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Survey on species distribution of moths (Insect: lepidoptera) in Chemancheri panchayath, Calicut, Kerala, India

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Abstract
Lepidoptera remain the critical thing of earthly ecologies owing to their part as nourishment assets intended for natural world and small animals, nightly time pollinators, food recyclers and capability signs. The recent aspect at could be situated the first document on lepidopteran categories identity, populace, species arrangement, profusion, in addition their reputation primarily founded on *International Union for Conservation of Nature* group of lepidopterans-moth in Chemancheri, Calicut, Kerala. On these observe duration, absolutely 104 sample moths have been saved from the 4 specimen places. Available of these, 61 specimens remained recognized, 37 varieties are combined in to 11 households and 22 samples are united in to 18 species. Based at the outcome indicates the uppermost range of night butterfly species, the *Erebidae* family turned into the maximum leading, on behalf of 36 species and accompanied by means of *Crambidae* representing 7 species, *Noctuidae* and *Limacodidae* with 4 species each, *Notodontidae* representing three species, *Dripanidae* representing 2 species, *Eupterotidae*, *Zygaenidae*, *Geometridae*, *Bombycidae*, *Saturnidae* with one species each. So, as of this we are able to finish that species within the family *Erebidae* and *Crambidae* are dominant within the places when as compared to other households. The present-day look at turned into intended to locate the variety and species arrangement of moth's current at exceptional trophic stage in nourishment food chain for maintainable biological procedure in Chemancheri, Calicut district, Kerala.

Keywords: Lepidoptera, *Asota tortuosa*, moths species, profusion, nightly time pollinators, nourishment food chain

Introduction

Insects are the maximum neglected institution with regards to the sector of flora and fauna and conservation. Were aware in their vital parts in effective environment facilities. Other than that, their effects on agriculture farming, social fitness in addition economical are well value stating. In India it's far anticipated that about 12,000 kinds of lepidopterans have its place to 41 households be located (Chandra, 2007) [1]. Lepidoptera exceptionally dependent awake on the nearby farm sample. Weather thing similar temperature, moisture, rain, breeze velocity, wind course likewise a completely vital to being of the species, and also nourishment obligation. It has been foretold that weather heating concluded the path of the following period determination motive and growth in international regular temperature of among 1.4^0 C too 5.8^0 C Intergovernmental Panel on Climate Change, 2007). India, presence a sizeable by extensive difference in bodily capabilities, weather and plants, owns single of the greatest profligate and maximum numerous plants and animals within the ecosphere.

Lepidoptera is the maximum wonderful order of herbivorous insects with the range of described species starting from 1,46,277 to 1, 74,250. Lepidoptera type consisting of circumscribed family-wise variety of recognized species under 15,578 genera of 133 households. Order Lepidoptera comprises suborders *viz*. Rhopalocera, butterflies (18,768 species below 1815 genera of 7 households in one superfamily, Papilionoidea) and Heteroceraie, moths (together with almost 30 superfamilies). In India, despite the fact that an estimated 15,000 Lepidopteran range is largely uneven across the united states (Ramakrishna and Alfred, 2007). Order Lepidoptera is the second one biggest insect order of holometabolous, endopterygotes, scaly winged bugs, most of them are moths with approximately 1,60,000 species global (Thakur & Pawan Kumar. 2014) [2].

Butterflies and moths that are remembered for the order Lepidoptera are maximum normal insect of the forest environments and farming fields and are regularly named because the natural marks of the organic gadget. Butterflies and moths are extremely sensitive to change in crops and structure, and specific sorts of vegetation display specific species compositions. So, butterflies and moth assemblages may be used to symbolize one-of-a-kind habitats. Plants are the vital source of nutrients for butterflies and moths: some precise plant species provide the trophic sources for caterpillars, while others offer nectar for adults. The plants also can play a vital role in their survival, offering particular structural elements for sun-basking or mating and determining certain appropriate microclimates. Therefore, it would be predicted that butterflies and moth will respond extra strongly to plants gradients than to edaphic gradients (Sawchik *et al*., 2003) [4].

Moths (Lepidoptera) are a vital issue of terrestrial ecosystems because of their function as food resources for birds (Wilson *et al*., 1999) [3] and small mammals, as pollinators (Proctor *et al*., 1996 Devoto *et al*., 2011) [12, 14], and nutrient recyclers (Merckx *et al*., 2013) [13]. Moths are cosmopolitan in distribution happening in every plausible habitat the front plane to deserts, forests and valleys of hills and mountains.

The variety and distribution of moth fauna may also reflect the biodiversity of plant life of the area being sampled.

All activities of moths aren't prompted with the aid of sure ecological adjustments, so susceptibility varies amongst taxonomic category. Therefore, the tracking of lepidopteran groups that answers to variations in habitation excellent establishes a crucial device for organic protection. Extensive farming and cropping speeds up modifications inside species arrangement in addition plenty of vegetal groups, and the ensuing damage of widespread crowd vegetation principals to a consistent lack of expert night-butterfly(moth) animals (Faiz *et al*.2018) [5]. Moths, like other insects, contain a very good organization of studies on conservation and ecology. Maximum moth species are nocturnal and can be without problems interested in mild traps, bearing in mind efficient estimates of relative abundance and geographical styles of species richness (Choi 2008) [6] greater over moths are bio signs of habitat great, and that they reply to human disturbances (Kitching *et al*., 2000) and successional techniques.

Materials and methods

Sampling Site

The survey be located in four places in Chemancheri village, Calicut, Kerala organizes 11.411590° N 75.734990° E: site A (LA 11° 23′ 55” N, LO 75 °44′ 14” E and AL 14.88 meters), Site B (LA 11° 23′ 58” N, LO 75° 44′ 14” E and AL 17.99 meters), Site C (LA 11° 23′ 40” N, LO 75° 43′ 36” E and AL 14.48 meters) Site D (LA 11.40′20”, LO 75°.74′85” and AL 77.8 meters) (Fig. 1).

Test group method

Maximum of the lepidoptera is night-time in habit only a limited are cellular in night-time in addition to daytime. Night butterfly group was completed as of nighttime forwards till early morning on subsequent diurnal experiential by mild trap technique (Fig. 2). Mild –supply changed into hardened sample moths at many places. Not any additional sample approach remains additional positive for shooting moths equally huge number of individuals and qualitatively (Muirhead-Thomson 1991) [16]. Group of night butterflies by means of the use of actinic blue tubes. Mostly handy technique used is sheet technique. The white color cloth material sheet stands droopy among dual perpendicular poles. Mild basis has to be positioned in such manner that whole sheet luminously reacts the mild. The night butterfly composed had been killed by the usage of the chemical properties of ethyl acetate and conserved in airtight covered insect container, consuming naphthalene balls and camphor as fumigant.

Identification of Moths

Documentation of butterflies remained passed available with the assistance of a skilled, proof of identity key, standard reference records, based on books and web sources. The samples were collected during the study were too acknowledged with reference to the related literature (Gurule *et al*., 2010, 2011a, b, 2013a, b, 2014, Hampson, 1891, 1892, 1895, 1896; Holloway, 1985, 1988, 1998;) [15, 17, 18, 19, 20, 21, 22, 23, 24, 25].

Data analysis

The Data analysis was done by using Microsoft excel,

Molecular analysis

Genomic DNA isolation from the insect

DNA isolation was executed by the usage of Qiagen DNeasy kit standardized protocol via setting up to 50-100mg moths in a mortar and pestle and was homogenized without any chemicals (partial homogenizer). The homogenized sample was then transferred to one.5ml centrifuge tube, brought with a hundred and eighty – 2 hundred µl of PBS and was homogenized the use of the tissue ruptor. 20-30 µl of proteinase okay and two hundred µl AL Buffer (without introduced ethanol) changed into delivered and mixed very well via vortexing and become incubated at 75° c for 15 minutes. After incubation the pattern was mixed thoroughly by means of vortaxing and became centrifuged at 10,000rpm for 2 mins. The supernatant from the 4th step turned into pipetted out and changed into transferred to at least one.5ml centrifuge tube and three hundred µl of 99-100% ethanol became added and mixed thoroughly via vortexing. The mix from 5th step (consisting of any precipitate) was pipetted into DNA spin column which changed into placed in a 2ml accumulating tube and become centrifuged at 8000rpm for 1 minute. The float changed into discarded in an accumulating tube. DNeasy spin column was positioned in a brand new 2ml accumulating tube and 500µl of AW 1 Buffer was brought and centrifuged at 8000rpm for 1 minute. The flow via become discarded. 5oo µl of AW 2 Buffer changed into brought and centrifuged for 3 minutes at 14,000 rpm to dry the DNeasy membrane. DNeasy mini spin column become placed in a smooth 1.5 or 2ml micro centrifuge tube and 100 µl of AE buffer turned into brought at once into the DNeasy membrane. Incubation changed into finished at working room temperature for 1 minute and become at that time centrifugated for one minute at 8000rpm and DNA was eluted. DNA affirmation turned into examined by the use of Agarose Gel Electrophoresis.

Agarose gel electrophoresis protocol

Prepare 365ml of 1X TAE Buffer (working solution) i.e., 365ml = 350ml (tank potential) + 15ml (boat ability). Put together 1% Agarose gel by means of dissolving 150mg in 15ml of TAE Buffer. melt the above agarose and buffer mixture at 1 min with the aid of retaining it in micro-oven and also add a pinch of Ethidium bromide to it. Cool the combination at room temperature and pour it into the boat and wait until the gel casts. Then restoration the properly comb in it. After casting, put off the brush cautiously without negative the wells. The boat is constant into the tank packed with 350 ml of 1X TAE Buffer. Mix the loading dye and the sample DNA (2:6) ratio and cargo it carefully into the wells of the casted gel. Finally, the gel is administered at 100 volts for half-hour and it is appropriately removed and located in UV Transilluminator or Gel doc to affirm whether the DNA is present or now not.

PCR- Polymerase Chain Reaction Protocol

PCR amplification was done using the following primers for mitochondrial DNA, CO1 gene. Amplification was performed in a total volume of 50µl containing 4µl of DNA template, 20 pmol of each primer 400µm of DNTP and 0.4µl of Taq DNA polymerase (Qiagen). The Thermocycler conditions were slightly modified from Swapnil *et.al*., 2009 and are as follows; 1 initial cycle of 1 minute at 94°C followed by 5 cycles of 94°C for 1 minute, 45°C for 1minute 30 seconds, 72°C for 1minute 15 seconds, then 30 cycles of 94°C for 1minute, 51°C for 1minute 30 cycles, 72°C for 5 minutes. The obtained PCR product was checked using 1% Agarose gel.

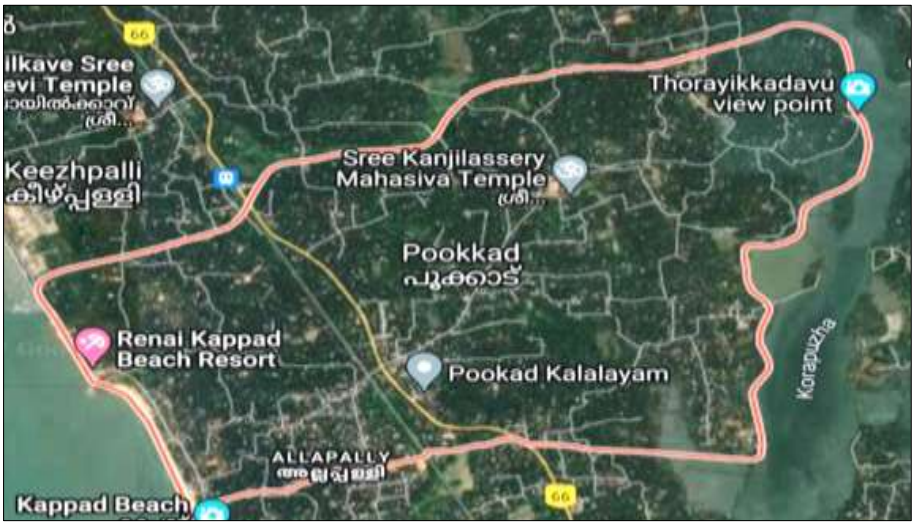

Fig 1: Topographic map of Chemancheri panchayath, Kozhikode, Kerala

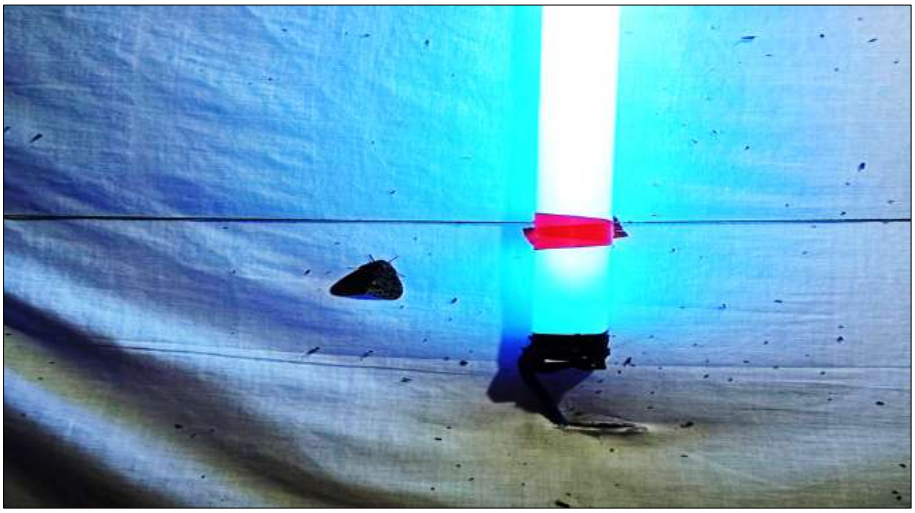

Fig 2: Acting light trap

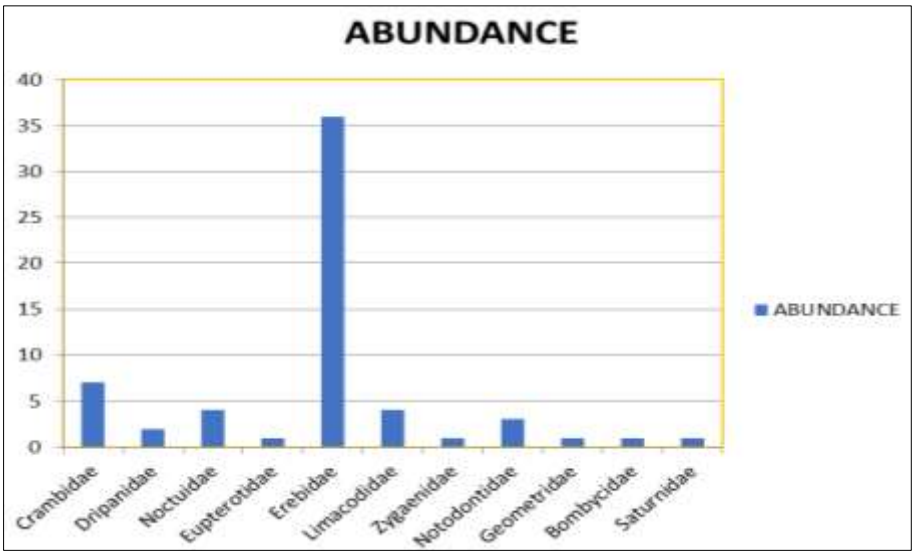

Fig 3: The abundance of moth species

Table 1: Details of the primer used in this study

Gene and Name of the Primer	Primer Sequence	Organisms
COI: LEP-F1 (Forward)	5'ATTCAACCAATCATAAAGATATTCG 3'	Butterflies (Hebert *et al*, 2004)
COI: LEP-R1 (Reverse)	5'TAAACTTCTGGATGTCCAAAAAATCA'	

Table 2: List of moth species identified

S.no	Genus	Species	Family
1.	*Achaea*	*Serva*	Erebidae
2.	*Aethaloessa*	*Sps*	Crambidae
3.	*Asota*	*heliconia*	Erebidae
4.	*Asota*	*producta*	Erebidae
5.	*Asota*	*tortuosa*	Erebidae
6.	*Amata*	*passalis*	Erebidae
7.	*Attacus*	*Atlas*	Saturniidae
8.	*Conogethes*	*Sps*	Crambidae
9.	*Creatonotos*	*gangis*	Erebidae
10.	*Ctenoplusia*	*Sps*	Noctuidae
11.	*Cyclosia*	*Sps*	Zygaenidae
12.	*Diaphania*	*Indica*	Crambidae
13.	*Erebus*	*ephesperis*	Erebidae
14.	*Eudocima*	*hypermnestra*	Erebidae
15.	*Eudocima*	*phalonia*	Erebidae
16.	*Eupterote*	*Sps*	Eupterotidae
17.	*Euproctis*	*Sps*	Erebidae
18.	*Egnasia*	*participalis*	Noctuidae
19.	*Glyphodes*	*Sps*	Crambidae
20.	*Herpetogramma*	*Sps*	Crambidae
21.	*Lymantria*	*Sps*	Erebidae
22.	*Leucoma*	*Sps*	Erebidae
23.	*Miresa*	*Sps*	Limacodidae
24.	*Miltochrista*	*Sps*	Erebidae
25.	*Mocis*	*undata*	Erebidae
26.	*Nepita*	*conferta*	Erebidae
27.	*Olepa*	*Ricini*	Erebidae
28.	*Phalacra*	*Sps*	Drepanidae
29.	*Perina*	*Nuda*	Erebidae
30.	*Polytela*	*gloriosae*	Noctuidae
31.	*Psalis*	*pennatula*	Erebidae
32.	*Sameodes*	*Sps*	Crambidae
33.	*Syntomoides*	*Sps*	Erebidae
34.	*Turnaca*	*Sps*	Notodontidae
35.	*Thalassodes*	*Sps*	Geometridae
36.	*Trilocha*	*Sps*	Bombycidae
37.	*Trichoplusia*	*Sps*	Noctuidae

Table 3: The abundance of moth species in each family

S.no	Family	Abundance
1.	Crambidae	7
2.	Dripanidae	2
3.	Noctuidae	4
4.	Eupterotidae	1
5.	Erebidae	36
6.	Limacodidae	4
7.	Zygaenidae	1
8.	Notodontidae	3
9.	Geometridae	1
10.	Bombycidae	1
11.	Saturnidae	1

Table 4: Number of species in four different habitats in 11 different families

Family	Number of species	Site A	Site B	Site C	Site D
Crambidae	7	6	-	1	-
Dripanidae	2	1	1	-	-
Noctuidae	4	1	2	-	1
Eupterotidae	1	1	-	-	-
Erebidae	36	10	10	8	8
Limacodidae	4	1	3	-	-
Zygaenidae	1	-	1	-	-
Notodontidae	3	1	-	2	-
Geometridae	1	1	-	-	-
Bombycidae	1	-	-	1	-
Saturnidae	1	-	-	-	1

Results

The end result proven that the at some stage in the prevailing survey on species of Lepidoptera within the Chemancheri village, Calicut, Kerala. (Figure:1.). Here in observe definitely 104 specimens had been amassed from the 4 sampling sites. The number of individuals of different species of moths are presented in Table: 2. Out of those, 61 specimens have been recognized, 37 specimens are consolidated in to 11 households and 22 specimens are consolidated in to 18 species. The result suggests the very best quantity of moth species (36) belonging to Erebidae family (Table: 3; Figure: 2) and observed by using Crambidae (7), Noctuidae (4), Limacodidae (4), Notodontidae (3), Dripanidae (2), Eupterotidae (1), Zygaenidae (1), Geometridae (1), Bombycidae (1), Saturnidae (1) (Fig 2 and Fig 3). From Table 4 suggests that commonly Erebidae circle of family greater in number in all sites. Next to own family Crambidae, the species are more in variety in site A. Subsequent to circle of family Crambidae households inclusive of Noctuidae, Limacodidae, Notodondidae, Eupterotidae, Zygaenidae, Geometridae, Bombycidae, Saturniidae are not a whole lot in number. So, from this we will conclude that species inside the family Erebidae and Crambidae are dominant in the sites when as correlated to different families.

Sequencing of PCR Product

The sequencing mix composition and PCR conditions are as follows, (Table: 1).

>Sample 1 (*Asota tortuosa* 658 bp)

TACATTATATTTCATTTTTGGTATTTGAGCAGGAAT
AGTAGGAACATCTTTAAGACTATTAATTCGAGCTG
AATTGGGTAATCCAGGATCTTTAATTGGAGATGAT
CAAATTTATAATACTATTGTTACTGCTCATGCTTTT
ATTATAATTTTTTTTATAGTAATACCTATTATAATT
GGAGGTTTTGGTAATTGATTAGTACCTCTTATATTA
GGAGCCCCTGATATAGCTTTCCCCCGAATAAATAA
TATAAGTTTTTGACTACTTCCCCCATCATTAACTTT
ATTAATCTCGAGAAGAATTGTAGAAAATGGAGCAG
GAACAGGATGAACAGTTTACCCCCCACTTTCATCT
AATATTGCCCATAGAGGGAGATCCGTTGATTTAGC
TATTTTTTCATTACATTTAGCTGGAATTTCTTCAATT
TTAGGAGCCATTAACTTCATTACTACAATTATTAAC
ATACGATTAAATAATTTATCATTTGATCAGATACCT
TTATTTGTTTGAGCAGTAGGAATTACAGCATTCTTA
TTACTTTTATCATTACCAGTTTTAGCAGGAGCTATT
ACTATACTTCTTACTGATCGAAACTTAAATACATCT
TTCTTTGATCCTGCTGGAGGTGGAGATCCAATTCTA
TATCAACATTTATTT

>Sample 2 (*Creatonotos gangis 632bp*)

GAGCAGGAATAGTAGGAACTTCATTAAGATTATTA
ATTCGAGCAGAATTAGGAACTCCTGGTTCTCTAATT
GGTGATGATCAAATTTATAATACTATTGTAACAGC
TCATGCTTTTATTATAATTTTTTTTATAGTTATACCT
ATTATAATTGGTGGATTTGGAAATTGATTAGTACCT
TTAATATTAGGAGCTCCTGATATAGCTTTCCCTCGA
ATAAATAATATAAGTTTTTGACTTTTGCCCCCATCT
TTAACTTTATTAATTTCAAGAAGAATTGTAGAAAA
TGGAGCAGGAACAGGATGAACAGTTTATCCTCCTC
TTTCCTCTAATATTGCTCACGGAGGTAGATCTGTTG
ACTTAGCTATTTTCTCCCTTCATTTAGCTGGAATCT
CTTCTATTTTAGGGGCCATTAATTTTATTACTACAA
TCATTAATATAAAACTAAATAATCTTTCATTTGATC
AAATACCTTTATTTGTATGAGCAGTAGGCATTACA
GCCTTTTTACTTTTATTATCTCTTCCTGTTTTAGCTG
GAGCTATTACTATACTTTTAACAGATCGAAATTTAA
ATACTTCATTTTTTGACCCTGCTGGAGGGGGTGACC
CTATTCTTTATCAACATTTATTT

>Sample 3 (*Psalis pennatula 586 bp*)

ATAATCGGAACTTCATTAAGTTTATTAATTCGAGCA
GAATTAGGTACTCCTGGATCATTAATTGGAAATGA
TCAAATTTATAACACTATTGTAACAGCTCATGCATT
TATTATAATTATTTTCATAGTAATACCAATTATAAT
TGGAGGATTTGGTAACTGATTAGTTCCTTTAATATT
AGGAGCCCCAGATATAGCATTCCGCCGAATAAATA
ATATAAGATTTTGACTTTTACCTCCCTCTTTAACCC
TACTTATCTCTAGAAGAATTGTAGAAAATGGAGCG
GGAACAGGATGAACAGTTTACCGCCCTTTATCTTCT
AATATTGCTCATGGTGGAAGATCTGTAGATTTAGC
TATTTTTTCTTTACATTTAGCTGGAATTTCTTCAATT
TTAGGAGCAATTAACTTTATCACTACAATTATTAAC
ATACGATTAAATAACTTATCATTTGATCAAATACCT
TTATTTGTTTGAGCTGTAGGAATTACAGCATTCTTA
CTTCTTCTTTCTCTTCCTGTATTAGCTGGAGCAATT
ACTATACTTTTAACAGACCGAAATCTTAATACTTCA
TTTTTGACCCAGC

>Sample 4 (*Egnasia participalis 632bp*)

AACTTTATATTTTATTTTTGGAATTTGATCTGGAAT
AGTAGGAACTTCATTAAGATTATTAATTCGAGCTG
AATTAGGAACTCCTGGATCATTAATTGGAGATGAT
CAAATCTATAATACAATTGTTACTGGTCATGCTTTA
TTATAATTTTTTTTTATAGTAATACCTATTATAATTG
GAGGATTTGGAAATTGATTAGTTCCTTTAATACTTG
GAGCACCTGATATAGCTTTCCCCCGCATAAATAAC
ATAAGATTTTGACTTTTACCCCCTTCCTTAACTCTA
TTAATTTCAAGATCTATTGTAGAAAATGGAGCTGG
AACTGGATGAACGGTTTACCCCCCCCTTTCATCAA
ATATTGCTCATGGAGGAAGATCAGTTGATTTAGCT
ATTTTTTCCCTACATTTAGCTGGAATTTCCTCAATTT
TAGGAGCAATTAATTTTATTACAACTATTATTAATA
TAAAATTAAATGGAATAATATTCGATCAAATACCT
TTATTTGTTTGAGCTGTAGGAATTACAGCCCTTCTT
CTTCTTCTATCCCTACCAGTTTTAGCGGGGGCAATT
ACTATACTTTTAACAGATCGCAATCTTAATACTTCT
TTTTTTGATCCTGCTGGAGGAGGAGATCCAATTCTA
TATCAACATTTATTT

Discussion

In the modern explanation changed into executed on species of Lepidoptera inside the 4 sites of Chemancheri village, Calicut district, Kerala. Here in observe 61 people are have its place to 11 familes (Crambidae, Drepanidae, Noctuidae, Eupterotidae, Erebidae, Limacodidae, Zygaeniadae, Notodontidae, Geometridae, Bombycidae, Saturniidae) and 18 Species. Painted apple lepidopterans are trades of moths that establish the household Erebidae. Erebidae are an own family of night butterfly in the supper own family Noctuoidea. Erebidae own family is essentially cosmopolitan. The family is amongst the biggest households of moths by means of species depend and incorporate a wide form of macro moth valuable business.

The up-to-date observe is a fast investigation which lead to inside the guidance of a preliminary impulse list of moths from Chemancheri village. The study zone is a semi-built-up

interplanetary with a wide type of farm with dispersed covers of reasonably profuse flora, lawns, bushes, and exposed region. It indicates a mild variety of moths in its numerous habitats. Amongst insects, the moths (*Lepidoptera*) species are economically essential number one herbivores in the forest environment and distinct surroundings. They may be numerous in their behavior and tailored to kind of natural situations.

Inside the studies shows that, the moth (*Lepidopterans*) species are useful for ecological and conservation research in view that maximum of them are nocturnal fauna and are letting notably inexperienced approximation of earthly styles of diversity and abundance. In addition, many researchers mentioned that the moths play an essential position in ecological indicators of plant variety in addition to nearby lands and forests administration due to their host-precise (Ricketts *et al*., 2001; Summerville *et al*., 2004; Usher and Kieller 1998; and Kitching *et al*., 2000;) [9, 10, 11] and they suggested that moth point to the nice of habitat in temperate of agronomic and forestry. Usher and Kieller (1998) [11] cited that there has been no difference amongst moth organizations of coniferous, deciduous, blended canopy woodlands and wooded vicinity edges, due to the fact moth species feeding on herbaceous flowers and non-affected forest environment.

Conclusion

The prevailing study have become intended to discover the variety and species arrangement of moth (*Lepidopterans*) present at delightful trophic grade in food chain for supportable ecological machine in Chemancheri village, Calicut, Kerala. The profusion of moth variety decided throughout this initial examines promises that the variety of moths on this zone is enormous. Insect being a mega numerous groups form a first-rate component of biodiversity in any location and therefore surveying and documenting this fauna will indispensably make contributions to many medical research and conservation programs (Kendrick 2002). A total of 61 species belonging to eleven households were collected from the examine place. The species were interested in mild at night time; generally, moth species had been present below mild. All moth species were herbivorous. Moths were found in specific habitats and consumed specific plants. All moths were drawn to light at night. The moths were determined to be very sensitive to alterations.

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