTGTCTACCCTC
CCTTAGCGTCCAACTCATTTCACAGAGGCCCATCAATCGACCTAACTAT
CTTTTCTCTTCATATTGCTGGAATATCATCAATTCTA