STUDIES ON THE ECOLOGY AND BEHAVIOUR PATTERNS OF ANTLION LARVAE (FAMILY: MYRMELEONTIDAE, ORDER: NEUROPTERA) OF SELECTED HABITATS OF KERALA

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For the award of the degree of

DOCTOR OF PHILOSOPHY in ZOOLOGY

By

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Under the Supervision of

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2022

DECLARATION

I hereby declare that the thesis entitled "**STUDIES ON THE ECOLOGY AND BEHAVIOUR PATTERNS OF ANTLION LARVAE (FAMILY: MYRMELEONTIDAE, ORDER: NEUROPTERA) OF SELECTED HABITATS OF KERALA**" submitted to University of Calicut for the award of Doctor Of Philosophy in Zoology is a bonafide research work done by me under the supervision and guidance of Dr. Francy K Kakkassery, Associate Professor & Head (Retd.), Research and Postgraduate Department of Zoology, St. Thomas College' (Autonomous), Thrissur.

I also declare that the findings presented in this thesis is original and does not form the basis for the award of any other degree, diploma or other similar titles of any other university

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CERTIFICATE

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Thrissur

Dr. Francy K Kakkassery

Research Guide

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Anila K

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CHAPTER -1

GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

Entomology is a branch of science which deals with the study of insects. Insects are a group of highly adaptable invertebrates coming under the largest animal Phylum Arthropoda (Arthro-joint or segment, poda-foot or appendage). The body of an insect is divided in to head, thorax and abdomen in which, thorax has three pairs of legs and one or two pairs of wings. Phylum Arthropoda divided into classes and Insecta is one of the biggest Class of Phylum Arthropoda. Class Insecta is divided into two subclass (Apterygota and Pterygota) based on the presence or absence of wings. Class Insecta again divided into thirty orders on the basis of the structure of wings, mouthparts and metamorphosis etc.

Previously Thysanura, Diplura, Protura and Collembola were included in Subclass Apterygota and the remaining 26 orders are again classified as exopterygotes and endopterygotes under Subclass Pterygota. Exopterygotes are insects with incomplete metamorphosis, wings develop externally and the immature stages resemble the adults. Exopterygotes includes Order Ephemeroptera, Odonata, Plecoptera, Grylloblattodea, Orthoptera, Phasmida, Dermaptera, Embioptera, Dictyoptera, Isoptera, Zoraptera, Psocoptera, Mallophaga, Siphungulata, Hemiptera and Thysanoptera. Endopterygotes are insects with a complete metamorphosis and the wings develop internally and the larvae differ from adults in structure and habitats. Endopterygotes includes Order Neuroptera, Coleoptera, Strepsiptera, Mecoptera, Siphonaptera, Diptera, Lepidoptera, Trichoptera and Hymenoptera (Imms *et al*., 1977). But in a recent classification Protura, Collembola and Diplura are upgraded from the status of Order to Class. The remaining 27 orders are classified under Class Insecta (Parsons, 2015).

Order Neuroptera is one of the Order of Class Insecta, known by about 5000 species from the world; most of them are represented in the temperate and subtropical regions. The members of this order includes a varied, though interrelated assemblage of holometabolous, carnivorous insects most commonly known as Antlions, Lacewings, Snake flies, Alder flies and Dobson flies (Nayar *et al.*, 1976). In earlier, Megaloptera, Raphidiodea, and Neuroptera were considered as Suborders of Order

Neuroptera (Borror and Delong, 1975), but now, these three are considered as three separate Orders. Order Megaloptera includes alder flies and dobson flies, Order Raphidiodea includes snakeflies and Order Neuroptera includes lacewings, mantispids and antlions (Gillot, 2005). The research work mainly focussed on Family Myrmeleontidae, one of the families of Order Neuroptera.

The term Neuroptera derived from two Greek words "Neuro" (nerve) and "ptera" (wings), and they are very similar in appearance to damsel flies (Order Odonata) with long, narrow, many veined wings and a slender abdomen (Borror and Delong, 1975). There are approximately 5000 species of Neuropteran recorded worldwide in which 335 species belonging to 125 genera and 13 families are known from India (Chandra *et al.*, 2014).

1.1. GENERAL CHARACTERS OF ORDER NEUROPTERA

As a group, the Neuropteroids are characterized by the nature of their wings which are membranous and almost subequal. The front and hind wings of most Neuroptera are similar in shape and venation, and are held roof like over abdomen when at rest. They are usually weak fliers. They have chewing type of mouth parts and antennae long, many segmented (Borror and Delong, 1975). These insects undergo complete metamorphosis and the eggs are laid singly or in masses in various situations. Generally the larvae are compodeiform type. The larvae of lacewings and antlions with sickle shaped mandibles suck the body fluids of the victim through a narrow channel formed between the mandibles and the maxillae. Pupation usually occurs in a silken cocoon; this silk is not derived from modified salivary glands as in most insects, but is produced by the malpighian tubules and is spun from the anus. The study of malpighian tubules of *Myrmeleon uniformis* larval instars shows the presence of precursors of silk fibres for the secretion of silk fibres for a cocoon (Pacheco *et al.*, 2014).

Order Neuroptera is divided into three suborders namely, Megaloptera, Raphidiodea and Planipennia. Family Myrmeleontidae is coming under Suborder Planipennia and Superfamily Myrmeleontoidea. The name Myrmeleontidae is rooted in the Greek words 'myrmex' (ant) and 'leon' (lion).

1.2. GENERAL CHARACTERS OF FAMILY MYRMELEONTIDAE

The adult of this group are rather feeble fliers and are often attracted to lights. The wings are clear in some species and irregularly spotted in others. Antlion larvae, or doodle bugs are queer looking creatures with long, sickle like jaws. Some species have an interesting method of capturing their prey, they conceal themselves at the bottom of a small conical pit, made in dry sand or dust, and feed on ants and other insects that fall down into this pits. The pit digging antlions are called 'doodlebugs' in the United States because of the designs they make in the sand. A doodlebug seeks an ideal location to dig its pit, it leaves meandering trails that resemble the random "doodles" of a preoccupied artist. When it finally finds the right place to dig, the doodlebug draws a series of concentric spirals, each deeper than the last, until the pit is excavated.

The adult antlion usually emerges from its cocoon in the evening. It is not yet able to fly, so it climbs at the nearest plant, where it waits for its wings to expand and harden. The adult antlion may eat small flies or water, but its real purpose is reproduction, not feeding. The development is comparatively slow and requires a year or so to become adult (Kapoor, 1985).The sex can be determined by the presence of oviposition setae on females and the pilula axilaris at the base of the hind wing on males. The general morphology of pit building antlion larvae and adult were given in Plate 1, Plate 2 and Plate 3. An idea about doodle marks, larval pits and life cycle are given in Plate 4. Family Myrmeleontidae divided into five subfamilies which include 201 genera and 14 tribes. The Subfamilies are Stilbopterigynae, Palparinae, Myrmeleontinae, Araripeneurinae, and Paleoleontinae (Stange, 2004). Only two subfamilies are seen in India (Subfamily Myrmeleontinae & Subfamily Palparinae) and the classification is described below,

Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Neuroptera

Suborder	:	Planipennia
Super family	:	Myrmeleontoidea
Family	:	Myrmeleontidae

1.2.1. Behaviour of larval Myrmeleontids

Larval myrmeleontids possess a number of behavioural patterns like pit building, predation and feeding. For predation, they opt a strategy called conical shape pit making. They make conical pits in sand or soil and wait inside the pit for the arrival of prey. Mostly the ants (Order Hymenoptera) are the victims of antlion predation. Again the pit making is also a beautiful process in which the larvae move backward with the flickering of soil or sand with its mouths part. The mouth parts also have a mechanism which helps in the feeding of larval antlion. Their mouthparts have a hollow mandible which helps to suck the body fluid of prey.

1.2.2. Natural enemies/Predators of antlion larvae

Successful natural ecosystems consist of predators, prey and its specific habitats and the population of each species was controlled and maintained by these preys and predators. Diptera, Hymenoptera, Neuroptera, Arachnida and Lizards are the main natural enemies of larval antlions (Stange, 2004). For increasing the survival rate, the larval antlions relocate its pits in the presence of natural enemies and make steeper pits, but it is different in different species. *Myrmeleon hyalinus* larvae reduce its pit building activity in the presence of its predator wolf spider (Loria *et al*., 2008).

1.2.3. Habitat

The habitat of antlion larvae was different in different places and countries, and the habitat preference is species specific too. The common pit building antlion is coming under Genus *Myrmeleon*, but the species of this genus has different habitats. *Myrmeleon carolinus* found in hot open areas but *Myrmeleon crudelis* found in cooler shaded areas (Lucas, 1985). *Myrmeleon immaculatus* found along the beach close to the vegetation, and under bank or log overhangs (Heinrich and Heinrich, 1984). *Myrmeleon obscures* found in shaded areas, but *Cueta pallens* present in open areas (Fisher, 1989).

Plate 1

General Morphology -Adult

Antenna-Clubbed

Wing -Apical portion

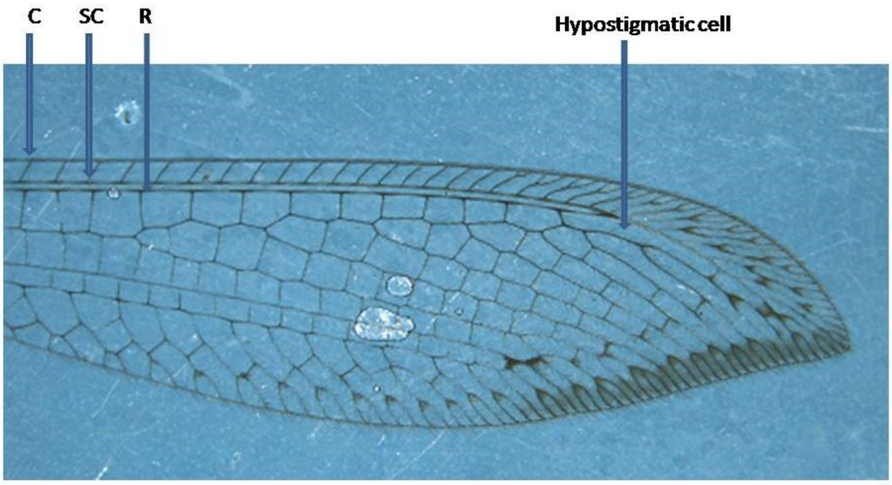
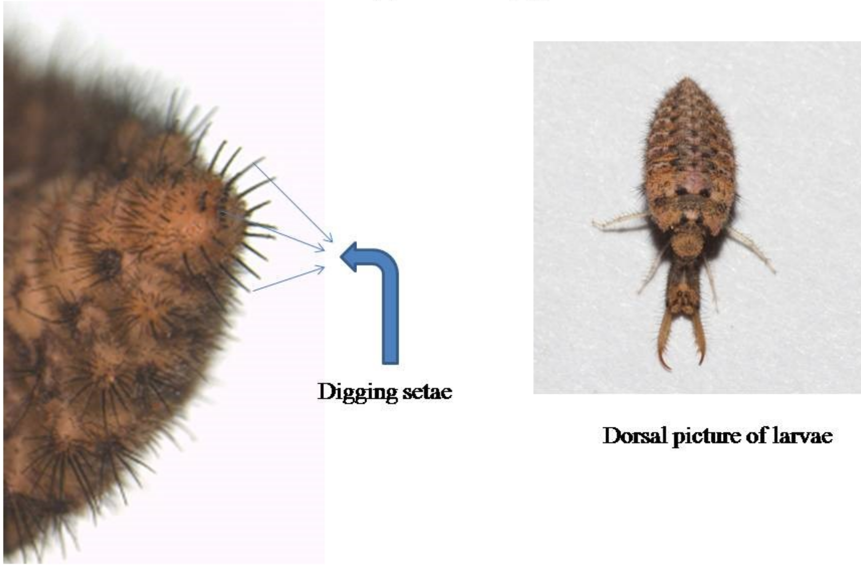


Plate 2

General Morphology- Larvae



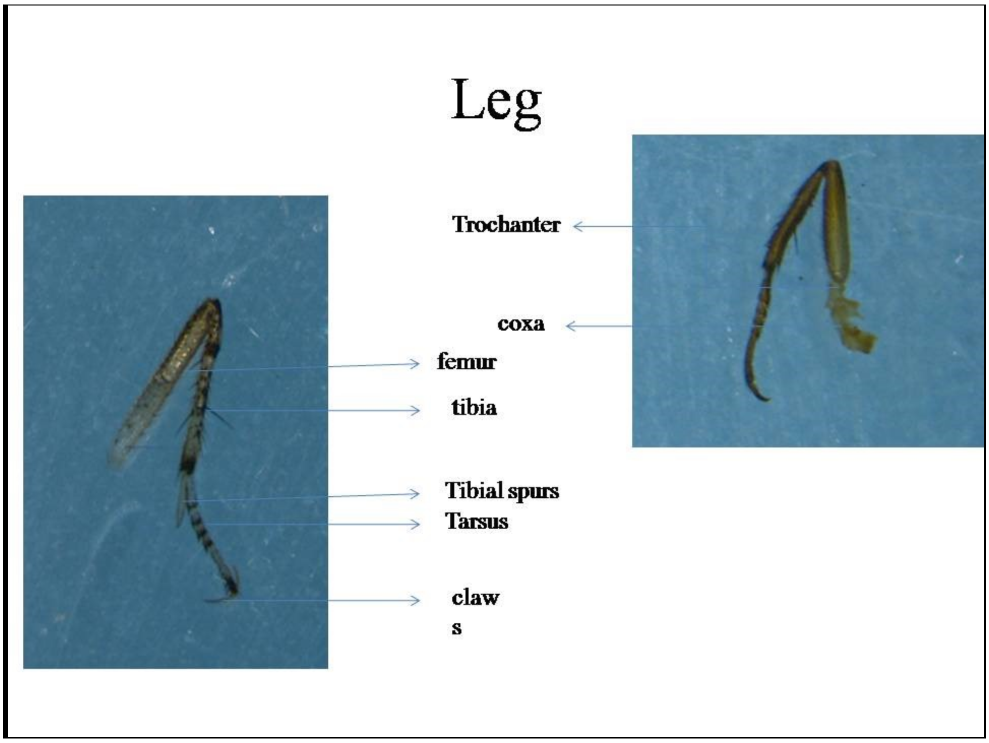
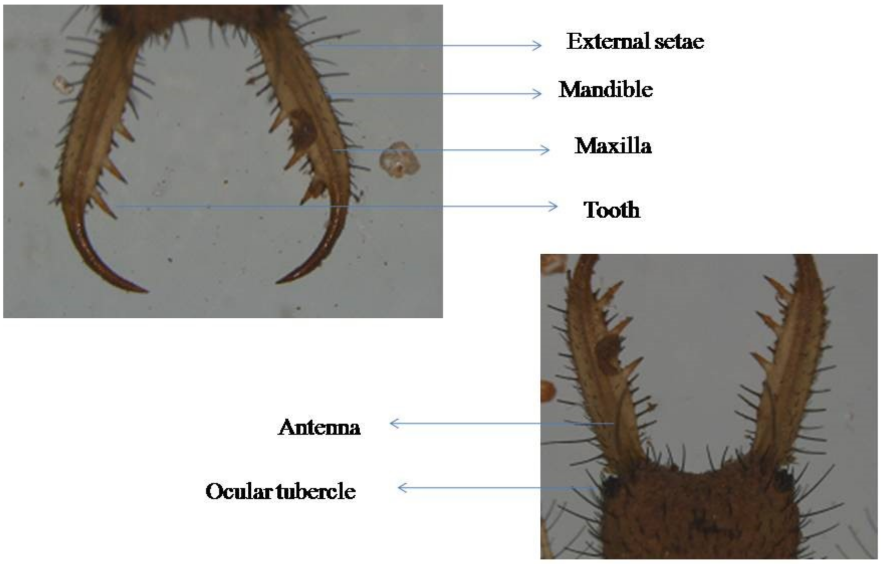


Plate 3

Head portion

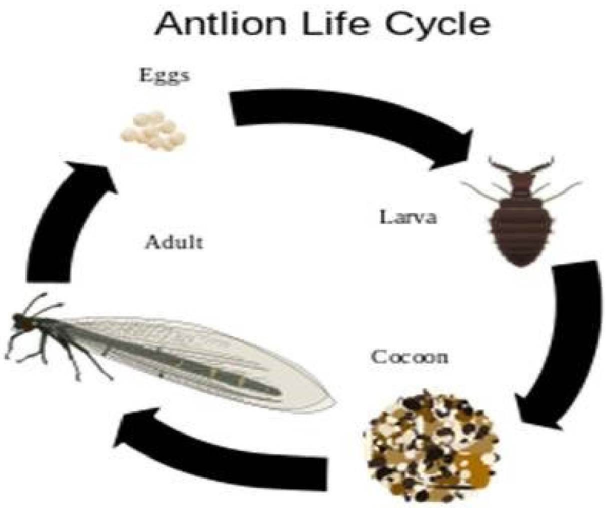


Ventral side of larvae

Plate 4

Doodle marks

Antlion larval pits



1.2.4. Parasites of antlion

The main parasites of antlions are coming under Order Hymenoptera, Diptera and Protozoa. Chalcididae, Eulophidae and Ichneumonidae are the parasites coming under Order Hymenoptera (Stange, 2004). *Itamuton stangei* is an Ichneumonidae which parasitize Central Chilean antlion *Elicura litigator* (Porter, 1989*)*. The species *Hockeria eriensis* and *Paravilla sp* parasitize larvae of *Myrmeleon brasiliensis* from Brazil (Uchoa and Missirian, 2014).

1.3. SIGNIFICANCE OF THE PRESENT STUDY

Neuroptera being a predator devouring over other insects which may include serious pests of crops and forest vegetations, it is of immense importance economically as well as ecologically. But, no serious attempts have been made to study the diversity, ecology and behaviour of this group in Southern parts of Kerala. Antlions are often included in lists of beneficial insects, no doubt because they prey upon ants, a common pest to humans.

The significance of the study relies on the pit building strategy of antlion larvae and its predation. It has an inherent architectural ability to make its steep conical pits like the honey comb made by honey bee. The exceptional behaviour as a predator, make this group a very interesting, but the studies were in initial stage in Kerala. The number and species of Myrmeleontids were decreasing gradually because of different anthropological activity, so if the study hasnt done now, after some years we cant get this much data.

It took up to two years to emerge an adult from larvae, so that the life stages studies were very difficult. Though there is an absence of enough taxonomic keys regarding Family Myrmeleontidae, the identification purpose is too hard; it makes to relay on molecular taxonomy than classical taxonomy. Sometimes antlions are categorized under bio indicators of hot condition (xerophilous) because they are living in dry places which indicates the global warming (Ngamo *et al.*, 2015)

The reasons for the selection of the group as study organisms and its significance are as follows,

- Considered as Bio indicators of global warming, because they are xerophilous insects, its presence is an indication of hot condition
- It is a model organism for studying the soil vibration and soil stability
- The architectural strategy was something different and new to science
- The ecology of antlion larvae and how they adapted to different types of habitat in Kerala was not studied yet
- Least studied group in India
- No studies in kerala till date

Lots of plants and animal species are disappearing from the earth due to anthropogenic activity and encroachment of lands for human life styles. So the natural habitats of several species are destroyed. According to the ZSI reports different species of antlions were reported from different states of India. Although the study of Southern species of antlions are still in upbringing stage.

The study was somewhat challenging because it is a preliminary study of antlions in the southern parts of India. Even though the collection of larvae was easy, but the identification of the species was difficult in Indian scenario due to the scarcity of experts in the same field. In order to increase the authenticity of species level identification both morphology and molecular sequencing was used as tools. There are lots of sequence deposits in NCBI and BOLD, but most of them are under the tag of Genus *Myrmeleon*. The numbers of species reported from India in 1984 were not caught now because of the habitat destruction due to human interaction.

1.4. OBJECTIVES OF THE PROPOSED WORK

The main objectives of the study include

- To study the different habitat of antlion larvae
- To analyze the physical and chemical structure of antlion inhabited soil
- To investigate the antlion larval behaviour patterns
- To examine the intraspecific and interspecific interactions

The first two objectives were studied under ecology and the remaining two coming under behaviour studies.

CHAPTER –2

ECOLOGY OF MYRMELEONTID LARVAE

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ECOLOGY OF MYRMELEONTID LARVAE

2.1. INTRODUCTION

Ecology is the study of interactions among organisms and between organisms and their environment. The interactions can be biotic and abiotic; biotic interactions are interactions among living organisms and those between organisms and their physical environment are called abiotic interactions. The four main areas of ecology are Organismal, Population, Community and Ecosystems ecology. The organismal ecology divided into three main subdivisions; that is evolutionary ecology, behavioural ecology and physiological ecology. Here the behavioural ecology of antlions was explained in two separate sections as ecology and behaviour. Behavioural ecology explains how the behaviour of an organism contributes to its survival and reproductive success which inturn affects the abundance of a population (Stiling, 2012). Antlion larvae have some strategy to increase its survival rate in its environment in which seasonal adaptability, high fecundity rates are some of the examples.

The study of ecological aspects of an individual or a species denotes the habit and habitat study in detail. In the case of ecology study of antlions, the different species present in a particular area (Kerala), the number of species present in one habitat, morphological peculiarities, physical parameters of habitat, natural enemy, seasonal adaptability, habitat choice are to be mentioned. Though the antlion larvae are terrestrial in nature, the organizing component of terrestrial ecosystems has more importance. For the study of abiotic factors and organism a new branch is present called ecophysiology. Ecophysiology is the part of ecology deals with the response of individual organisms or species to abiotic factors such as temperature, light, moisture, atmospheric gases and other factors in the environment (Barrick *et al*., 2005).

According to the latest classification work done in India (Ghosh, 2000) and World antlion Catalogue (Stange, 2004) a comparison of presence of antlions were made for understanding the status of antlion species both in India and World.

Stange-2004 (World Antlion)

Family Myrmeleontidae

1. Subfamily Stilbopteryginae

2. Subfamily Palparinae

Tribe Palparidini , Tribe Palparini , Tribe Pseudimarini , Tribe Dimarini

3. Subfamily Myrmeleontinae

Tribe Maulini, Tribe Dendroleontini, Tribe Nemoleontini, Tribe Brachynemurini Tribe Gnopholeontini, Tribe Lemoleontini, Tribe Myrmecaelurini, Tribe Nesoleontini, Tribe Myrmeleontini, Tribe Acanthaclisini

4. Subfamily Araripeneurinae

5. Subfamily Paleoleontinae

6. Subfamily Unknown

Ghosh-2000 (North East India)

Family Myrmeleontidae (Table 1 and Table 2)

1. Subfamily Palaparinae

Tribe Palparini (1 genus)

2. Subfamily Myrmeleontinae

Tribe Dendroleontini (4 Genus), Tribe Acanthaclisini (2 Genus), Tribe Myrmeleontini (3 genus), Tribe Distoleontini (5 Genus), Tribe Glenurini (1Genus)

Table 1. Subfamily Myrmeleontinae- Species reported from India

Sl No	Tribe	Genus	Species
1	Dendroleontini	*Layahima*	*L. nebulosa Navas*
		Indoclystus	*I. singularis (Westwood)*
		Dendroleon	*D. regius (Navas)*
		Gatzara	*G.jubilaea Navas*
2	Acanthaclisini	*Centroclisis*	*C. horridus (Walker)*

		Stiphroneura	*S. inclusa (Walker)*
3	Myrmeleontini	*Myrmeleon*	*M. clothildeBanks,* *M. montanus Navas,* *M. assamensis Ghosh,* *M. berenice Banks*
		Hagenomyia	*H. sagax (Walker),* *H. marginicollis(Gerstaecker),* *H. eurystictus (Gerstaecker),* *H. nigrinus (Esben-Petersen),* *H. monticolla (Navas),* *H. jamduarensis Ghosh*
		Talosus	*T. oberthurai Navas*
4	Distoleontini	*Creoleon*	*C. griseus (Klug)*
		Allogama	*A.irene (Banks)*
		Neuroleon	*Neuroleon sp.*
		Distoleon	*D. verendus (Walker),* *D. bivittatum Banks,* *D. sambalpurensis Ghosh,* *D. audax (Walker)*
		Dolicholeon	*D. substigmalis Navas*
5	Glenurini	*Negrokus*	*N. lebasi Navas*
	Uncertain position	*Baga*	*B. montana Navas*

Table 2. Subfamily Palparinae- Species reported from India

Sl No	Tribe	Genus	Species
1	Palparini	*Palpares* Rambur	*P. pardus Rambur* *P. contrarius (Walker)*

Though the larval antlions are present in soil, the development of larvae into adult was highly influenced by the soil texture and composition of that particular area. Soils of different ecosystems or climate have special properties such as different colours and compositions (Colinvaux, 1986). Soil formation is influenced by the climate, vegetation and hydrological conditions of the particular area and according to this, lots of soil classifications are present. Here, the classification of soil types were taken from Kerala agriculture website (http://www.keralaagriculture.gov.in) and the comparison of soil type and antlion larval presences were studied (Table 3).

Table 3. The six type of soils in Kerala

Sl No	Soil Type	Description
1	Coastal Alluvium	80% sand content and up to 15% clay content , The water holding capacity is poor
2	Alluvial Soil	Kuttanad and Kole lands of Thrissur district. Sandy clay loam to clay
3	Kari soil	Chittur/Palakkad. Clay loam to clay
4	Laterite soil	p^H-5.0-6.2
5	Red soil	Thiruvananthapuram. Sandy clay loam to clay loam. With red to dark red colour
6	Hill soil	Loam to clay loam

The soil is composed of sand, silt and clay particles. According to the International Union of Soil Sciences (IUSS), the soil particles were classified as follows (Table 4).

Table 4. Soil particle classification by IUSS

Particle	Diameter (mm)
Coarse sand	2.0-0.2
Fine sand	0.2-0.02
Silt	0.02-0.002
Clay	<0.002

Like habitat, another important aspect of ecological study rely on how an organism get its food and how they were food by another organism. The former is called feeding habit and the latter termed as natural enemy. The ecology study of an organism will complete only by mentioning these areas in a proper way. In the case of antlions, the major importance is given to the larval forms because of the presence of specific feeding habits, natural enemies, specific habitat etc.

2.2. REVIEW OF LITERATURE

2.2.1. Myrmeleontids of World

Numerous studies were done by using different species of pit building antlions, but in the case of diversity or quantifying different species, the previous works were less when compared to behaviour study. Iran, Malesia, China, United Arab Emirates, Pakistan are some countries in which quantification of antlion species were done efficiently. New (1982) described Newzealand species of antlion, *Weeleus acutus* with structure of wings, genitalia and its larval stage. In the case of India, after 1984 not much work was conducted and from Southern part of India unfortunately not a single species was reported. New and Sudarman (1988) described the Neuropterans of Krakatau Islands, Indonesia in which *Myrmeleon frontalis* was the only species present there, it is one of the earlier study and report of a species. Poinar and Stange (1996) gave knowledge about fossil records of Myrmeleontids and also described a new Dominican Amber antlion species that is *Porrerus dominicanus*.

In 2002, Mirmoayedi presented a list of 23 species of Neuroptera from Iran, in which 9 species are coming under Family Myrmeleontidae. Stange (2004) consolidated the data regarding the world antlion species and the works of all eminent scientists in the field of antlion research were included. In this book he explained about 1522 extant species, 13 fossil species, 201 genera, 14 tribes and 5 subfamilies including 2 fossil subfamilies. The Subfamilies are Stilbopteryginae, Palparinae, Myrmeleontinae and the remaining Araripeneurinae and Paleoleontinae are fossil subfamilies. It is considered as the latest consolidation of family Myrmeleontidae, with valid taxonomic keys, distribution, parasites, predators, which includes the works from the year 1700 to 2000.

With a gap of 4 years Mirmoayedi (2006) again contributed to antlion fauna of Iran with 7 new records. In the same year Bao and Wang reviewed the species of *Euroleon* from China. Instead of Mirmoayedi, Abraham (2007) also presented a new species of *Macronemurus* from Iran. In the year of 2008, Saji and Whittington recorded 27 species of antlion from Abudhabi Emirate followed by Dong and Engel, they described a new fossil antlion from North Eastern China.

Scudder and Cannings (2009) made a checklist of Neuroptera of British Columbia, in which 5 species were coming under Family Myrmeleontidae. It includes 3 species of genus *Brachynemurus*, *Dendroleon* and *Myrmeleon*. Abraham (2010) described a new *Palpares* species from Middle East Asia and Devetak *et al*., (2010a) studied the morphology of non pit building antlion larvae *Neuroleon microstenus* in the same year. Devetak *et al*., again contributed about the morphology of *Myrmeleon yemenicus* Holzel in the same year (2010b). Pantaleoni *et al*., (2010) described a new Mediterranean species *Myrmeleon mariaemathildae* from Sardinia and Tunisia.

The next decade starts with the work of Miller and Stange (2011), and they published a paper about the antlions of Hispaniola; Abraham and Dobosz listed 27 antlion and 7 owl fly species from Madagascar in this year. Michel and Akoudjin (2012) reviewed the *Neuroleon Navas* of West Africa with 4 new species descriptions. Zhan *et al.*, (2012) presented a synopsis of the genus *Deutoleon Navas.* Pantaleoni and Badano reported a new species of pit building antlion *Myrmeleon punicanus* from Sicily and Pantellaria (2012). In the same year Zhan and Wang (2012) described a new species of *Bankisus Navas* (*Bankisus sparsus sp*) and also provided a Key to Bankisus. A huge work was come from Miller and Stange (2012) about cave mouth antlions of Australia in which they described 12 new species.

Devetak *et al.*, (2013) studied about the antlions of Albania with 14 species and Acevedo *et al.*, (2013) described the larvae of European species *Distoleon Banks* in the sam year. Krivokhatsky *et al*., (2014) got three species of antlion from Curonian spit Russia from bird traps and the species includes- *Myrmeleon tschernovi*, *M. Formicarius* and *Euroleon nostras* in the ratio 100:3:2. In the same year four rare species of antlion from middle Asian countries were reported by Khabiev and Krivokhatsky (2014). Badano and Pantaleoni (2014) published a most important work which is helpful for many emerging scientists in the field of Myrmeleontids that is larvae of European Myrmeleontids with taxonomic key. Acevedo *et al.*, (2014) in the same year described the larvae of *Tricholeon relictus* Holzel. Michel (2014) revised the Genus *Solter* Navas 1912 of Maghreb and West Africa with descriptions of 5 new species. Myrmoayedi *et al*., in (2015) made a check list of antlions of Iran.

Devetak (2016) contributed a checklist of Lacewings of Albania and Abraham (2017) described *Myrmeleon* species from Sichuan, China. Krivokhatsky *et al.*, (2017) in the same year described *Palpares turcicus* in the Iranian fauna. Akhtar *et al*., (2018) reported 5 species of Genus *Myrmeleon* from Pakistan and Badano *et al.*, reviewed the antlions of Cyprus with 7 new reports. Hamouly *et al.*, (2019) reviewed subfamily Palparinae of Egypt and Krivokhatsky (2019) noted two size ranges of *Myrmeleon hyalinus hyalinus* from UAE. The larval morphology of 3 Afrotropical pit building antlion genus *Myrmeleon* by Badano published in 2020 is considered as the latest work published regarding antlions.

2.2.2. Myrmeleontids of India

An earlier knowledge about Neuroptera was found in ZSI, in which a book named 'Animal resource of India' (1991) has found as a historical resume of Neuropterida and estimated 37 genera of Myrmeleontidae and 125 species. The Records of the ZSI gave knowledge about Neuropteran species of Himachal Pradesh. A total of six species were described which includes *Palpares pardus* (Tribe *Palparini*), Tribe *Distoleontini*, Tribe *Acanthaclisini* and Tribe *Myrmeleontini*. Ghosh and Sen in 1977 published a checklist of Indian Planipennia (Order Neuroptera) and Ghosh (1983) reported Neuropteran from North-West Himalayan and Northern peninsular sectors of India. Ghosh again reported Neuroptera from Rajasthan (1977), Orissa (1987) and Lakshadweep Islands (1990).

Alfred *et al*., in 1998 wrote a book named 'Faunal diversity of India' depict that 335 species, 125 genera and 13 families of Neuropterans are recorded from India in which 40 genera and 126 species are coming under family Myrmeleontidae. In the same year Ghosh again contributed about collection, and preservation of Neuroptera, external morphology and terminology and systematic accounts. Here, Key to the genera of *Palpares, Centroclisis, Cueta, Nesoleon, Hagenomyia, Myrmeleon, Creoleon, Gatzara, Neuroleon* and *Distoleon* were presented. A consolidated list of 73 species of neuropteran from West Bengal was recorded in this paper.

Ghosh (2000) consolidated the Neuroptera of North-east India (Arunachal Pradesh, Assam, Meghalaya, Manipur, Nagaland, Mizoram, Tripura, West Bengal, and Sikkim) and 128 species in 69 genera and 11 families were reported. In the book named 'Insects of India', Sengupta (2005) found that among the 100 species of antlion recorded from India, 45 species was from North East India and approximately 12 families and 35 species of Neuropterids were recorded from India. Sharma and Chandra (2012) recorded 60 species of Neuropterans from Maharashtra in which family Myrmeleontidae consists of two subfamilies (Palparinae and Myrmeleontinae) and 5 tribes and a checklist was made. Three species of antlion were reported from Chattisgarh such as *Siphoneurainclusa* coming under Subfamily Myrmeleontinae and *Stanaresimprobus*, *palparespardus* coming under Subfamily Palparinae with detailed description (Chandra *et al*., 2014). Kaur *et al.*, (2019) studied about the female and male genitalia of *Myrmeacaelurus acerbus*.

2.2.3. Abiotic factors

Abiotic factors like temperature and humidity have some effects on every organism, sometimes the abiotic factors influence the lifecycle and behaviour also. Arnett and Gotelli (2001) studied pit building behaviour of *Myrmeleon immaculatus* larvae, and how temperature influences the pit building of the species. The optimum temperature of *Myrmeleon obscures* and connection between the temperature and life cycle were analysed by Bakoidi *et al.*, (2019). The influence of soil temperature and soil illumination in *M. Immaculatus* larvae were studied by Klein (1982).

There are only limited studies were present regarding the altitude and pit building. Bozdogan *et al*., (2013) observed some peculiarities of *Myrmeleon formicarius* in forest areas and non forest areas of Kahramanmaras in Turkey.

2.2.4. Soil preference

The main characteristic features of soil include soil temperature, soil moisture and soil texture. The different species of genus *Myrmeleon* was used for most of the studies and the pit building of *Myrmeleon pictifrons* in moisture condition and different grain size were studied by Kitching (1984). Sand preference study reveals the preference of find sand (Lucas, 1986) for pit building in *Myrmeleon sp*. Instability of sandy soil was studied by Halloran *et al.*, (2000) and the antlion larvae (*Myrmeleon crudelis)* considered as bioindicator of soil stability. Farji-Brener (2003) determined whether the soil condition or ant acacia clearings influence the pit trap construction and the results shows that soil condition is most significant than ant acacia. The pit building strategy in different conditions like sand depth, soil type and thermal conditions were studied byAlcalay *et al.*, (2014). Maoge *et al*., (2014) analysed the chemical composition of the media and the role in the pit building of antlion larvae. In a three year survey, *Myrmeleon quinquimaculatus*, *M. obscures* and *Hagenomya tristicis* are the most abundant species of Northern part of Cameroon.

2.3. RESEARCH METHODOLOGY

2.3.1. Study Area- Kerala

Kerala lies between northern latitude of 8º.17’.30”N and 12º.47’.40”N and east longitudes 74º.27’.47”E and 77º.37’.12”E (Map-1). Geographically Kerala is situated between Arabian sea to the west and the Western ghats to the east. Physiographically the land has three regions, such as lowlands (0-7.5m altitude), midlands (7.5-75 m altitude) and highlands (>75 m altitude). The forest types in Kerala includes dry deciduous, moist deciduous, semi evergreen, evergreen and shola forests (Champion and Seth, 1968). Ten types of soils were noted from Kerala such as red soil, laterite soil, coastal alluvial, riverine alluvial soil, greyish onattukara soil, brown hydromorphic soil, hydromorphic saline soil, acid saline soil, black soil and forest soil.

2.3.2. Meteorological data of Kerala

In Kerala, there are four seasons such as summer (March to May), South west monsoon (June to September), North east monsoon (October to December) and winter (January and February) and the average temperature and rainfall is given in Table 5. The mean maximum temperature is seen in the month of March in Kerala, which is about 33ºC. The minimum temperature noted in the month of July is about 28.5ºC. From January to March, the humidity varies from 35% to 71% in the state, and during the monsoon, it is about 85%. The South west monsoon (June to October) comprises the 70% of annual rainfall in Kerala (Table 6).

Table 5. Seasons in kerala with average temperature and rainfall

Seasons in Kerala	Average temperature		Average rainfall (mm)
	Max (ºC)	Min (ºC)	
Winter	28	18	25
Summer	36	32	135
South west monsoon	30	19	2250-2500
North east monsoon	35	29	450-500

Table 6. Average monthly rainfall in Kerala

Average monthly rainfall in Kerala												
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rain Fall (mm)	14.6	16.6	36.1	110.9	252.6	653.2	687.2	404.7	252.3	270.7	158.6	45.9

(ENVIS: Kerala state action plan on climate change, source: Economic review 2013-2018, IMD TVRM)

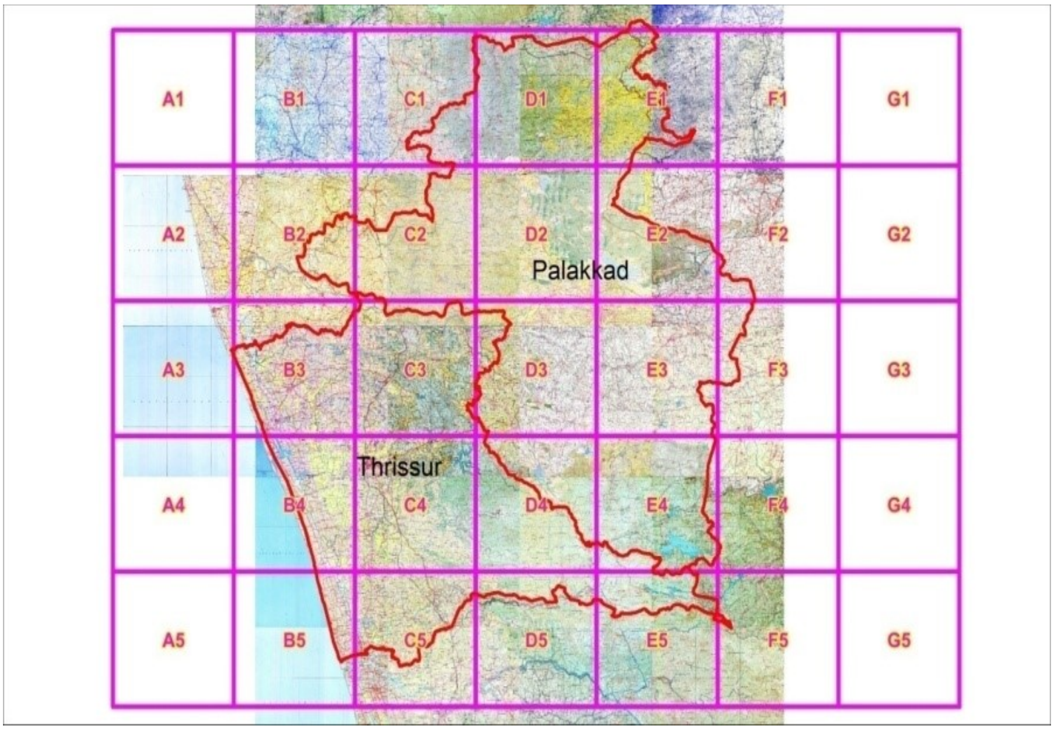
2.3.3. Sampling

A pilot study was carried out from June 2015 to December 2015 for identifying the presence of antlion species in Thrissur and Palakkad districts of Kerala. These two districts were divided into 20 grids and in each grid the area was set to 625 km^2 (25X25 km) (Map-2). Out of the 20 grids surveyed, 15 grids were randomly selected for the collection purpose. From this initial survey it was understood that the number of species and individuals were not sufficient for behavioural and ecological studies. Therefore it was decided to cover all districts for maximum species collection and identification of habitat.

Genus *Myrmeleon* is the common pit building antlion in India (176 species worldwide), and the study concentrated on Genus *Myrmeleon* and the behaviour study also done by using the common species of this genus. The collection of antlion was carried out from January 2016 to December 2019 and for behaviour study focussed on the pit building antlion species. Care was given to choose at least one study area in each district for the collection of antlion.

Random collection was done and the collection methods include visual encounter survey and active search due to the unavailability of previous studies regarding the habitat in southern India.

Map 1- Study Area- Kerala



Map 2- Map plotted for pilot study

2.3.4. Collection Techniques (Both adult and larvae)

Collection techniques adopted for the study includes handpicking, sweeping and light trap.

Handpicking: Antlion larvae were collected by handpicking or by using a spoon (Maoge *et al*., 2014) in the day time between 7 am to 4 pm. Larvae were scooped by the help of a spoon and it was transferred to a paper cup (diameter 6 cm and height 6 cm) filled with sand/soil.

Sweeping: This method was used to collect adult antlions during the day time. The bushes and plants in different localities were disturbed using a wooden stick. The antlions present were collected using a hand net.

Light trap: As the antlion adults are attracted to light during the early morning and evening periods, light traps were set up. Light traps consisted of a white cloth hung near a 9wt LED (Light emmiting diode) bulb. The presence of antlion adult was observed in active time (5.30 to 7.30 am and 6.30 to 8.30 pm) near light trap.

2.3.5. Killing and Preservation

The collected adults were killed using chloroform and spread on a spreading board. The spread, pinned and labelled specimens were stored in insect boxes for the purpose of identification. Specimens used for morphological studies were carefully taken and slides of legs, antennae and wings were prepared by mounting in DPX without staining.

2.3.6. Rearing of Antlion larvae

For rearing of antlion larvae, individuals were observed and collected after measuring the pit depth and diameter. Field collected individuals were brought to the laboratory and the morphometric measurements of larval body parts were taken. Measured individuals were transferred to a paper cup (diameter 6 cm and height 6 cm) filled with sand or dry soil (Liang *et al*., 2010; Guillette *et al*., 2009; Maoge *et al*., 2014). The paper cups were covered by using a cloth in order to avoid the escape of adult after emergence. The larvae were given one ant

(*Anoplolepis gracilipes*) per day as food. When the larva stops feeding, they are allowed to moult or pupate. After emergence of the adult, it was spread, dried and kept in the insect box for further studies.

2.3.7. Depository

The curated adult antlion specimens were deposited in the Research and Postgraduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur) for further reference.

2.3.8. Identification of collected Specimens

The collected specimens of antlion adult and larvae were identified by using standard taxonomic keys and for substantiating the results molecular sequencing were performed (Stange, 2004 and Ghosh, 2000) by the following protocol.

2.3.8.1. DNA sequencing using universal primers of CO1

Genomic DNA Isolation: The genomic DNA isolation was done by using NucleoSpin® Tissue Kit (Macherey-Nagel).Tissue was placed in 1.5ml microcentrifuge tube. Added 180µl of T1 buffer and 25µl of Proteinase K and incubated at 56°C in a water bath. Then added 5µl of Rnase A (100mg/ml) and incubated at room temperature for 5 minutes. Added 200µl of B3 buffer and incubated at 70°C for 10 minutes. 210µl of 100% ethanol was added followed by vortexing. The mixture was pipetted in to NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000xg for 1 minute. NucleoSpin® Tissue column transferred to a 2 ml tube and washed with 500µl of BW buffer. This step was repeated using 600µl of B5 buffer. NucleoSpin® Tissue column placed in 1.5ml tube and DNA eluted using 50µl of BE buffer.

Agarose Gel Electrophoresis for DNA Quality Check: 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. Samples loaded to 0.8% agarose gel prepared in 0.5 X TBE (Tris-Borate-EDTA) buffer containing 0.5µg/ml ethidium bromides. Electrophoresis performed with 0.5X TBE as buffer at 75V. Gels were visualized in UV transilluminator (Genei) and the image captured under UV light using Gel documentation system (Bio-Rad).

PCR (Polymerase chain reaction) Analysis: PCR amplification was done in a 20 µl reaction volume containing 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl PhireHotstart II dNA polymerase enzyme, 0.1mg/ml BSA and 3% DMSO, 0.5 M Betaine, 5pM of forward and reverse primers (Table 7). Amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

Table 7. Primers used for PCR

Target	**Primer Name**	**Direction**	**Sequence(5'→3')**
COX1	LCO	Forward	GGTCAACAAATCATAAAGATATTGG
	HCO	Reverse	TAAACTTCAGGGTGACCAAAAAATCA

PCR amplification profile

COX1

98°C- 30 sec

98°C-5 sec
45°C- 10 sec
72°C- 15 sec } 10 cycles

98°C- 5 sec
50°C- 10 sec
72°C- 15 sec } 30 cycles

72°C- 60 sec

4°C- ∞

Agarose Gel electrophoresis of PCR products: The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

ExoSAP-IT Treatment: ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37ºC for 30 minutes followed by enzyme inactivation at 80ºC for 15 minutes. Sequencing using BigDye Terminator v3.1: Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)- 10-20 ng

Primer - 3.2 pM (either Forward or Reverse)(Table 7)

Sequencing Mix - 0.28 µl

DMSO - 0.30 µl

5x Reaction buffer - 1.86 µl

Sterile distilled water - make up to 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96ºC for 2 minutes followed by 30 cycles at 96ºC for 30 sec, 50ºC for 40 sec and 60ºC for 4 minutes.

Post Sequencing PCR Clean up: Master mix I of 10µl milli Q and 2 µl 125mM EDTA per reaction and master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol were prepared. 12µl of master mix I was added to each reaction containing 10µl of reaction contents and was properly mixed. 52 µl of master mix

II was added to each reaction. Contents were mixed by inverting and incubated at room temperature for 30 minutes. Spun at 14,000 rpm for 30 minutes. Decanted the supernatant and added 100 µl of 70% ethanol. Spun at 14,000 rpm for 20 minutes. Decanted the supernatant and repeated 70% ethanol wash. Decanted the supernatant and air dried the pellet. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

Sequence Analysis: The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010). The final sequence was run in BOLD systems (Barcode of Life data system) and NCBI BLAST (National Centre for Biotechnology Information, Basic Local Alignment Search Tool) and the maximum similarity showing species in the database was used to confirm the species. Phylogenitic trees (Neighbour tree without distance corrections) were prepared by using Clustal Omega.

2.3.9. Habitat

The latitude, longitude and the physical parameters of the study area was noted for understanding the habitat of antlion larvae. According to the data, antlion larval habitats were classified as abandoned area, human dwelling area, forest boundary area and riparian. The area in which no disturbance of animals and human being are considered as abandoned area. Mainly it includes abandoned buildings and land without the presence of human activity (under the shades of trees). The area near to houses, schools, bus stops etc are considered as human dwelling area. The areas up to 5 kms from forest boundaries were considered as forest area, which included abandoned areas, buildings, and shaded areas due to the difficulty of collecting antlion larvae inside the forests. River banks were considered as riparian habitat.

2.3.10. Morphometric Analysis-Larvae

Morphometric measurements of two species of genus *Myrmeleon* were made. *Myrmeleon hyalinus* and *Myrmeleon pseudohyalinus* were used for this study.

For this purpose the larval body length, larval body width, larval head length, larval head width, mandible length were calculated by using measurement tool of leica stereozoom research microscope (LEICA S8APO) (Plate 15) attached with digital camera (LEICA MC170HD). The statistical analysis of the data was performed in PAST software (3 & 4.2 versions).

In order to identify the relationship between the antlion larval size and pit size, observations were conducted in the sunshade of an abandoned house in Parli, Palakkad district, from April 1 - May 30, 2015. A natural population of *M. pseudohyalinus* larvae was selected for this experiment and the depth and diameter of the pits were noted without disturbing the antlion larvae. Pit diameters and depth were measured to the nearest millimeter. The soil temperature of the study area also recorded.

For understanding the correlation between the pit size and larval size in natural condition the following parameters were studied; pit depth, pit diameter, larval head length, larval head width, larval body length and larval body width. These above measurements were taken on site. The non-resident pits or abandoned pits of antlion larvae were also noted. The correlation between larval size and pit parameters were examined to understand whether its larvae used to predict larval instar.

2.3.11. Morphometric Analysis-Cocoon

The cocoons of genus *Myrmeleon* were collected by sieving the soil through a net from antlion habitats in which the presence of pit was noted. After the collection, it was labelled and stored in zip covers without any damage in the field itself.

The cocoons were examined and the presence /absence of pupa was determined by noting the emergence hole (Plate 14). Cocoon with hole indicated that the adult had emerged and escaped from the cocoon. The absence of emergence holes were indicated the presence of pupae inside cocoon and it was shifted to paper cups filled with dry soil for ensuring the emergence of the adult antlions. Cups were covered with nets and kept in laboratory under room temperature. The cocoon morphology was analysed by carefully measuring its diameter, weight, and circumference (Plate 16). The circumference was measured using a vernier calliper and weight of cocoon was noted by using Shimadsu digital weighing balance.

2.3.12. Morphometric Analysis- Adult

The following morphometric measurements of *Myrmeleon pseudohyalinus* were taken for this study: length from head to the tip of abdomen, forewing length, forewing breadth, hindwing length, hindwing breadth and length of antennae. The length of the wings was measured from the base to the apex, and the width was taken as the maximum width perpendicular to the length measurement line (Pantaleoni *et al.*, 2010).

2.3.13. Physical Parameters

From each study area the physical characters of the habitat was noted by using a digital temperature and humidity checker and the soil temperature was noted by using a thermometer. Temperature, humidity, dew point, pressure, UV index, visibility and wind speed were noted for understanding the habitat character of antlion larvae by using gadgets and from weather.com. Pearson’s correlation performed in Past3 software for statistical analysis and the significant level was set at 5%. Significant positive correlations are marked in blue and boxed. Significant negative correlations are marked in red and boxed in the correlation plot. Those not boxed are not significant.

2.3.14. Soil Texture Analysis

Soils samples were collected from selected habitats based on the availability. 100 grams of soil is needed for the texture analysis and again a 100 gram is needed for chemical analysis. So the samples collected from antlion inhabited area are taken for the above purpose in a zip cover and carried to Research lab (Plate 15). The collected soil samples were weighed and sorted for the collection of all the prey species from that area. After that 100 grams were packed tightly and the texture was analysed in the labs of IRTC (Integrated Rural Technology Centre), Mundur, Palakkad and Soil Science Department of KFRI (Kerala Forest Research Institute), Peechi, Thrissur. The methodology used for the analysis of texture was Hydrometer method.

Hydrometer method: It is based on the principle of dispersion and sedimentation techniques employed to a given weight of soil sample. Sedimentation refers to the settling rates of the dispersed particles in water, which is function of particle size and is governed by Stoke's law. Theoretically the hydrometer measures the density of soil suspension. In practice, an average density of the depth of the inserted hydrometer is taken. The hydrometer is based on the fact that the suspension at a given depth decreases as an initially homogeneous dispersed suspension settles. The rate of decrease in density, at any given depth, is directly related to the settling velocities of the particles, which in turn are related to their size. The hydrometer reading indicates that 4 minutes after sedimentation particles greater than 0.02 mm settle which after 2 hours, particles of size less than 0.002mm are left in soil suspension.

Procedure:-Weighed 50 g fine textured soil or 100 g coarse textured soil (>75-80% sand) which have been passed through a 2 mm sieve based on oven dry condition in to a beaker. Added 50 ml of 6% H_2O_2 (Hydrogen peroxide) and covered the beaker with a watch glass and placed it on a water bath until oxidation of organic matter is completed (indicated by the presence of effervescence), removed the beaker and cooled. After cessation of frothing transferred the contents in to a dispersing cup with about 400 ml of distilled water. Added to it 100 ml of calgon (a combination of sodium hexametaphosphate and sodium carbonate) solution. Stirred the suspension for 10 minutes by an electric stirrer. Transferred the suspension into a litre graduated cylinder and made up the suspension up to 1 litre mark with distilled water. Stopper the mouth of the cylinder and shaked vigorously upside down and back several times for about 1 minute. Placed the cylinder on a table and note the time immediately. Dipped the hydrometer in to the suspension and take the first reading after 4 minutes when particle >0.02mm have settled (Start inserting the hydrometer 10 seconds in advance of the reading time). Carefully removed the hydrometer and washed with distilled water and noted down the temperature of the suspension.

Note- The hydrometer is calibrated at 67°F (19.4°C). If the suspension temperature is above 67°F, the correction is added, and if below, the correction is subtracted. The correction is equal to the difference between the experimental temperature and 67°F multiplied by 0.2.

For conversion of °F to °C the following equation is used $\frac{C}{5} = \frac{F-32}{9}$

Kept the suspension undisturbed and dip the hydrometer again at the end of 2 hours after initial shaking was stopped. Now, the particles greater than 0.002 mm (sand+silt) have settled. Recorded the hydrometer reading. Calculated the percentage of sand, silt and clay and determined the textural class using ISSS textural triangle.

2.3.15. Soil Chemical Analysis

Though antlion larvae spent most of its life cycle in soil or sand, the chemical composition or chemical parameters may influence the larvae. For this purpose analysed the following chemical component of the antlion larvae inhabited soil. Soil pH, Electrical Conductivity, Organic Carbon, Available Phosphorus, Available Potassium, Available Calcium, Magnesium, Available Sulphur, Iron, Manganese, Zinc, Copper, Available Boron, Nitrogen and Chlorine were analysed and the various methodology used for the analysis were explained below.

2.3.15.1. Soil pH

It is a measure of hydrogen ion concentration of the soil water system and indicates whether the soil is acidic, neutral or alkaline in reaction.The reagents used as follows, Standard buffer solutions: Buffer solutions of pH 4.0, 7.0, 9.2 were prepared using commercially available buffer tablets. Dissolved the respective tablets in freshly prepared distilled water and made up the volume to 100 mL. Calibrated the pH meter using buffer solutions. The pH of soil is determined in 1:2.5 soil water suspensions. Took 10 g sample of soil sifted through 2 mm sieve in a 50 or 100 mL beaker. Add 25 mL of distilled water, stir well for about 5 minutes and keep for half an hour. Stir well again and take the reading using the pH meter.

2.3.15.2. Electrical conductivity (EC)

Electrical conductivity in soil water system is a measure of concentration of soluble salts and extent of salinity in the soil and is measured using a conductivity meter. The clear supernatant of 1:2.5 soil water suspension prepared for pH measurement can be used for estimation of EC. Calibrated the conductivity meter using 0.01N KCl (Potassium chloride) solution prepared and determined the cell constant. Determined the conductivity of the supernatant liquid.

2.3.15.3. Organic carbon in soil (OC)

Soil organic matter has been defined as the organic fraction of soil, including plant, animal and microbial residues, fresh and at all stages of decomposition and the relatively resistant soil humus. However, soil organic matter estimate includes only those organic materials that accompany soil particles through a 2 mm sieve. Carbon is the chief element present in soil organic matter, and forms 48-58% of the total weight. Therefore organic carbon determinations are often used as a basis for estimation of organic matter.The principle behind this procedure was according to Schollenberger (1927) and the methodology used for the estimation was Walkley-Black Wet Digestion Method (Walkley, 1947)

The Procedure is as follows**,** Grinded soil upto pass through a 0.5 mm sieve and transferred a weighed sample (approximately 0.5 to 1 g soil) into a 500 ml wide mouth conical flask. Added 10 ml of 1*N* $K_2Cr_2O_7$ (Pottassium dichromate) and swirled the flask gently to disperse the soil in the solution. Then rapidly added 20 ml of concentrated H_2SO_4 (Sulfuric acid). Immediately swirled the flask gently until the soil and the reagents are mixed, then more vigorously for a total of one minute. Allowed the flask to stand on an asbestos sheet for about 30 minutes. Then added 200 ml of water to the flask. Added o-phenanthroline indicator and titrated the solution with 0.5*N* ferrous ammonium sulphate ($Fe(NH4)_2SO_4$). As the endpoint approaches, the solution takes on a greenish cast and then changes to a dark green colour. The ferrous ammonium sulphate was added drop by drop until the colour changes sharply from blue to red.

Calculation

$$\text{Organic carbon (\%)} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq } Fe(NH_4)_2SO_4) \times 0.003 \times 100 \times 1.3}{\text{Weight of soil}}$$

O C (%) = {10 x 1 – Titre Value(mL) x Normality of $Fe(NH_4)_2SO_4$} x 0.003 x 100 x 1.3

Weight of soil

O C (%) = {10 x 1 – Titre Value(mL) x Normality of $Fe(NH_4)_2SO_4$} x 0.39

Weight of soil

2.3.15.4. Available phosphorus (P)

Determination of plant available P in soil has two distinct phases – first, the extraction of plant available pool of phosphorus, present in soil, and second the quantitative determination of the P in the extract. The choice of a colorimetric method for determining P depends on the P concentration in the solution, the concentration of interfering substances in the solution to be analysed and the particular acid system involved in the analytical procedure. The molybdenum blue method is the most sensitive and widely used one for soil extracts containing small amounts of P. The available pool of P varies depending on the pH of the soil, reagents used for extraction of this pool also are different.

Procedure includes Extraction and Estimation by reduced molybdate blue colour methods. The Extraction: Weighed out 5 g of soil to a 100 mL conical flask and added 50 mL of Bray No.1 reagent (Bray and Kurtz, 1945) and shaked for exactly 5 minutes. Filtered through Whatman No.42 filter paper. To avoid interference of fluoride, 7.5 mL of 0.8 *M* boric acid (50 g of H_3BO_3 per litre) can be added to 5 mL of the extract if necessary. Estimated phosphorus in the extract by ascorbic acid method (Watanabe and Olsen, 1965).

Estimation by reduced molybdate blue colour method: Pipetted out 5 mL of the extract into a 25 mL volumetric flask and diluted it to approximately 20 mL. Added 4 mL of Ascorbic acid (1.056 g of ascorbic acid in 200mL of Ammonium paramolybdate). Made up the volume with distilled water and shaked the contents well. Read the intensity of colour after 10 minutes at 660 nm. The colour is stable for 24 hours and the maximum intensity develops within 10 minutes. The concentration of P in the sample is computed from the standard curve (Plot the concentration vs Absorbance curve on a graph paper)

Calculation

$$\text{Available P (mg kg}^{-1}\text{ soil)} = \mu\text{g P mL}^{-1}\text{ of the aliquot} \times \frac{50 \times 25}{5}$$

$$\text{Available P (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for sample}}{\text{Slope of Std. Curve}} \times 50$$

Available P (kg ha^{-1} soil) = Available P (mg kg^{-1} soil) x 2.24

2.3.15.5. Available potassium (K)

A relatively small portion of the total K in soils is exchangeable (approximately 1%). Exchangeable K generally ranges from <100 to 2000 µg mL^{-1} or more when compared with total K values which is in the order of 1 to 2%. Water soluble K seldom exceeds a few parts per million except in the case of certain saline soils.

The Procedure is as follows**,** Extraction: Shaked 5 g of soil with 25 mL of neutral normal ammonium acetate for 5 minutes and filtered immediately through a dry Whatman No.42 filter paper. First few mL of the filtrate may be discarded. Potassium concentration in the extract is determined using flame photometer after necessary setting and calibration of the instrument.

Standard curve for potassium: Diluted measured aliquots from the standard solution using ammonium acetate solution to give concentrations of 5 to 20 µg mL^{-1} K. After attaching the appropriate filter and adjusting the gas and air pressure, set reading in the flame photometer as zero for the blank (ammonium acetate) and at 100 for 20 µg mL^{-1} K. The curve is obtained by plotting the readings against the different concentrations (5, 10, 15 and 20 µg mL^{-1}) of K. Fluctuation in gas and air pressure does not allow steady reading in the meter and must be taken care of.

Calculation

$$\text{Available K (mg kg}^{-1}\text{ soil)} = \mu\text{g K mL}^{-1}\text{ of the aliquot} \times \frac{25}{5}$$

Available K (mg kg^{-1} soil) = µg K mL^{-1} of the aliquot x 5

Available K (kg ha^{-1}) = Available P (mg kg^{-1} soil) x 2.24

2.3.15.6. Available calcium and magnesium (Ca and Mg)

As in the case of potassium, exchangeable plus water soluble calcium and magnesium contribute to the plant available pool. The neutral normal ammonium acetate extracts the pools of calcium and magnesium also along with potassium and sodium.The principle is, the cations Ca^{2+}, Mg^{2+} and Na^{+} along with K^{+} appear to be completely exchangeable in the absence of excess of $CaCO_3$ (Calcium carbonate) by neutral normal ammonium acetate.

Extraction of available Calcium and Magnesium: Shaked 5 g of soil with 25 mL of neutral normal ammonium acetate for 5 minutes and filtered immediately through a dry Whatman No.42 filter paper. First few mL of the filtrate may be discarded.

Estimation of Calcium and Magnesium by Atomic Absorption Spectrophotometry: From the soil extract Ca and Mg can be estimated by Atomic Absorption Spectrophotometry (AAS). The chemical interference, resulting from the formation of stable compounds between Ca and Mg ions and the accompanying anions may reduce the absorption. This interference may be overcome by using a realising agent such as Lanthanum or Strontium.

Calculation

$$\text{Available Ca/Mg (mg kg}^{-1}\text{ soil)} = \mu\text{g Ca/Mg mL}^{-1}\text{ of the aliquot} \times \frac{25}{5}$$

$$\text{Available Ca/Mg (mg kg}^{-1}\text{ soil)} = \mu\text{g Ca/Mg mL}^{-1}\text{ of the aliquot} \times 5$$

2.3.15.7. Available sulphur (S)- (By $cacl_2$ Extraction)

Different reagents have been proposed for extracting plant available sulphur from the soil. These include water, salt solutions such as 0.15% $CaCl_2$ (Calcium chloride), 500 ppm P as $Ca(H_2PO_4)_2$ (Calcium phosphate) or KH_2PO_4 (Potassium dihydrogen phosphate) and acidic solutions such as 0.5 *N* ammonium acetate plus 0.25 *N* acetic acid and Bray No. 1. Generally phosphate solutions extract more sulphate sulphur from soils than can be extracted with water or salt solutions because phosphate ions displace the adsorbed sulphate, which is known to be readily available to plants.

Extraction (Tabatabai, 1982): Shaked 10 g of air-dried processed soil with 50 mL of 0.15% $CaCl_2$ solution in a 250 mL conical flask for 30 minutes. Filtered the extract through Whatman No. 42 filter paper and estimated the sulphate content by turbidimetric procedure.

Preparation of standard curve: Pipetted out 0, 0.25, 0.5, 0.75, 1.0, 1.25, and 2.5 ml of standard sulphate solutions in seven different volumetric flasks (25 mL) and added 10ml of extracting solution (0.15% $CaCl_2$). Prepared fresh standards each time when a batch of sample is analysed. Added 1 g of $BaCl_2$ (Barium chloride) crystals to each flask and dissolve it. Add 1 mL of 0.25% gum acacia solution (Dissolved chemically pure gum acacia in hot water and filtered the hot solution through Whatman No.42 filter paper, cooled the filtrate and diluted it to 100 mL), made up the volume with distilled water and shake well. After the development of turbidity (Within 5-30 minutes), read the absorbance at 440 nm on a spectrophotometer. Draw the standard curve with absorbance on Y axis and concentration on X axis.

Turbidimetric estimation of Sulphur (Massoumi and Cornfield, 1963): Pipetted out10 mL of the soil extract into a volumetric flask (25 mL). Added 1 g of $BaCl_2$ crystals and allow it to dissolve. Added 1 mL of 0.25% gum acacia solution, made up the volume with distilled water and shake well. After the development of turbidity (Within 5-30 minutes), read the absorbance at 440 nm on a spectrophotometer.

Calculation

$$\text{Amount of sulphur (mg kg}^{-1}\text{ soil)} = \text{Concentration from the instrument} \times \frac{25}{10} \times \frac{50}{10}$$

$$\text{Amount of sulphur (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for the sample}}{\text{Slope of Std. Curve}} \times 12.5$$

2.3.15.8. Iron, manganese, zinc and copper (Fe, Mn, Zn and Cu)

The major categories of micronutrient extractants presently in use are dilute acids, and solutions containing chelating agents, such as DTPA or EDTA. Among the chelating agents, DTPA is the most commonly used one. The DTPA soil test, developed for near neutral and calcareous soil by Lindsay and Norvell (1978) illustrates the evolution of a soil test extractant from theoretical principles. The extracting solution consists of 0.005 *M* DTPA (Diethylenetriamine pentaacetate) and 0.01 *M* $CaCl_2.2H_2O$, (Calcium chloride dihyadrate) buffered at pH 7.3 by 0.1 *M* triethanolamine (TEA). The DTPA extractant offered the most favourable combination of stability constants necessary to simultaneously extract four micronutrient cations (Fe, Mn, Cu and Zn). The buffered pH and presence of soluble Ca^{2+} prevent excessive dissolution of calcium carbonate avoiding the release of unavailable micronutrients occluded by this solid phase. At pH 7.3, 70-80% of the buffering capacity provided by TEA has been consumed. Therefore use of DTPA extractant on acidic soils, will result in neutralisation of remaining buffer capacity and unpredictable extraction pH.

Estimation of Fe, Mn, Zn, and Cu in acid soils (pH < 6.5)

Extraction and estimation*:* Shaked 2 g of soil with 20 mL of 0.1 *M* HCl (Hydrochloric acid) for 5 minutes. Filtered through Whatman No. 42 filter paper. Collected the filtrate and estimated the contents of Fe, Mn, Zn and Cu using an Atomic Absorption Spectrophotometer.

Calculation

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \frac{\text{Concentration from the instrument x 20}}{2}$$

Amount of micronutrient (mg kg^{-1} soil) = Concentration from the instrument x 10

Estimation of Fe, Mn, Zn, and Cu in near neutral to alkaline soils (pH > 6.5)

Extraction and estimation: Shaked 10 g of soil with 20 mL of DTPA for 2 hours. Filtered through Whatman No. 42 filter paper. Collected the filtrate and estimated the contents of Fe, Mn, Zn and Cu using an Atomic Absorption Spectrophotometer.

Calculation

Amount of micronutrient (mg kg^{-1} soil) = $\frac{\text{Concentration from the instrument x 20}}{10}$

Amount of micronutrient (mg kg^{-1} soil) = Concentration from the instrument x 2

2.3.15.9. Available boron (B) - (Hot-water soluble Boron (Gupta, 1967))

Although there are a variety of chemical tests for predicting crop response to boron, the hot water extraction procedure developed by Gupta (1967) is the easiest method.

Extraction and Estimation: Weighed 20 g of air-dried processsed soil in a 250 mL quartz or other boron-free conical flask and added 40 mL distilled water. Added 0.5 g of activated charcoal and boiled for 5 minutes on a hot plate, filter immediately through Whatman No.42 filter paper. Cooled the contents to room temperature and transferred 1 mL aliquot of blank, diluted boron standard, or sample solution into 10 mL polypropylene tubes**.** Added 2 mL of buffer and mixed. Added 2 mL of azomethine-H reagent (0.45 g of azomethine-H dissolved in 100 ml of 1% L-ascorbic acid solution), mix, and after 30 minutes, read the absorbance at 420 nm on a spectrophotometer. Prepared a standard curve plotting B concentrations (0 to 10 µg B mL^{-1}) on X-axis and absorbance on Y-axis.

Calculation

Amount of B in soil (mg kg^{-1} soil) = $\frac{\text{Absorbance reading}}{\text{Slope from curve}} \times \frac{40}{20}$

Amount of B in soil (mg kg^{-1} soil) = $\frac{\text{Absorbance reading}}{\text{Slope from curve}} \times 2$

2.3.15.10. Available Nitrogen (N)

Nitrogen in the soil sample was determined by Alkaline permanganate method. Weighed and transferred 20 g of soil in to a distillation flask. Added 30ml of distilled water just to moist the soil and 1 ml of liquid paraffin or 1 g of paraffin wax (to avaid frothing). Added few pieces of glass beads also to avoid bumping. Added 100 ml of freshly prepared 0.32% $KMnO_4$ (Potassium permanganate) and 100 ml of 2.5% NaOH (Sodium hydroxide) to the soil in the distillation flask. Kept a 100 ml beaker containing approximately 20 ml of 2% boric acid with double indicator below the delivery end of the condenser in the distillation set. Distilled the contents at a steady rate and collected the liberated ammonia in boric acid. Continued the distillation until the release is free of ammonia and about 30 ml of distillate is collected. Titrated the ammonia collected in boric acid with N/50 H_2SO_4.

Calculation

Weight of the soil taken= 20g

Volume of N/50 H_2SO_4 consumed=X ml (titre value)

1 ml of N/50 H_2SO_4= 0.00028 gN

X ml of N/50 H_2SO_4= 0.00028 x XgN

Therefore Nitrogen present in Kg/ha= $\frac{0.00028(X)2\times10^6}{20}$

2.3.15.11. Available Chlorine (Cl)

Chloride determination is based on the formation of nearly insoluble silver salts. Silver nitrate in presence of potassium chromate indicator is used for precipitating Cl^-. Pipetted out 50 ml aliquot from the same soil-water extract or 5 ml of the filtered water sample. Added 5-6 drops of K_2CrO_4 indicator and titrated the solution with 0.02 (N) AgNo3 (Silver nitrate) solutions with stirring until the first reddish brown tinge appears. The ml of $AgNo_3$ required corresponds to the amount of chloride present.

2.3.16. Seasonal Adaptability and Habitat choice

The natural habitat of antlion larvae (Genus *Myrmeleon*) is mainly terrestrial in nature. Though antlion larvae inhabiting in the soil/sand, natural calamity like rain or flood affects more quickly than drought/high temperature condition. Experiments were conducted for analyzing the seasonal adaptability of soil inhabiting antlion larvae in the rainy condition and also analyzed the temperature tolerance or preferred soil temperature.

M.pseudohyalinus larvae were collected from soil without causing any damage. The second and third instar larvae were transferred to plastic trays (30X25cm) filled with loose soil (5 cm thickness). Five larvae were released in each tray and allowed to make its pits. Then larvae were given the rainy condition by spraying water in required quantity and noted the behaviour of the larvae and number of days taken for pit rebuilding. 20 ml of water was used for each spray and 3 experiments were performed such as two sprays per day, four sprays per day and six sprays per day in regular intervals (Freire and Lima, 2019.). After each spray the temperature of soil was noted and the pit building temperature was identified. The experiment repeated 10 times to decrease the bias.

Table 8. Water Sprays given and the time interval

	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Two sprays	8 am	8 pm				
Four sprays	8 am	12 am	4 pm	8 pm		
Six sprays	7 am	9.30 am	12 pm	2.30 pm	5 pm	7.30 pm

The quantity of water sprayed in a day in two sprays condition, four spray condition and six spray conditions were 40 ml, 80 ml and 120 ml respectively. Also conducted an experiment with wet and dry condition in a single tray. Tray (30X25 cm) filled with dry soil with 5 cm thickness, and partitioned it to two equal halves by using a cardboard. Then one part sprayed with 20 ml of water and the other side remained in the dry condition. After removing the cardboard five larvae released in the centre of the tray and pit building behaviour was noted. Repeated the experiment in ten times.

2.3.17. List of abbreviations used

A- Attack

A D-Adult Antlion

AA- Abandoned area

AN-Antennae

CD-Cocoon diameter

CW- Cocoon weight

E- Emergence

F- Feeding

FB- Forest boundaries

FWB- Forewing breadth

FWL- Forewing length

G- Grooming

H- Holding

HDA-Human dwelling area

HR- Head roll

HTA- Head to the tip of abdomen

HWB- Hind wing breadth

HWL- Hind wing length

JS- Jaw set

KNR-Kannur

LBL-Larval body length

LBW-larval body width

LHL-Larval head length

LHW-larval head width

L-Larvae

Max-Maximum

Min-Minimum

ML-Mandible length

MLPM- Malappuram

PB- Prey beating

PC- Pit clearing

PD-Pit diamter

PKD-Palakkad

PR- Prey clearing

PTMA- Pathanamthitta

PT-Pit Depth

Q- Quiescence

RB- River banks/Riparian

S- Submergence

TCR- Thrissur

TVRM- Thiruvanathapuram

WYND-Wayanad

2.4. RESULTS

A total of 64 adults (31 collected as adult and the remaining emerged from larval rearing), 315 larvae and 75 cocoons were collected from the study areas all over Kerala. Specimens were grouped under species using DNA barcoding. For this purpose one or two specimens from each study area were kept in absolute alcohol before the specimen was killed.

2.4.1. Collection- Sampling sites

The 68 study sites which were surveyed all over Kerala for identifying the presence of pit building antlion larvae genus *Myrmeleon* are provided in Table 9. Palakkad (25 sites), Thrissur (12 sites), Thiruvananthapuram (3 sites), Kozhikode (5 sites), Ernakulam (4 sites), Wayanad (6 sites), Malappuram (6 sites), Kannur (1 site), Idukki (1 site), Kottayam (1 site), Kollam (1 site), Alappuzha (1 site), Kasargod (1 site) and Pathanamthitta (1 site) and the larvae were collected from Fifty study areas of kerala except Kozhikode district (Plate 5, 6 and 7).

Table 9. Visited study sites for antlion collection in Kerala

Sl No.	Name of place	Latitude	Longitude
1	Parli	10º47’36”N	76º33’52”E
2	Edathara	10º47’29”N	76º33’58”E
3	Pezhumpara	10º34’41”N	76º36’13”E
4	Murukkumpara	10º49’62”N	76º32’94”E
5	Prakruthigramam	10º29’52”N	76º45’24”E
6	Tagore theatre	8◦50’34”N	76º96’08”E
7	Kanalpalam	10º70’51”N	76º41’26”E
8	Walayar	10º84’28”N	76º83’88”E
9	Pattikkad	10º54’96”N	76º33’52”E
10	Sarovaram park	11º26’86”N	75º79’27”E
11	Nedumbasserry	10º16’79”N	76º39’78”E
12	Athani	10º15’30”N	76º46’21”E
13	Sulthan Bathery	11º39’38”N	76º15’04”E
14	Kadamanchira	11º41’10”N	76º15’31”E

15	Thiruvizhamkunnu	11º02’23”N	76º22’16”E
16	Bengalow kunnu	11º17’17”N	76º14’17”E
17	Dhoni temple	10º51’11”N	76º37’29”E
18	Soochippara	11º50’95”N	76º16’06”E
19	Meenvallam	10º55’24”N	76º33’51”E
20	Mukkali	11º66’84”N	75º55’88”E
21	Conoly	11º26’53”N	76º20’69”E
22	Poabs Tea factory	10º32’49”N	76º42’05”E
23	Kakkayam	11º54’73”N	75º89’26”E
24	Thusharagiri	11º28’22”N	76º03’14E
26	Poomala	10º36’00”N	76º14’35”E
27	Ezhattumugham	10º17’40”N	76º27’09”E
28	Kuruva	11º50’48”N	76º04’20”E
29	Thumboormuzhi	10º17’44”N	76º27’33”E
30	Vettilappara	10º29’22”N	76º51’49”E
31	Meenkara	10º62’45”N	76º80’43”E
32	Kiriyathupara	11º53’81”N	75º89’51”E
33	Ottappalam river	10º46’22”N	76º22’45”E
34	Parli Manamthody	10º47’33”N	76º33’51”E
35	Wadakkancherry	10º39’52”N	16º15’05”E
36	Nedupuzha	10º29’34”N	76º12’46”E
37	Kodungallur	10º13’20”N	76º12’09”E
38	Vellayani	8º25’53”N	76º59’09”E
39	Brennan college campus	11º46’41”N	75º28’07”E
40	Thennal resort, Kattikulam	11º50’26”N	76º05’05”E
41	Nilambur Dippo	11º16’44”N	76º15’11”E
42	Irrigation office	11º16’18”N	76º13’43”E
43	Idimuzhikkal	11º09’48”N	75º52’39”E
44	Marthoma college, Tiruvalla	9º24’02”N	76º35’02”E
45	Moyan modal school	10º46’46”N	76º39’17”E
46	Vettikkattiri	10º43’53”N	76º16’56”E
47	Kanniyampuram	10º46’14”N	76º21’26”E

48	Kinavallur	10°48’32”N	76°33’56”E
49	Meenakshivilas	11°65’53”N	75°96’44”E
50	Thathamangalam	10°67’72”N	76°71’63”E
51	Thiruvalathur	10°74’25”N	76°68’78”E
52	Asarikkadu	10°50’94”N	76°32’55”E
53	Ramakrishnappadi	10°46’07”N	76°26’27”E
54	Melarankode quarters	8°29’12”N	76°57’57”E
55	Tanur	10°98’20”N	75°87’54”E
56	Kanimangalam	10°48’61”N	76°20’88”E
57	Vandithavalam	10°65’91”N	76°74’23”E
58	Mananchira square	11°25’37”N	75°77’64”E
59	Suvarnodyanam	10°14’99”N	76°36’90”E
60	Padur	10°65’52”N	76°46’21”E
61	CPCRI, Chowki	12°31’40”N	74°58’06”E
62	Nangiarkulangara	9°25’90”N	76°46’35”E
63	Kollam Railway station	8°53’09”N	76°35’42”E
64	Ettumanur	9°40’14”N	76°33’24”E
65	Vadakkencherry	10°35’32”N	76°29’05”E
66	Maneed	9°90’55”N	76°45’78”E
67	Adimali	10°03’02”N	76°87’84”E
68	Kulappulli	10°78’75”N	76°27’98”E

2.4.2. Identification

For identifying the presence of different antlion species in Kerala, both adult and larvae were collected. The specimens were identified by the help of both classical and molecular taxonomy. Two species namely *Myrmeleon pseudohyalinus* and *Myrmeleon hyalinus* of pit building antlion larvae were observed in this study. *Myrmeleon pseudohyalinus* was used for the ecology and behaviour studies as more specimens of this species were collected.

Plate 5

Wayanad

Thrissur

Palakkad

Malappuram

Plate 6

Kannur

Pathanamthitta

Thiruvanathapuram

Kollam

Plate 7

Ernakulam

Kasargod

Idukki

Alappuzha

Kottayam

Plate 8

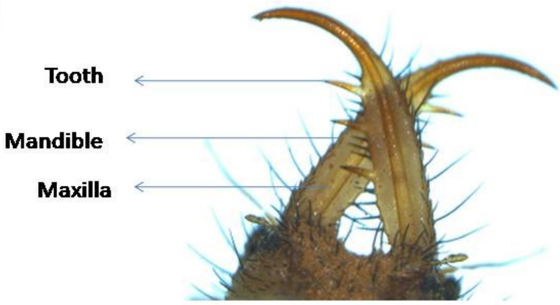
A

B

C

Fig. 1 A-C. General morphology of *M. pseudohyalinus* larvae A. Dorsal view, B. Ventral View, C. Abdomen

D

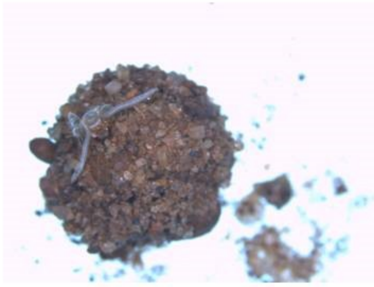


E

Fig 2. D . Dorsal portion of head, E. Mouth parts

Plate 9

F



G

H

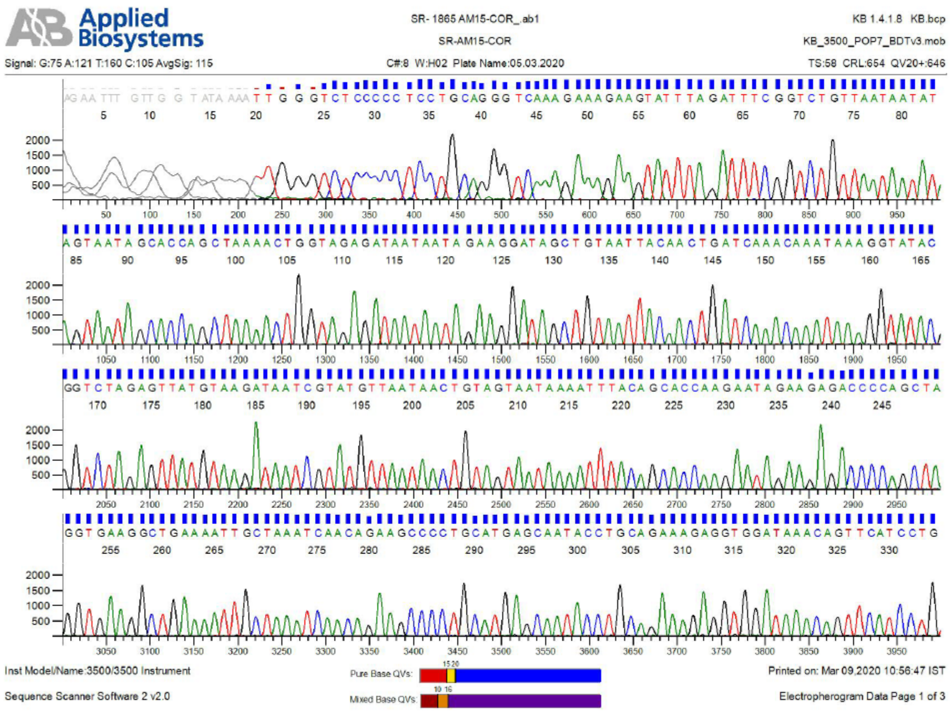
I

Fig. F-Exuviae, G- Cocoon with Exuviae, H- Adult, I- Tip of Abdomen

Adult- *M. Pseudohyalinus*

Plate 10

Sequence result of *Myrmeleon pseudohyalinus* collected from Adimali (Reverse Lap)



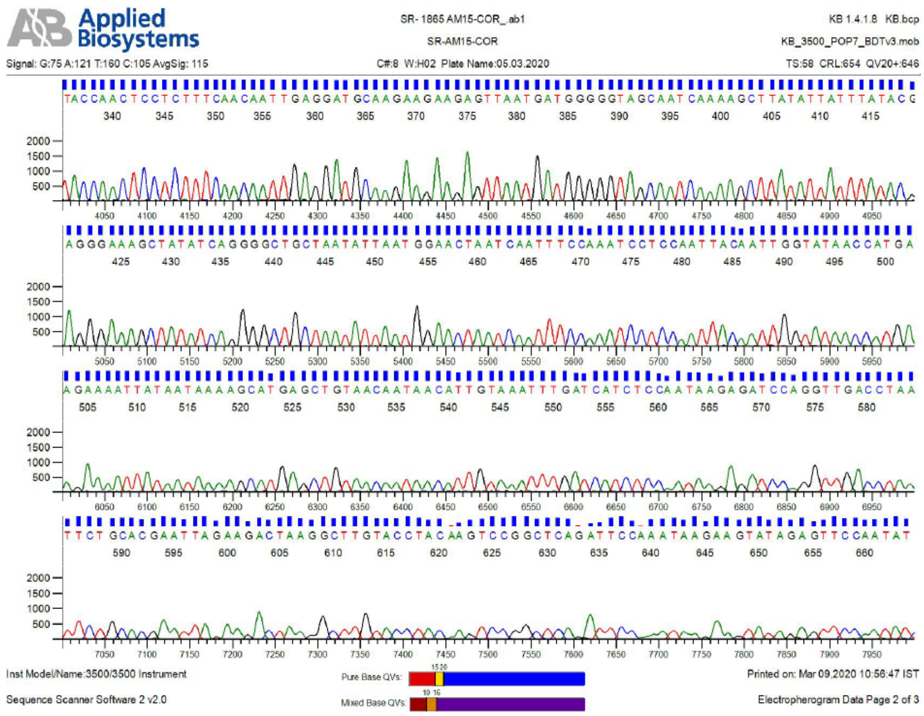
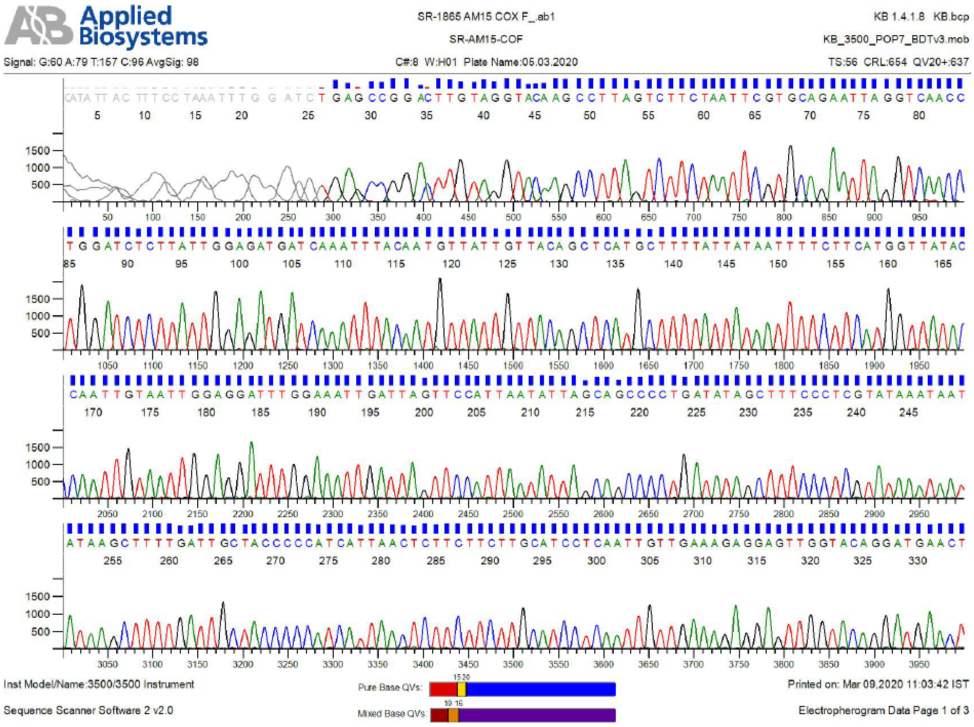


Plate 11

Sequence result of *Myrmeleon pseudohyalinus* collected from Adimali (Forward Lap)



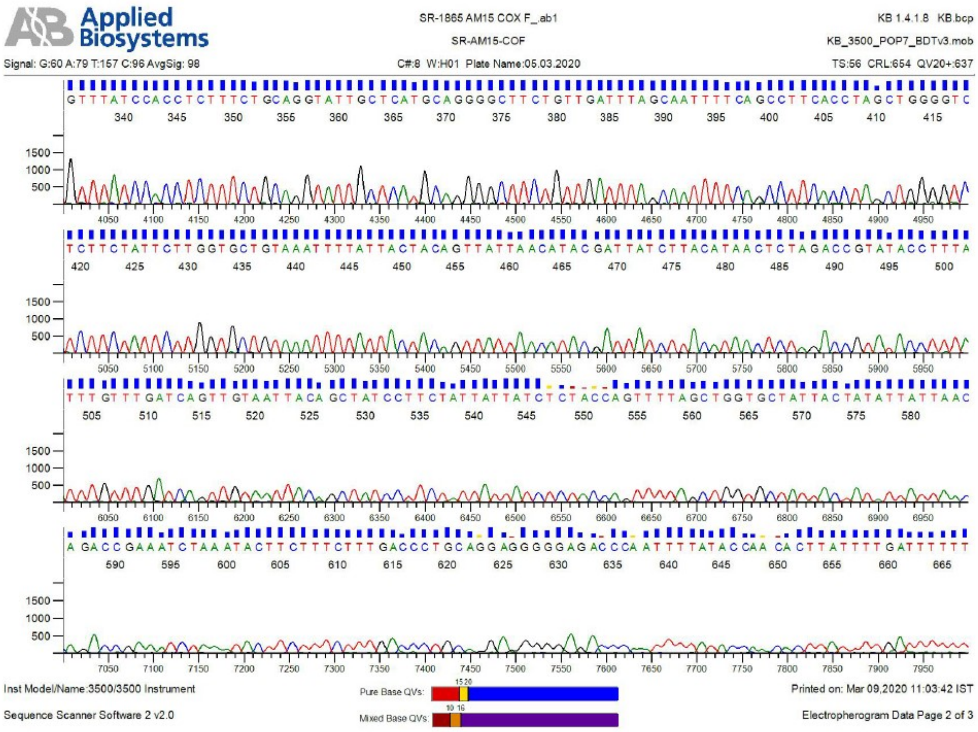
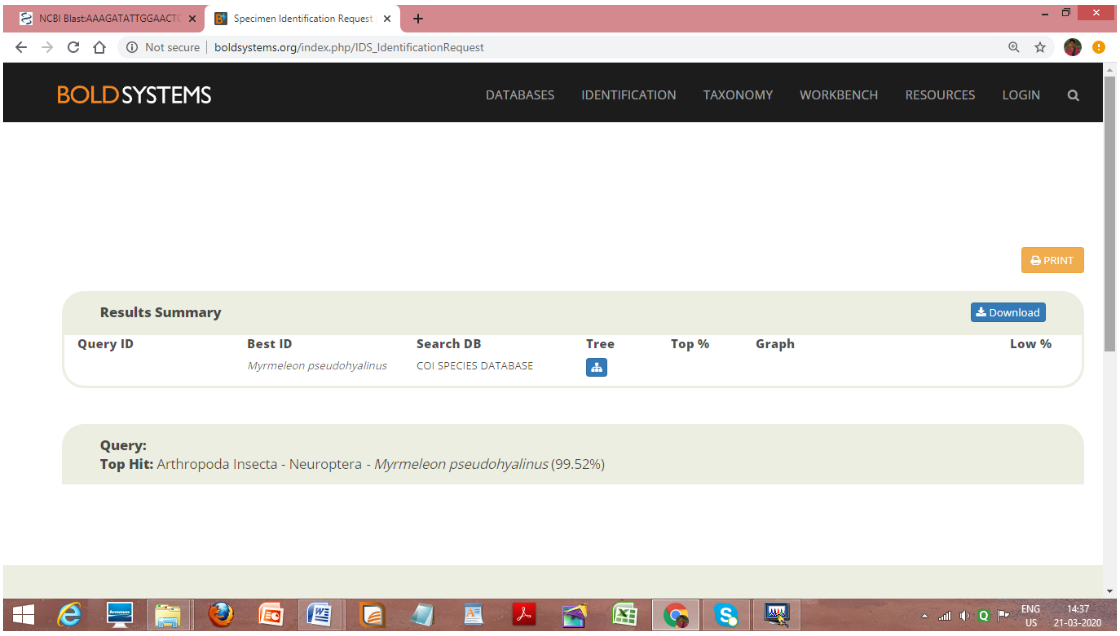


Plate 12

BOLD system result of *M.pseudohyalinus*



M. pseudohyalinus was collected from 9 study areas coming under 4 districts of Kerala. The detailed localities are specified in Table 10 and the general morphology of the larvae, cocoon, adult and exuviae are given in Plate 8 and 9.

Table 10. The collection sites of *M. pseudohyalinus*

Sl No	Locality	District	Latitude	Longitude
1	Meenavallam (L)	Palakkad	10º55'24"N	76º33'51"E
2	Dhoni (L)	Palakkad	10º51'11"N	76º37'29"E
3	Canoly (L)	Malappuram	11º26'53"N	76º20'69"E
4	Mukkali (L)	Palakkad	11º66'84"N	75º55'88"E
5	Kalady (AD)	Ernakuam	10º11'46"N	76º47'77"E
6	Kulappulli (L)	Palakkad	10º78'75"N	76º27'98"E
7	Vandithavalam (AD)	Palakkad	10º65'91"N	76º74'23"E
8	Maneed (L)	Ernakulam	9º90'55"N	76º45'78"E
9	Adimali (L)	Idukki	10º03'02"N	76º87'84"E

The DNA of nine samples of *M. pseudohyalinus* was extracted and amplified using standard methodology. The FASTA formats of DNA sequence of the species is described (Table 11) and chromatogram is given (Plate 10 and 11). Multiple sequence alaignment of sequences (Table 14) were performed in CLUSTAL O (1.2.4) and a phylogenetic tree was made (Fig. 1). The sequence was run in BOLD systems for the identification and confirmation. Also a phylogenetic tree was made with *M. pseudohyalinus* sequence and related species from NCBI (Fig. 2). From the tree it is clear that *M. formicarius, M. fasciatus, M. obscurus* and *M. quinquemaculatus* are species with common ancestry and *M. carolinus* and *M. crudelis* are another group of species with common ancestry. The remaining *M. mariaemathildae*, *M. caliginosus, M. hyalinus* and *M. pseudohyalinus* belongs to a common stalk.The sequence showed 99.52% similarity with the already existing sequence in the database (Plate 12). The sequence information of different populations of *M. pseudohyalinus* was given in Table 12.

Table 11. FASTA formats of *Myrmeleon pseudohyalinus* sequence of different populations

>AM05-Meenavallam ACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTC TTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCAATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATT CTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGT TTGATCAGTTGTAATTACAGCTATCCTT
> AM06-Dhoni CTGGTCAACAAATCATAAAAGATTATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCT TAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAG CTCATGCTTTTATTATAATTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCT TGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAG GGGCTTCTGTTGATTTAGCAATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTAT CCTTCTATTATTATCTCTACCAGTTTT
>AM07-Canoly TTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCTCT TATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTCATGGTTATACCAATTG TAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATA AGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAAC TGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCAATTTTCAGCCTTCACCTAGCTG GGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGT ATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCT
>AM08-Mukkali ATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGT GCAGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTAT AATTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATA TAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTT GAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTT AGCAATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATAC GATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCT CTACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCTTT
>AM09-Kalady GGTCAACAAAATCATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAG TCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTC ATGCTTTTATTATAATTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTA GCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGC ATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGG CTTCTGTTGATTTAGCAATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACA GTTATTAACATACGATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCT TCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTT
>AM12-Kulappulli TCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAAC CTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTCATGGTT ATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATAGCTTTCCCTCGTAT AAATAATATAAGCTTTTGATTACTACCCCCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTA CAGGATGAACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCAATTTTTAGCCTT CATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTATATAAC

TCTAGATCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTTTACCAGTTTTAGCTG GTGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGGGGGACCC
>AM13-Vandithavalam GGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGG TCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTCA TGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATAGCTTTCCCT CGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGT TGGTACAGGATGAACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCAATTTTTA GCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTAT ATAACTCTAGATCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTA
>AM14-Maneed AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGC AGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAA TTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTGGCAGCCCCTGATATA GCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGA AAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAG CAATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGA TTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCT ACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGG
>AM15-Adimali AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGC AGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAA TTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATA GCTTTCCCTCGTATAAATAATATAAGCTTTTGATTGCTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGA AAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAG CAATTTTCAGCCTTCACCTAGCTGGGGTCTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGA TTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCT ACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGG GAGA

Table 12. The Sequence information of different populations of *Myrmeleon pseudohyalinus*

Sl no	Population	Bp length	Base count				AT content (%)	GC Content (%)
			A	T	G	C		
1	Meenvallam	268	105	62	57	44	62.31	37.69
2	Dhoni	587	229	154	94	110	65.25	34.75
3	Canoly	555	219	139	104	93	64.50	35.50
4	Mukkali	622	247	165	112	98	66.24	33.76
5	Kalady	639	252	171	116	100	66.20	33.80
6	Kulappulli	633	161	249	107	116	64.77	35.23
7	Vandithavalam	532	137	209	89	97	65.04	34.96
8								

	Maneed	640	166	247	107	120	64.53	35.47
9	Adimali	644	167	246	111	120	64.13	35.87

Seasonal abundance of *Myrmeleon pseudohyalinus* larvae were noted from January 2016 to December 2017 from three spots of Parli, Palakkad and it was tabulated such a way that the presence of larval pits noted (Percentage of occurrence was calculated by the number of *M.pseudohyalinus* larvae identified divided by total number of antlion larvae multiplied by hundred) and given in Table 13. The highest number of larvae and pits were present in the month of January to March followed by April to June (2016 and 2017).

Table 13. Seasonal occurance of *M. pseudohyalinus* larvae

Sl. No.	Months (2016 & 2017)	Percentage of occurance of *Myrmeleon pseudohyalinus*
1	January to March	44%
2	April to June	36%
3	July to September	2%
4	October to December	18%

Table 14. CLUSTAL O (1.2.4) multiple sequence alignment

```
AM15-Adimali             ------------------AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          42
AM14-Maneed              ------------------AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          42
AM07-Canoly              ------------------------------------------TTATTTGGAATCTGAGCC
          18
AM05-Meenavallam         ------------------------------------------------------------
          0
AM06-Dhoni               CTGGTCAACAAATCATAAAAGATTATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          60
AM12-Kulappulli          --------------------------------TCTATACTTCTTATTTGGAATCTGAGCC
          28
AM08-Mukkali             ----------------ATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          44
AM09-Kalady              ---GGTCAACAAAATCATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          57
AM13-Vandithavalam       ---------------------------GGAACTCTATACTTCTTATTTGGAATCTGAGCC
          33


AM15-Adimali             GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          102
AM14-Maneed              GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          102
AM07-Canoly              GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          78
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AM05-Meenavallam        ------------------------------------------------------------
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AM06-Dhoni              GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
         120
AM12-Kulappulli         GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
         88
AM08-Mukkali            GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
         104
AM09-Kalady             GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
         117
AM13-Vandithavalam      GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
         93


AM15-Adimali            CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         162
AM14-Maneed             CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         162
AM07-Canoly             CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         138
AM05-Meenavallam        ------------------------------------------------------------
         0
AM06-Dhoni              CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         180
AM12-Kulappulli         CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         148
AM08-Mukkali            CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         164
AM09-Kalady             CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         177
AM13-Vandithavalam      CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
         153


AM15-Adimali            TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         222
AM14-Maneed             TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         222
AM07-Canoly             TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         198
AM05-Meenavallam        ------------------------------------------------------------
         0
AM06-Dhoni              TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
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AM12-Kulappulli         TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         208
AM08-Mukkali            TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         224
AM09-Kalady             TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         237
AM13-Vandithavalam      TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
         213


AM15-Adimali            TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTGCTACCC
         282
AM14-Maneed             TTGGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
         282
AM07-Canoly             TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
         258
AM05-Meenavallam        --------------------------------------------------------ACCC
         4
AM06-Dhoni              TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
         300
AM12-Kulappulli         TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
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AM08-Mukkali            TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
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AM09-Kalady             TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
         297
AM13-Vandithavalam      TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
         273
                                                                                ****

AM15-Adimali            CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
         342
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AM14-Maneed               CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        342
AM07-Canoly               CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        318
AM05-Meenavallam          CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        64
AM06-Dhoni                CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        360
AM12-Kulappulli           CCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        328
AM08-Mukkali              CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        344
AM09-Kalady               CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        357
AM13-Vandithavalam        CCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
        333
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AM15-Adimali              ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        402
AM14-Maneed               ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        402
AM07-Canoly               ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        378
AM05-Meenavallam          ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        124
AM06-Dhoni                ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        420
AM12-Kulappulli           ACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCA
        388
AM08-Mukkali              ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        404
AM09-Kalady               ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
        417
AM13-Vandithavalam        ACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCA
        393
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AM15-Adimali              ATTTTCAGCCTTCACCTAGCTGGGGTCTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        462
AM14-Maneed               ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        462
AM07-Canoly               ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        438
AM05-Meenavallam          ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        184
AM06-Dhoni                ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        480
AM12-Kulappulli           ATTTTTAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
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AM08-Mukkali              ATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        464
AM09-Kalady               ATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        477
AM13-Vandithavalam        ATTTTTAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
        453
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AM15-Adimali              ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
        522
AM14-Maneed               ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
        522
AM07-Canoly               ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
        498
AM05-Meenavallam          ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
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AM06-Dhoni                ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
        540
AM12-Kulappulli           ACAGTTATTAACATACGATTATCTTATATAACTCTAGATCGTATACCTTTATTTGTTTGA
        508
AM08-Mukkali              ACAGTTATTAACATACGATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGA
        524
AM09-Kalady               ACAGTTATTAACATACGATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGA
        537
AM13-Vandithavalam        ACAGTTATTAACATACGATTATCTTATATAACTCTAGATCGTATACCTTTATTTGTTTGA
        513
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AM15-Adimali            TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          582
AM14-Maneed             TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          582
AM07-Canoly             TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCT---
          555
AM05-Meenavallam        TCAGTTGTAATTACAGCTATCCTT------------------------------------
          268
AM06-Dhoni              TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTT-------------
          587
AM12-Kulappulli         TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTTTACCAGTTTTAGCTGGTGCTATT
          568
AM08-Mukkali            TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          584
AM09-Kalady             TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          597
AM13-Vandithavalam      TCAGTTGTAATTACAGCTA-----------------------------------------
          532
                        *******************

AM15-Adimali            ACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGGGA
          642
AM14-Maneed             ACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGG--
          640
AM07-Canoly             ------------------------------------------------------------
          555
AM05-Meenavallam        ------------------------------------------------------------
          268
AM06-Dhoni              ------------------------------------------------------------
          587
AM12-Kulappulli         ACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGGGG
          628
AM08-Mukkali            ACTATATTATTAACAGATCGAAATCTAAATACTTCTTT----------------------
          622
AM09-Kalady             ACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTT------------------
          639
AM13-Vandithavalam      ------------------------------------------------------------
          532


AM15-Adimali            GA---          644
AM14-Maneed             -----          640
AM07-Canoly             -----          555
AM05-Meenavallam        -----          268
AM06-Dhoni              -----          587
AM12-Kulappulli         GACCC          633
AM08-Mukkali            -----          622
AM09-Kalady             -----          639
AM13-Vandithavalam      -----          532
```

*indicates complimentary region, -- indicates missing nucleotides

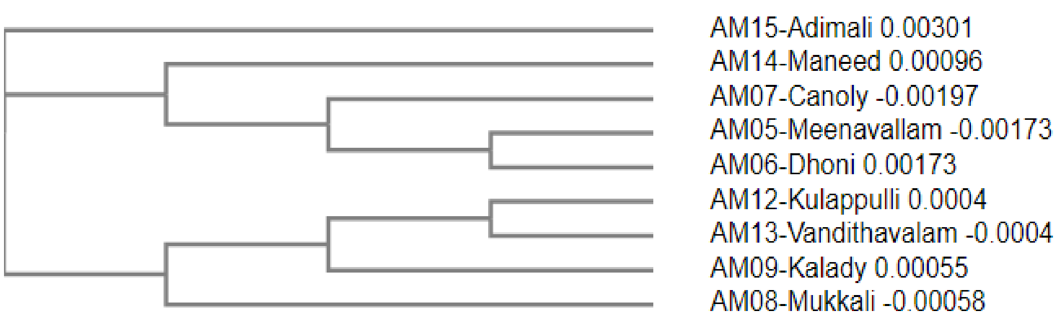


Fig. 1. Phylogenetic tree results of *Myrmeleon pseudohyalinus* populations from Clustal omega

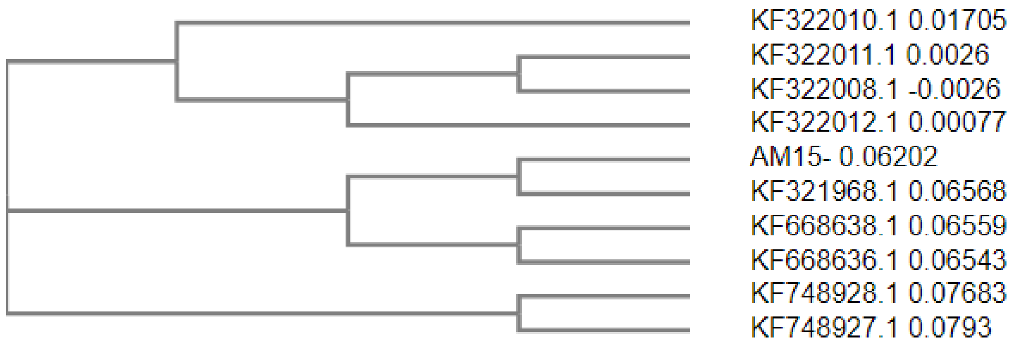


Fig. 2. Phylogenetic tree result of *M. pseudohyalinus* with related species from NCBI

2.4.3. Habitat of Antlion larvae (Genus Myrmeleon)

The four different habitats of antlion larvae Genus *Myrmeleon* classified from the study is described below

2.4.3.1. Abandoned area (9 study sites)

Here the soil vibrations were low when compared to human dwelling areas, so that they can make funnel shaped pits more cost effective and sense the arrival of even a small prey species. Here sand, soil and m-sand materials were observed as pit building media and the shaded areas were preferred by larvae. In abandoned areas, the soil texture was sand or fine sand. The mean percentage of sand, silt and clay was 89.5, 3 and 7.5 respectively. There are limited numbers of plants or trees present in these habitats because of the presence of walls and ceilings. The trees include coconut, teak etc with small canopy and mainly large trees with lesser quantity of small sized pollinators, the vegetation and antlion larval population was not that much correlated here. Also the direct light illumination was low in these areas.

2.4.3.2. Human dwelling area (27 study sites)

Here also antlion larvae preferred shaded areas but the presence of human being and other animals like goat, cattle, dogs, cats were high. But the designs of pits built in these areas were found without causing much damage by rain during monsoon. The light illumination was also less in these habitats but the soil vibration was high. The soil texture was sand (86%) or fine sand (14%).The mean percentage of sand, silt and clay was 86, 2 and 12 respectively in these areas.

The prey capture success was high in human dwelling areas because of the presence of ants in these areas in large quantity and also they are considered as the common pest of human beings. The plants and trees are more in human dwelling areas, so that the insect pollinators and small organisms were rich in these areas.

2.4.3.3. Forest boundary area (7 study sites)

In these habitats, lowsoil vibration and light illumination were noted. Here the vegetation was somewhat high and soil and atmospheric temperatue was low.The soil texture was sand (67%) and sometimes fine sand (33%). The mean percentage of sand, silt and clay was 90, 1 and 9 respectively.

2.4.3.4. Riparian (7 study sites)

River banks were considered as riparian habitat, also found antlion larval pits under large coconut trees beside the river. The soil texture was sand (75%) and fine sand (25%). The mean percentage of sand, silt and clay was 87, 2 and 11 respectively. The wind was somewhat high in these habitats and the soil vibrations were somewhat low. The vegetation was thick in these areas so that the insect pollinators were present at an increased level.

A total of 68 study sites were visited and the antlion larvae were collected from 50 sites which include 9 abandoned areas, 27 human dwelling areas, 7 forest areas and 7 riparian areas (Table 15 and 16). The district wise distribution of genus *Myrmeleon* was plotted in Map 3 (Palakkad), Map 4 (Thrissur), Map 5 (Thiruvananthapuram), Map 6 (Wayanad), Map 7 (Malappuram), Map 8 (Kottayam), Map 9 (Kasargod), Map 10 (Pathanamthitta), Map 11 (Kannur), Map 12 (Alappuzha), Map 13 (Ernakulam), Map 14 (Idukki), and Map 15 (Kollam).

Table 15. The different habitats in which the search of antlion larvae carried out (68 study sites).

Abandoned areas	Forest areas	Riparian
Parli	Thiruvizhamkunnu	Parali Riverbank
Edathara	Bengalow kunnu	Poomala
Pezhumpara	Dhoni temple	Ezhattumugham

Murukkumpara	Soochippara	Kuruva
Prakruthigramam	Meenvallam	Thumboormuzhi
Tagore theatre	Mukkali	Vettilappara
Kanalpalam	Canoly	Meenkara
Walayar	Poabs Tea factory	Kiriyathupara
Pattikkad	Kakkayam	Ottappalam river
Sarovaram park	Thusharagiri	
Nedumbasserry		
Athani		
Sulthan Bathery		
Kadamanchira		
Human dwelling areas		
Parli Manamthody	Kinavallur	Suvarnodyanam
Wadakkancherry	Meenakshivilas	Padur
Nedupuzha	Thathamangalam	CPCRI, Chowki
Kodungallur	Thiruvalathur	Nangiarkulangara
Vellayani	Asarikkadu	Kollam Railway station
Brennan college campus	Ramakrishnappadi	Ettumanur
Thennal resort, Kattikulam	Melarankode quarters	Vadakkencherry
Nilambur Dippo	Tanur	Moyan modal school
Irrigation office	Kanimangalam	Vettikkattiri
Idimuzhikkal	Vandithavalam	Kanniyampuram
Marthoma college, Tiruvalla	Mananchira square	Kulappulli
Maneed	Adimali	

Table 16. The habitats in which the presence of antlion larvae observed (50 study sites).

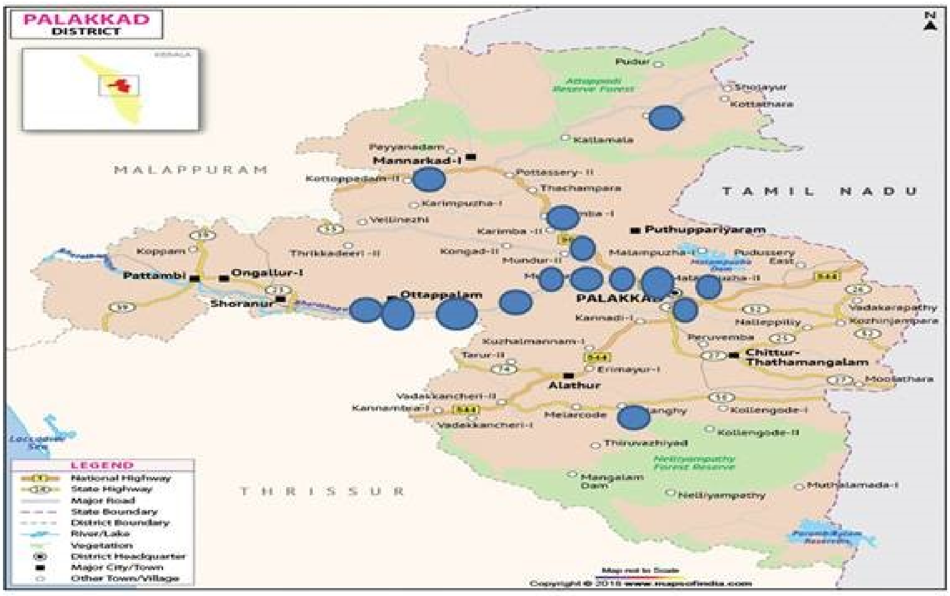
Abandoned	Forest	Riparian
Parli	Thiruvizhamkunnu	Parali Riverbank
Edathara	Bengalow kunnu	Poomala
Pezhumpara	Dhoni	Ezhattumugham

Murukkumpara	Soochippara	Kuruva
Prakruthigramam	Meenvallam	Thumboormuzhi
Tagore theatre	Mukkali	Vettilappara
Pattikkad	Canoly	Ottappalam river
Sulthan Bathery		
Kadamanchira		
Human Dwellings		
Parli Manamthody	Kinavallur	CPCRI, Chowki
Wadakkancherry	Thiruvalathur	Nangiarkulangara
Nedupuzha	Asarikkadu	Kollam Railway station
Kodungallur	Ramakrishnappadi	Ettumanur
Vellayani	Melarankode quarters	Moyan modal school
Brennan college campus	Tanur	Vettikkattiri
Nilambur Dippo	Idimuzhikkal	Kanniyampuram
Thennal resort, Kattikulam	Adimali	Irrigation office
Marthoma college, Tiruvalla	Maneed	Kulappulli

2.4.4. Morphometric Analysis-Larvae

M. pseudohyalinus larvae were collected from seven study areas (Table 10) coming under four districts of Kerala namely Palakkad, Malappuram, Ernakulam and Idukki. Morphometric measurements of the second and third instar of the species were taken, the LBL, LBW, LHL, LHW and ML (Table 17 and 18) were measured and compared with the habitats. Collections were taken from forest boundaries (Meenvallam, Dhoni, Canoly, and Mukkali) and human dwelling areas (Kulappulli, Maneed and Adimali). The specimens were brought to laboratory for rearing purpose (Plate 13) and the photographs were taken and given in Plate 18.

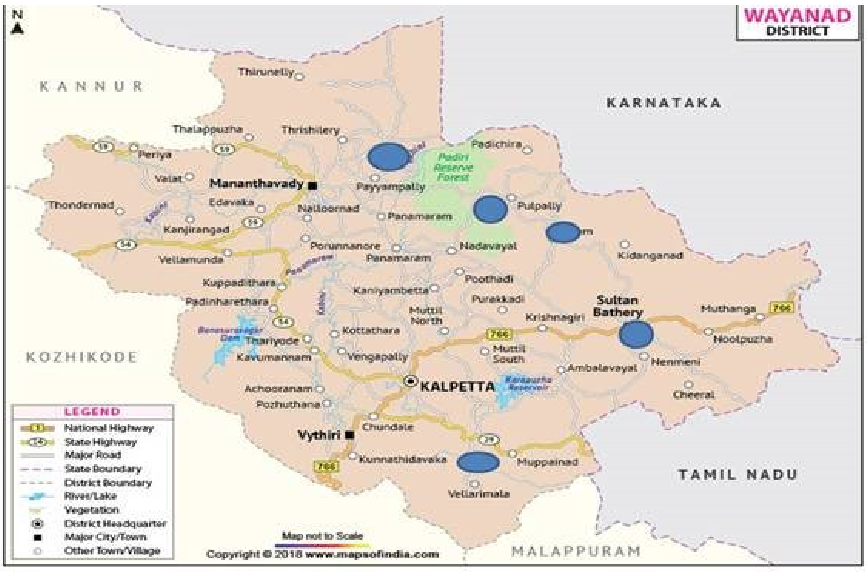
The second instar larvae with highest value of mean LBL were collected from Canoly (MLM) and lowest values of mean LBL from Dhoni (PKD). The values of mean LBL ranged from 0.5 to 0.8 cm. The mean LBL from seven different sites showed slight difference as indicated by the standard deviation of 0.098. Mean LBW, LHL, LHW and ML also showed similar trend with the values collected



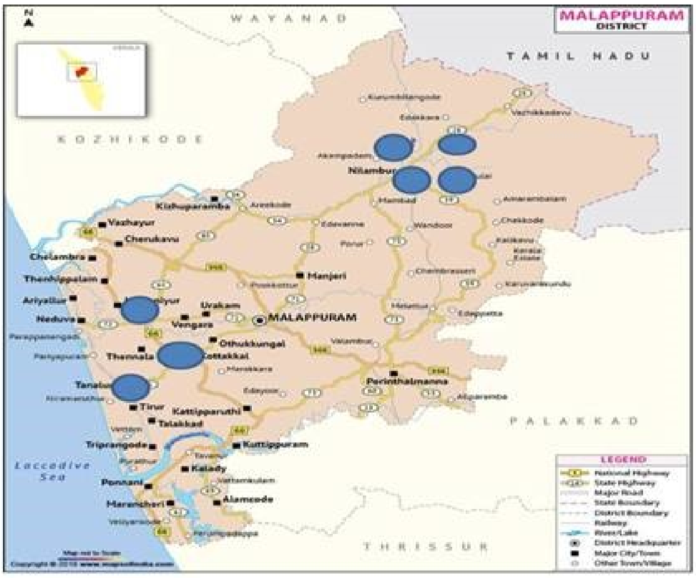
Map 3- Distribution of Genus *Myrmeleon* in Palakkad Dstrict

Map 4- Distribution of Genus M*yrmeleon* in Thrissur District

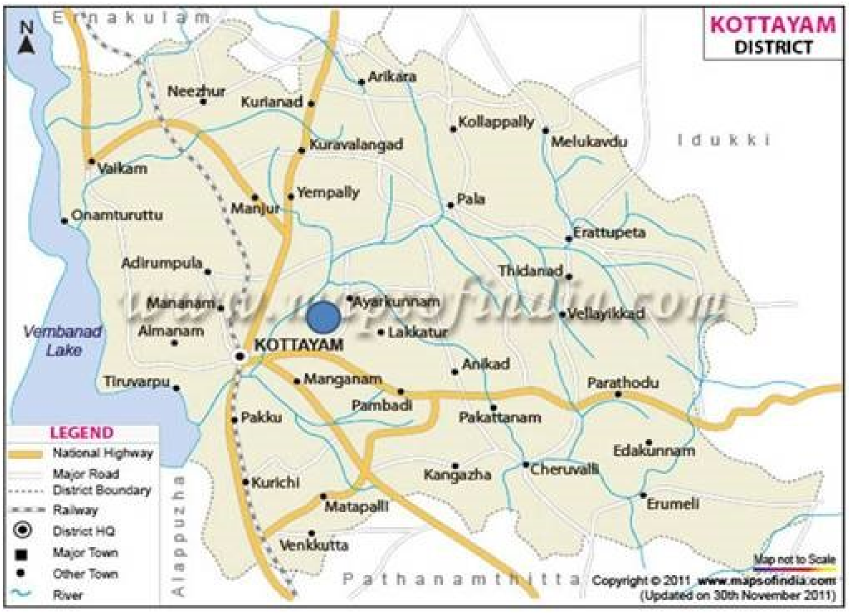
Map 5- Distribution of Genus *Myrmeleon* in Thiruvananthapuram District



Map6- Distribution of Genus *Myrmeleon* in Wayanad District



Map 7- Distribution of Genus *Myrmeleon* in Malappuram District



Map 8- Distribution of Genus *Myrmeleon* in Kottayam District

Map 9- Distribution of Genus *Myrmeleon* in Kasargod District

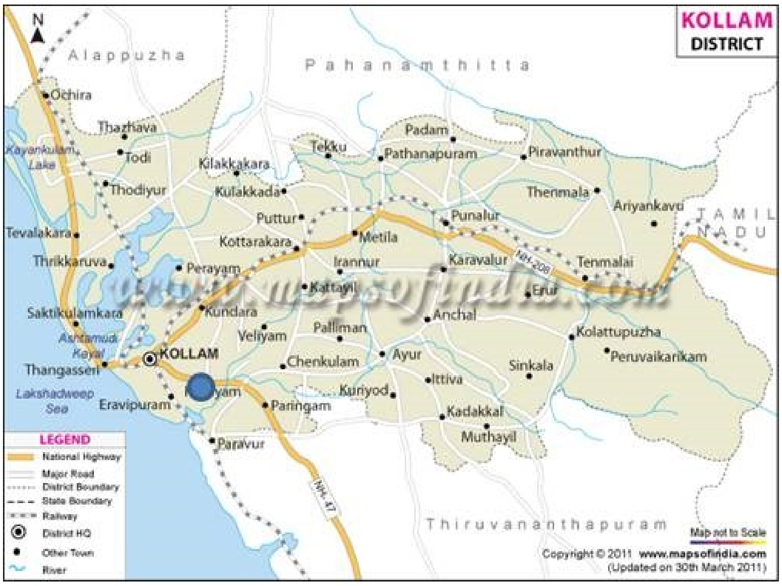
Map 10- Distribution of Genus *Myrmeleon* in Pathanamthitta District

Map 11- Distribution of Genus *Myrmeleon* in Kannur District

Map 12- Distribution of Genus *Myrmeleon* in Alappuzha District

Map13- Distribution of Genus *Myrmeleon* in Ernakulam District

Map14- Distribution of Genus *Myrmeleon* in Idukki District



Map15- Distribution of Genus *Myrmeleon* in Kollam District

from different sites having almost similar.Standard deviation for LBW, LHL, LHW and ML were 0.038, 0.079, 0.053 and 0.053 respectively. Therefore it may be assumed that *M. pseudohyalinus* second instar larvae from study area would follow the above morphometric measurement range (Fig.3).

The third instar larvae with highest value of mean LBL were collected from Meenvallam (PKD) and Canoly (MLM) and lowest values from Mukkali (PKD), Maneed (ERN) and Adimali (IDKI). The values of LBL ranged from 0.8 to 1 cm. The LBL from seven different sites showed very little difference as indicated in the standard deviation of 0.089. Mean LBW, LHL, LHW and ML also showed similar trend with the values collected from different sites having almost similar. Standard deviation for LBW, LHL, LHW and ML were 0.049, 0.053, 0 and 0.053 respectively. Therefore it may be assumed that *M. pseudohyalinus* third instar larvae from study area would follow the above morphometric measurement range (Fig. 4).

The five body measurements of larvae of *Myrmeleon sp.* and two measurements of cocoon were compared by using Pearson correlation for parametric variables at 5% significant level (Appendix 1). Here, positive correlation were found between body width and body length , body length and head length, body width and head length, body width and head width (Fig. 7).

The shape and size of a body and its body parts both in absolute and relative term are a function of its ecological role. The shape and size determines how efficiently an organism can perform different activities both general and specialized. Correlation between certain body parts or traits would indicate a symmetry between them which in turn would determine its efficacy in its chosen habit and habitat.

Since this is a preliminary study, there is no data available on these species regarding body shape, size and relative size of body parts. The objective in presenting the data here is to provide a baseline data from where other studies can take off.

Plate 13

Rearing of Antlion larvae

Adult spreading

collection

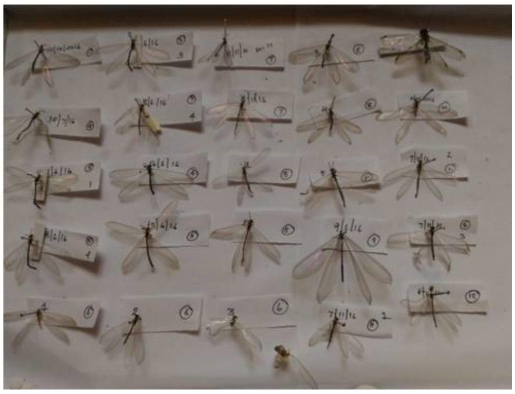
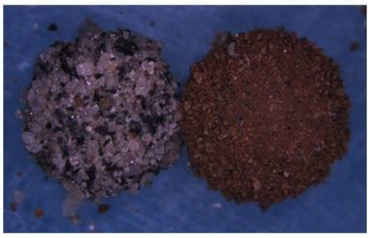


Plate 14

Larvae



Types of cocoons

Adult

Cocoon with exuviae

Cocoon with out exuviae

Exuviae

Plate 15

Collected soil samples

Microscope

Plate 16

Weighing of Antlion Cocoon

Slide making

Correlation

	PDE	*PDI*	*LHL*	*LHW*	*LBL*	*LBW*
PDE	1					
PDI	-0.08251	1				
LHL	-0.22056	0.216193	1			
LHW	-0.16066	0.355969	0.681227	1		
LBL	0.021572	0.397212	0.371056	0.596104	1	
LBW	-0.1565	0.587012	0.58401	0.513509	0.518773	1

Fig. 5. Correlation results showing relationships between the different parameters of pit size and larval size. (Krishnan & Kakkassery, 2016)

Relationship between pit size and larval size were studied (Fig. 5) and from the correlation results it was noted that, there is a negative correlation between larval head length and pit depth (Fig. 7). That is, the pit depth increased with the decrease in larval head length. So smaller the head length of antlion larvae, bigger the depth of pits. Also, there is a high positive correlation between larval body width and pit diameter (Fig. 6). That is the pit diameter increases with the increase in larval body width (Plate 17). There is no relationship between the larval body length and pit depth. The larval body width may help the larvae to make the pits with larger diameter. From this study, it is clear that other parameters of pit do not have any relationship with the larvae dwelling inside. Also there is a high positive correlation between pit depth and diameter (fig. 8). The pit depth increases with the increase in diameter.

The high positive correlation results of larval body width and pit diameter were used for the instar determination study. Here the pit diameter classified into three classes according to their values, that are 1.2-2.2, 2.2-3.2, 3.2-4.2, from these classes the mean larval body width in each diameter was calculated by taking its average (Table 17). The result is furnished below.

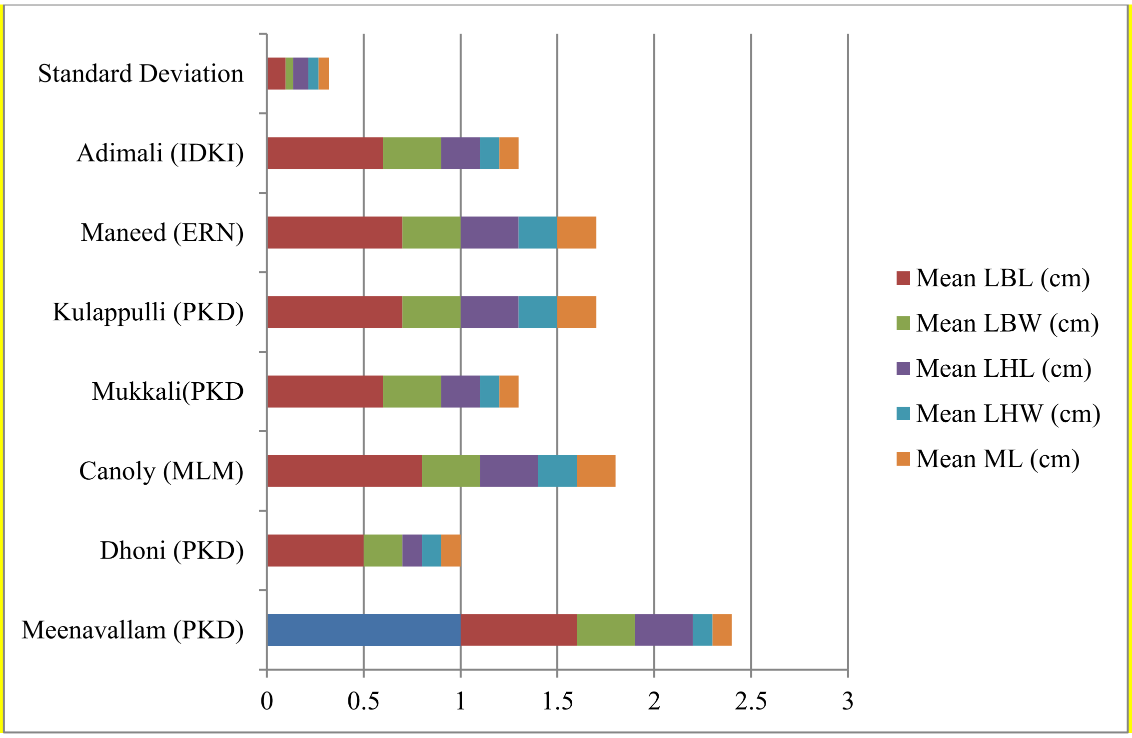


Fig.3. Comparison of body measurements of second instar *Myrmeleon pseudohyalinus* larvae in different study areas of Kerala

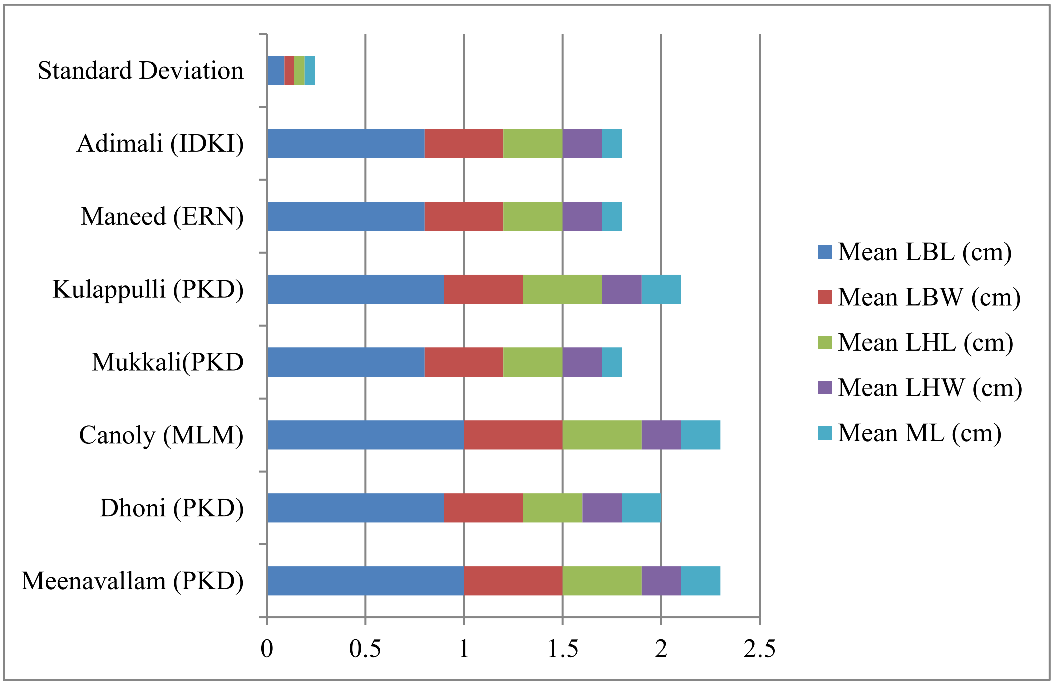


Fig.4. Comparison of body measurements of third instar *Myrmeleon pseudohyalinus* larvae in different study areas of Kerala

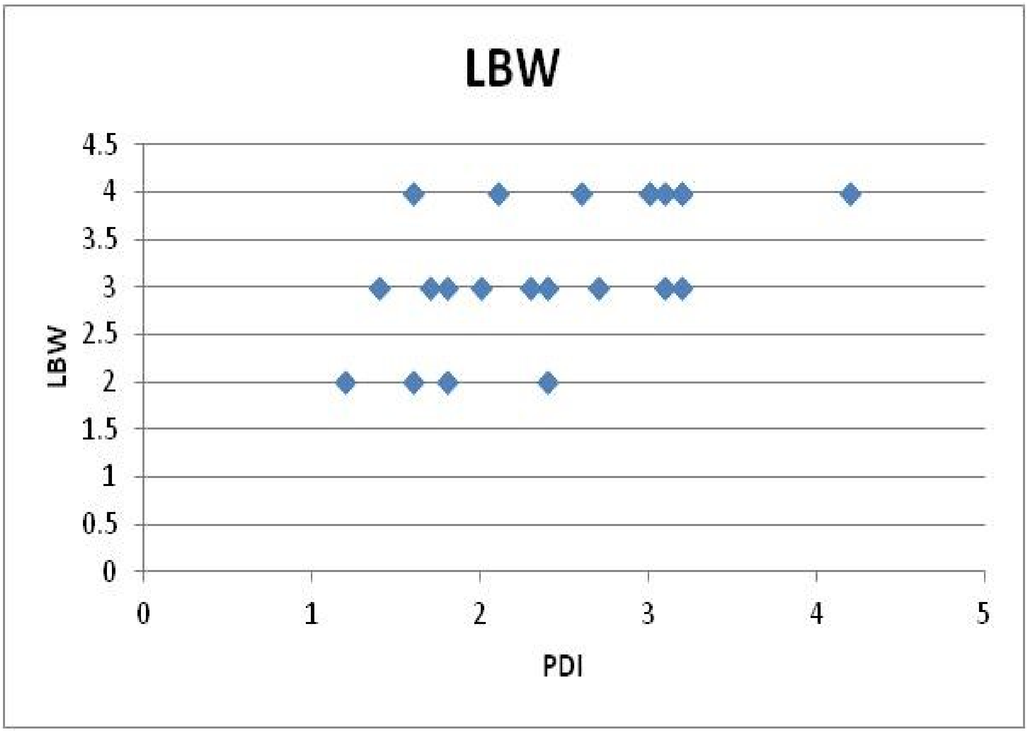


Fig. 6. Positive correlation between larval body width and pit diameter.

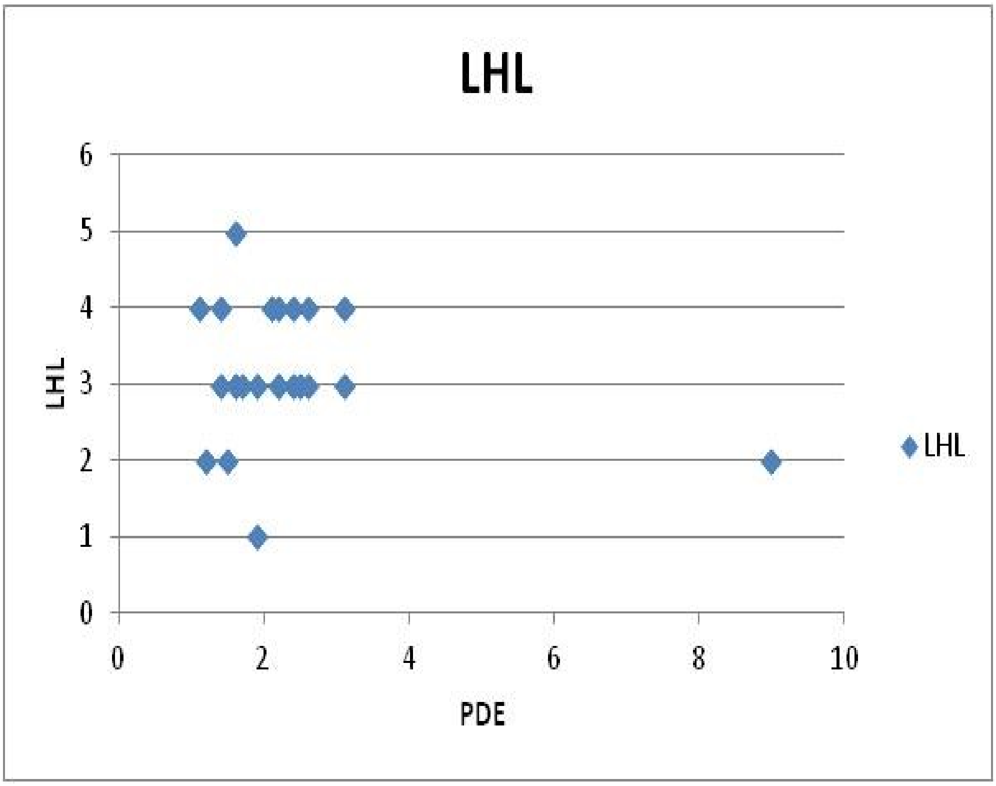


Fig.7. Negative correlation between larval head length and pit depth

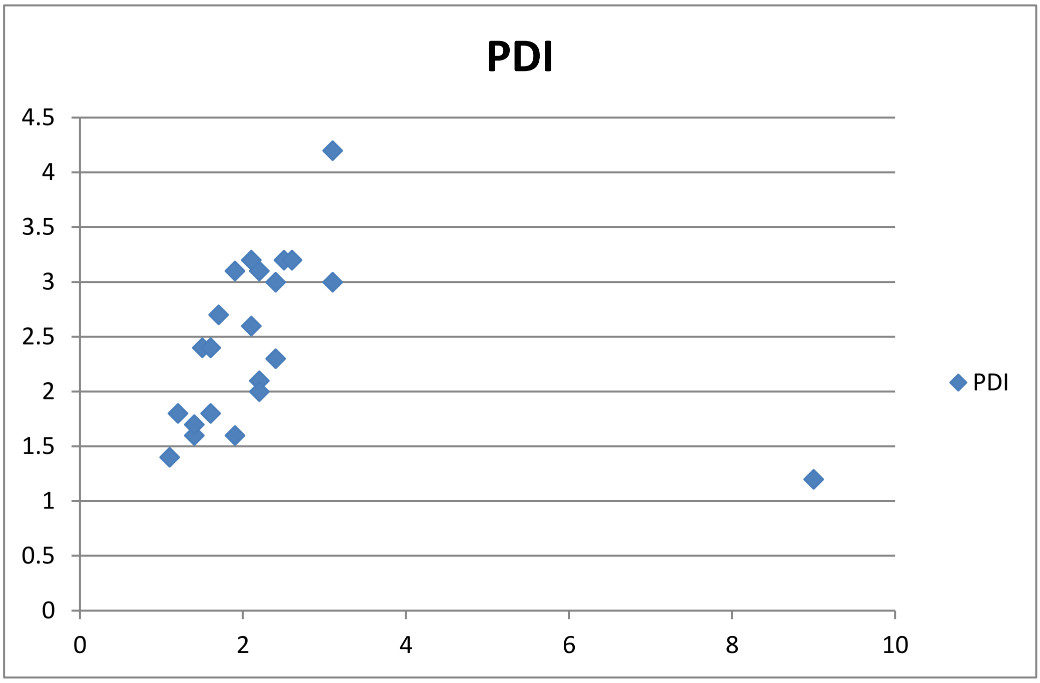


Fig. 8. showing the positive correlation between pit depth and diameter

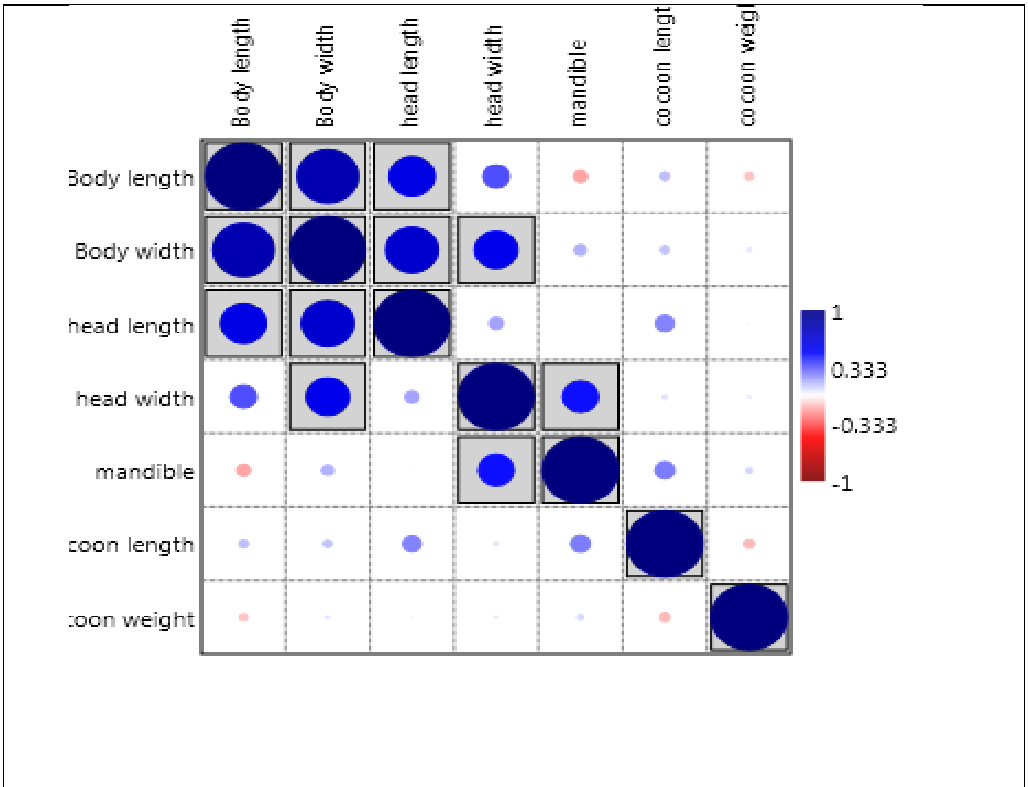
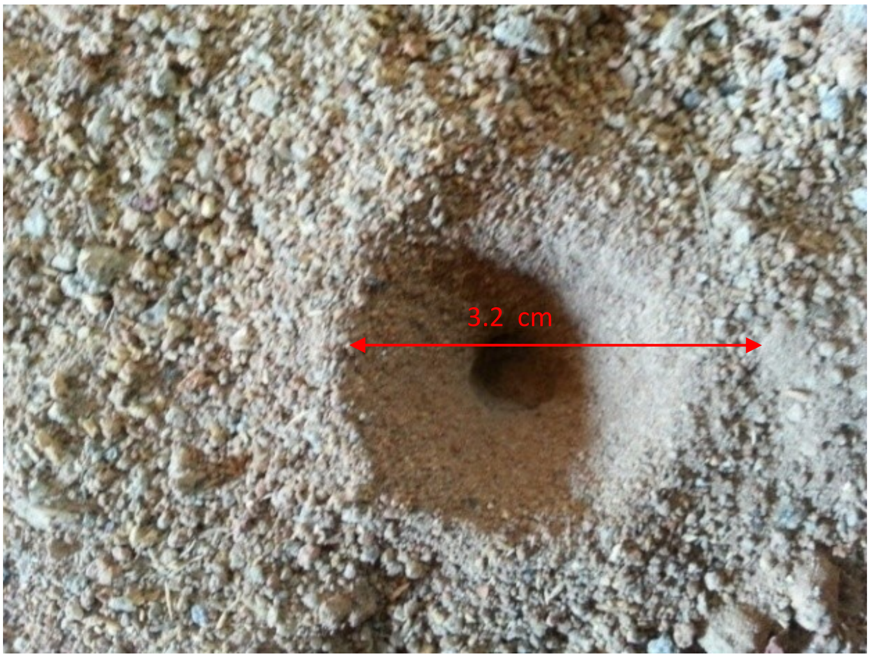


Fig. 9. Correlation plot of Genus Myrmeleon (larvae and cocoon)

Plate 17



The correlation between pit diameter and larval body width.

Plate 18

Dorsal View

Ventral View

Antennae

Compound eye

Abdomen

Abdominal tip

Table 17. The expected larval body width as per diameter of pits

PDI (cm)	LBW (number)	Mean LBW (mm)
1.2-2.2	18	2.88
2.2-3.2	20	3.35
3.2-4.2	10	3.80

From the result, we can predict the larval size of pit- dwelling larvae by measuring its diameter. It is known that antlions have three larval instars during its life cycle. Here the average size of each larva in each instar is determined. The average body width of first instar larvae is approximately 2.88 mm seen in the pit with diameter ranging from 1.2-2.2 cm. The average body width of second and third intar larvae is approximately 3.35 and 3.80mm respectively which is seen in pit diameter ranging from 2.2-3.2and 3.2-4.2 cm. Simply it can be determined that, the pits with diameters ranging from 1.2-2.2, 2.2-3.2, 3.2-4.2 cm have the larvae with body width 2.9, 3.4, 3.8 mm respectively.

Both *M. pseudohyalinus* and *M. hyalinus,* the size of all body parts showed significant positive correlation with each other (Appendix 2 and 3) (Fig. 10 and 11). Oneway Anova was performed to compare means of different body part measurements of different species. It was found that there was no significant difference in the mean LBL and mean LBW (Appendix 4 and 5) of the *M. pseudohyalinus* and *M.hyalinus* (F=0.1739; df 2, 69 at 5%). Thus it was concluded that the larval body length and larval body width were not enough to distiguish between the larvae of the two species.There was a significant difference in the mean LHL (F=3.645, df 2, 69) (Appendix 6), mean LHW (F=17.7, df 2,69) (Appendix 7) and ML (F=6.694, df 2,69) (Appendix 8) at 5% significance. This indicate that the major differentiating character between the larvae of the two species are LHL, LHW and ML.The *Myrmeleon hyalinus* has longer head (0.27 cm), wider head (0.17 cm) and longer mandible (0.16 cm) than *M. Pseudohyalinus*

2.4.5. Morphometric Analysis-Cocoon

A total of 75 cocoons of the genus *Myrmeleon* were collected from five districts of Kerala and the district wise data are given in Table 18. The photographs were taken and given in Plate 19.

Table 18. District wise collection data of cocoon coming under genus *Myrmeleon*

Sl No.	District	Number of cocoon
1	Palakkad	54
2	Thrissur	14
3	Wayanad	1
4	Thiruvananthapuram	4
5	Kannur	2
	Total	75

Dry soil, sand, m sand and brick dust were used by antlion larvae to make their cocoon for pupation. The smallest cocoons were collected from Wayanad district and largest cocoons were from Kannur district. The type of materials which forms the cocoon of antlion larvae was sand in Wayanad and dry soil in Kannur. The detailed description of circumference, diameter and weight of cocoon were given in Fig.12 and Fig.13.

2.4.6. Morphometric Analysis- Adult

The adult of genus M*yrmeleon* was small in size when compared to *Palparinae*, and *Distoleon* species*,* The morphometric measurements were done using a scale and it was given in Fig.14.

2.4.7. Physical Parameters

The average temperature was high in human dwelling areas and low in river banks. The larvae prefer high temperature, and thus show preference for human dwelling areas with shades. The soil and atmospheric temperature, humidity, dewpoint, pressure, uv index, visibility, and wind were measured in the study areas. The average soil temperature was 32°C and the average atmospheric temperature was 30.1°C. The maximum and minimum atmospheric temperatures were 39°C and

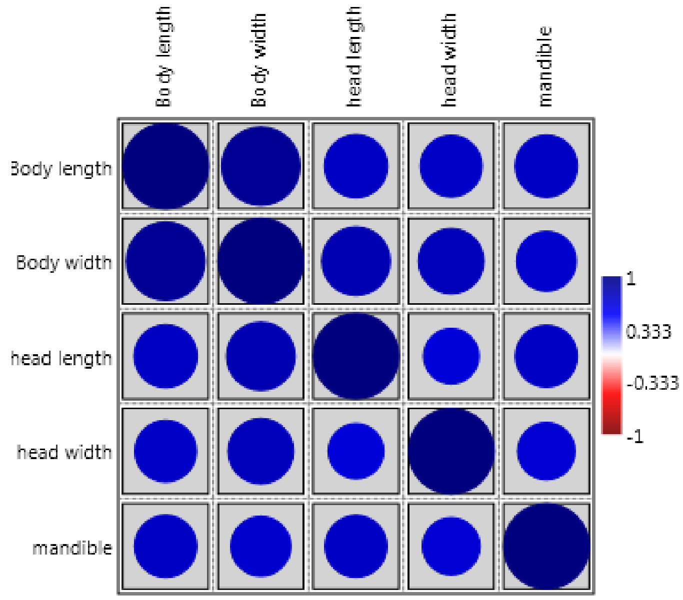


Fig. 10. Correlation plot of larval morphometry of *M. pseudohyalinus*

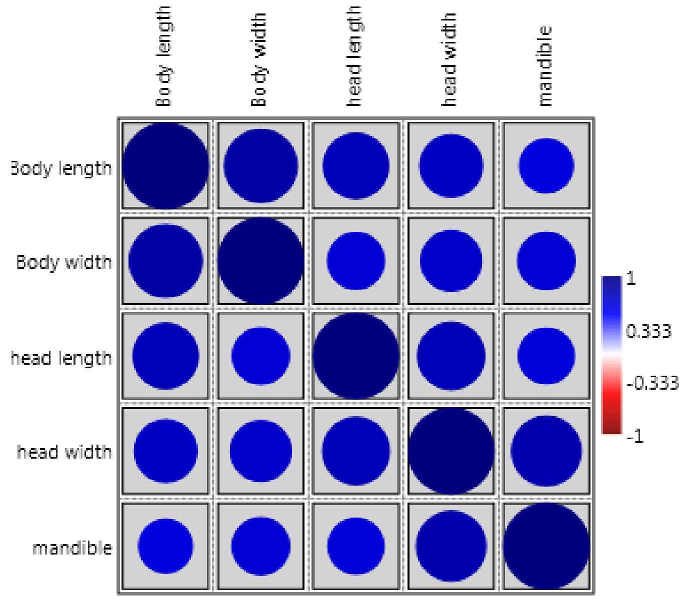


Fig. 11. Correlation Plot of larval morphometry of *M. hyalinus*

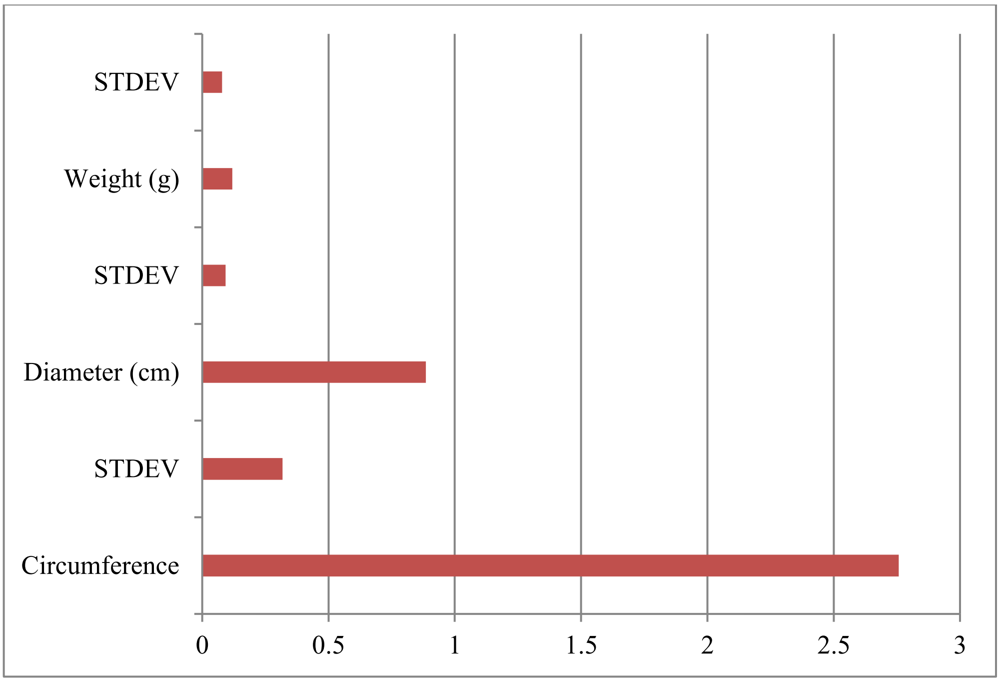


Fig.12. The average circumference, diameter and weight of cocoon collected from Kerala

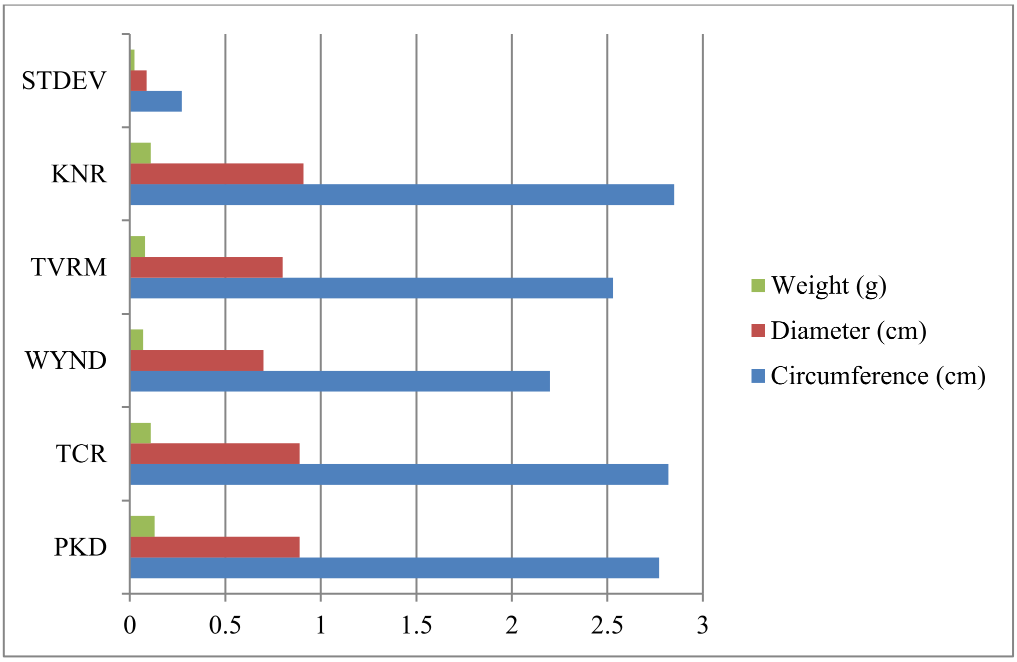
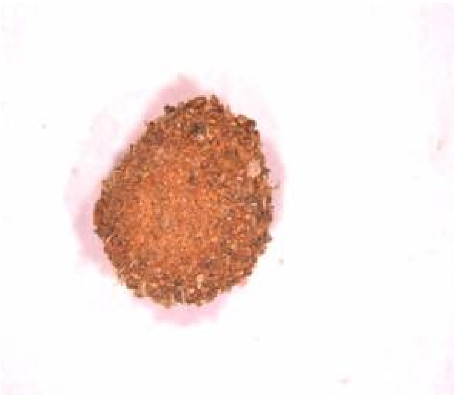


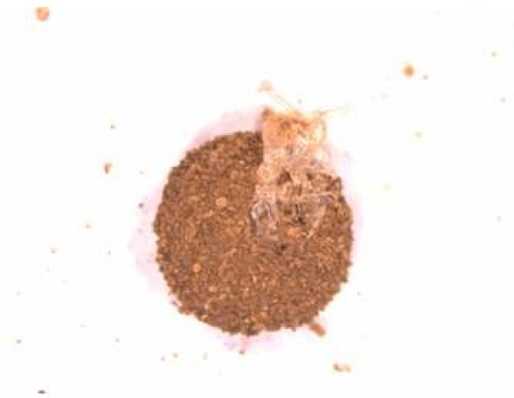
Fig.13. The average circumference, diameter and weight of cocoon collected from Different District of Kerala

Plate- 19



Cocoon in dry soil

Cocoon in dry sand



Cocoon with exuviae

Hole in cocoon

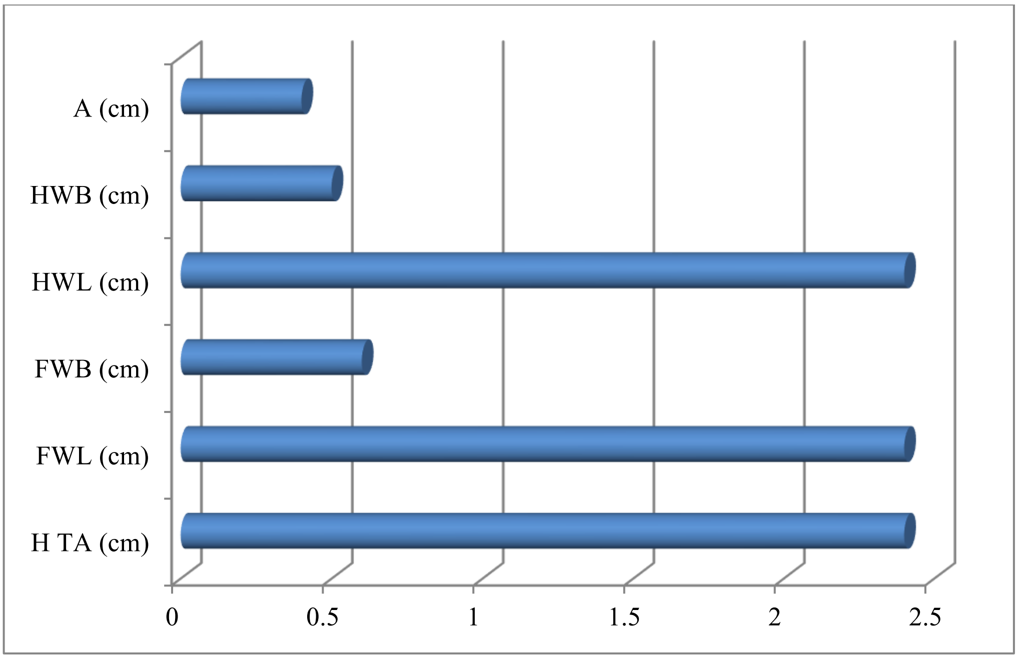


Fig.14. Average morphometric measurements of *Myrmeleon pseudohyalinus*

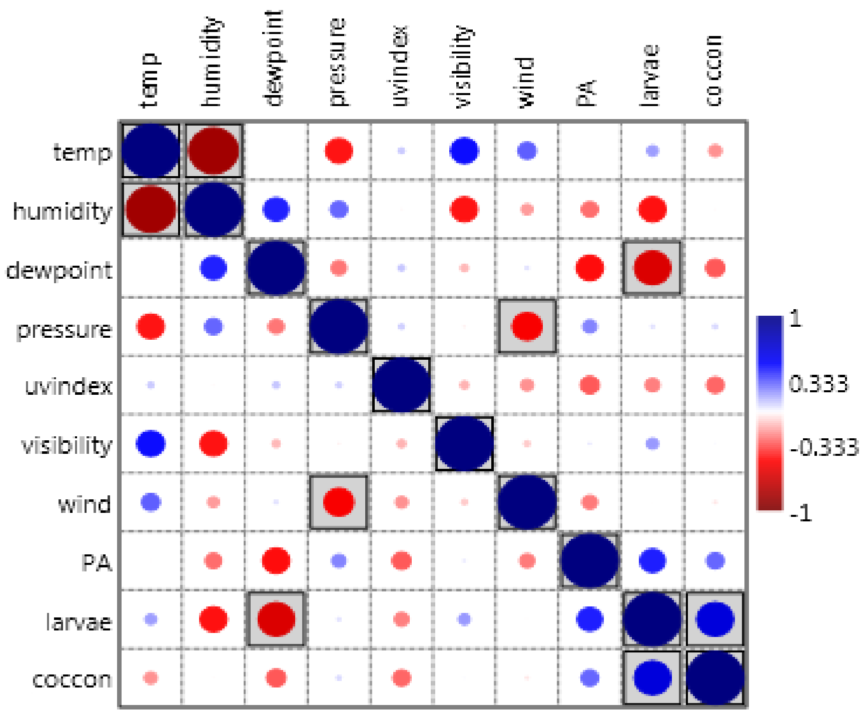


Fig.15. Correlation plot for different abiotic parameters

21°C respectively.The maximum and minimum humidity observed were 90% and 20 %. The average humidity was 64.8%. The dew point ranges from 11°C to 26°C and the average was 22.3°C.

The larvae of genus *Myrmeleon* showed a significant negative correlation to dew point and a non significant negative correlation to humidity and uv index (Fig. 15). Non significant positive correlation to temperature, visibility was also observed. The detailed comparisons of different abiotic factors present in four habitats were given in Table 19.

Table 19. Comparison of different abiotic factors observed in four habitats

Parameters	Abandoned area	Forest	Riparian	Human dwelling
Mean temperature (°C)	31.29	30.42	29.8	30.92
Min temperature	27	20	22	22
Max temperature	36	37	36	39
Mean humidity (%)	57.25	65.17	59	59.92
Min humidity	46	42	45	20
Max humidity	65	90	81	82
Mean dewpoint (°C)	20	23	20.8	21.2
Min dewpoint	19	19	19	11
Max dewpoint	22	25	22	24
Mean pressure (milli bars)	1010.33	1009.17	1011.8	1010.5
Min pressure	1010	1008	1009	1007
Max presure	1011	1013	1017	1008
Mean uv index	6	5.67	6.6	4.66
Min uv index	0	1	1	1
Max uv index	6	11	11	11
Mean visibility	12.33	10	11.2	12.7
Min visibility	11	3	5	8

Max visibility	13	13	16	14
Mean wind speed (km/hr)	9	10.66	10.25	10.36
Min wind speed	8	3	5	5
Max wind speed	10	13	16	14

2.4.8. Soil Texture Analysis

A total of 27 soil samples were collected from study sites in which the presence of antlion larvae was observed. It includes the study areas coming under seven districts of Kerala namely Palakkad, Thrissur, Wayanad, Malappuram, Thiruvanathapuram, Kannur and Pathanamthitta. The twenty seven soil samples were analysed for understanding the texture of substrate of these twenty one soil samples were of the texture class sand and six soil samples were of the texture class fine sand (77.8% of soil samples were in texture class sand and 22.2% was fine sand).

Ten soil samples were collected and analysed the texture by examining the percentage of the soil components (sand, silt and clay) in Palakkad District. Seven soil samples are coming under the texture class sand and three samples coming under fine sand (Fig. 16). The soil samples collected from the study area Parali, Thiruvizhamkunnam, Parli river bank were fine sand in texture and that of Ottappalam, Manamthody, Moyan school, Kinavallur, Edathara, Dhoni, and Pezhumpara were sand in texture. Ottappalam, Kinavallur and Edathara were the study sites in which the absence of silt component noted.

Seven soil samples were tested from Thrissur district (Fig. 17) in which three samples were coming under texture class fine sand and four samples were texture class sand. The soil samples collected from Asarikkadu, Wadakkanchery and Nedupuzha were fine sand in texture and Thumburmuzhi, Vallathol Nagar, Kodungallur, Poomala and Ezhattumugham were sand in texture. Kuruva and Kattikulam of Wayanad District (Fig. 18), Benglakunnu, Nilambur Dippo, Nilambur Irrigation office and Idimuzhikkal of Malappuram District (Fig. 18) were tested and the result shows the sand texture in that place. The samples collected from Vellayani of Thiruvanathapuram district (Fig. 19), Brennan college

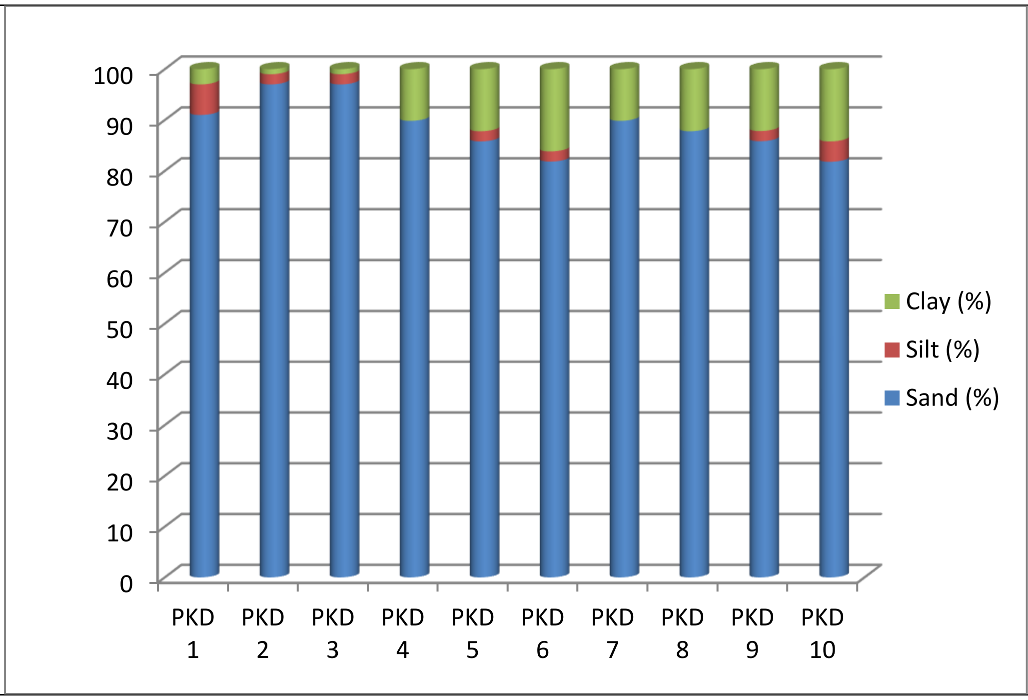


Fig. 16. The sand, silt and clay percentage of soil samples in Palakkad District PKD1=AA,PKD2=FB, PKD3=RA, PKD4=RB, PKD5=HDA, PKD6=HDA, PKD7=HDA,PKD8=AA, PKD9=FB, PKD10=AA

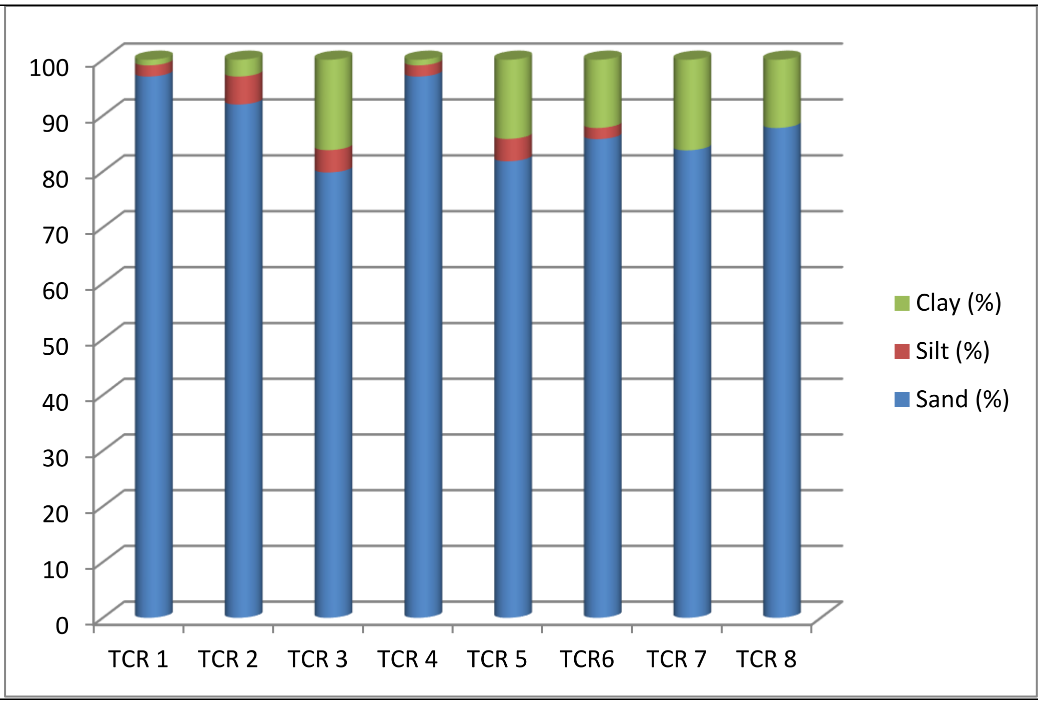


Fig 17. The sand, silt and clay percentage of soil samples in Thrissur District TCR1= HDA, TCR2=HDA, TCR3=RB, TCR4=HDA, TCR5=HDA, TCR6=HDA, TCR7=RB, TCR8=RB

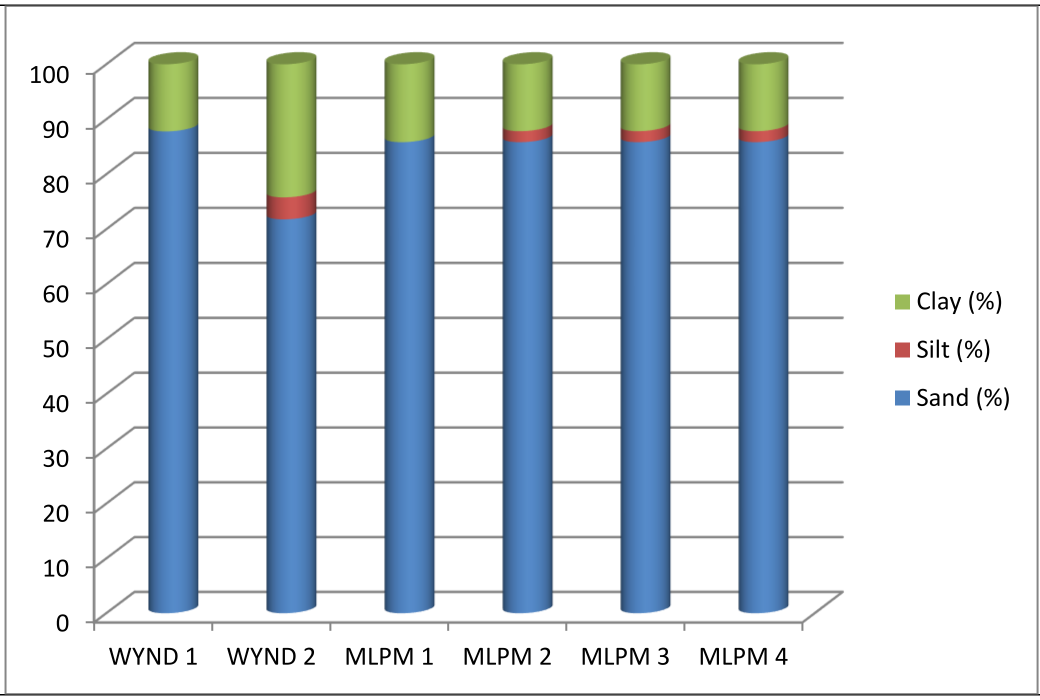


Fig. 18. The sand, silt and clay percentage of soil samples in Wayanad and Malappuram Districts. WYND1=RB, WYND2=HDA, MLPM1=FB, MLPM2=HDA, MLPM3=HDA, MLPM4=HDA

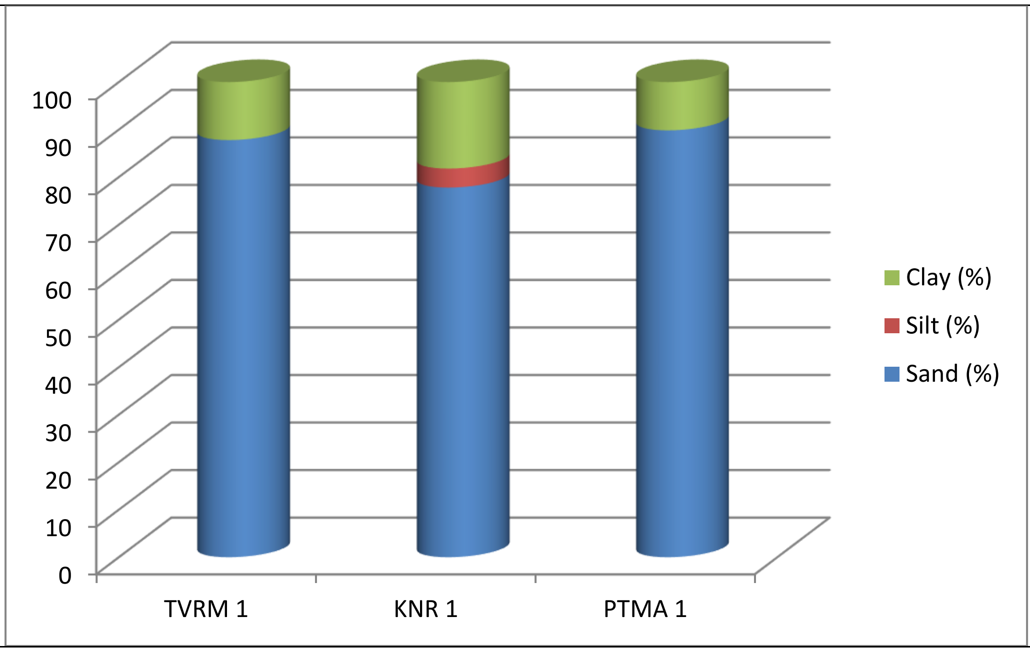


Fig. 19. Sand, silt and clay percentage of soil samples in Thiruvananthapuram, Kannur and Pathanamthitta Districts
TVRM1=HDA, KNR1=HDA, PTMA1=HDA

campus of Kannur District (Fig. 19), Tiruvalla of Pathanamthitta District (Fig. 19) were sand in texture.

In the case of Palakkad District the minimum and maximum sand percentage is noted from the soil samples collected from Pezhumpara & Moyan modal school area and Thiruvizhamkunnu & Parli river bank respectively. Pezhumpara is an abandoned area and Moyan modal school is a human dwelling area. Thiruvizhzmkunnu and Parli river banks are coming under forest and riparian respectively. In the case of Thrissur, the sand percentage was minimum in the soil collected from Thumboormuzhy and the sand percentage was maximum in the soil collected from Murikkumpara and Nedupuzha. In case of Malappuram district all the four samples were observed similar percentage of sand particle.The detailed sand, silt and clay content present in four habitats were given in Table 20 and Appendix 9.

Table 20. The sand, silt and clay content of four habitats of genus *Myrmeleon*

Sl No.	Habitat	Min sand %	Max sand %	Min silt %	Max silt %	Min clay %	Max clay %
1	**Abandoned area**	82	97	0	6	1	14
2	**Human dwelling**	72	97	0	5	1	24
3	**Riparian**	80	97	0	4	1	16
4	**Forest areas**	86	97	0	2	1	14

Number of individuals are significantly positively correlated with silt (Pearson's correlation, 5% significance) in sandy soil (Fig. 20). Soil samples collected from seven districts (total of 27 sites) indicated that sand was the predominant content, 80% of every soil sample was sand, and there was slight variation in the silt and clay content.

2.4.9. Soil Chemical Analysis

PCA for all 10 chemical parameters of soil where antlion larvae sampled and the number of individuals present were done. The soil chemical parameters are PH, EC, OC, N, P, K, Ca, Mg, S, Cl. The Mg, OC, EC, and P^H were influenced the number of individuals in a positive manner. But, Ca and K show a negative correlation with the number of individuals. The Eigen values and map were given in Table 21 and Fig. 21 respectively.

Table 21. Eigen values

PC	Eigenvalue	% variance
1	2.26E+06	97.286
2	56452.5	2.4302
3	5133.78	0.221
4	1137.15	0.048953
5	232.276	0.009999
6	75.0003	0.003229
7	6.51953	0.000281
8	2.93664	0.000126
9	0.0668348	2.88E-06
10	0.00245453	1.06E-07

2.4.10. Seasonal Adaptability and Habitat Choice

In the first, second and third experiment, the larvae made its first pit at an average of 23, 32 and 57 days respectively. In this experiment it is inferred that, in the rainy conditions they remain buried deep in the soil and waited for the right time to make its pits by analysing the soil temperature. This may also be considered as a seasonal adaptation of the species. The time taken for pit rebuilding were given in Table 22 and the pit building behaviour in natural and laboratory conditions were compared in Table 23.

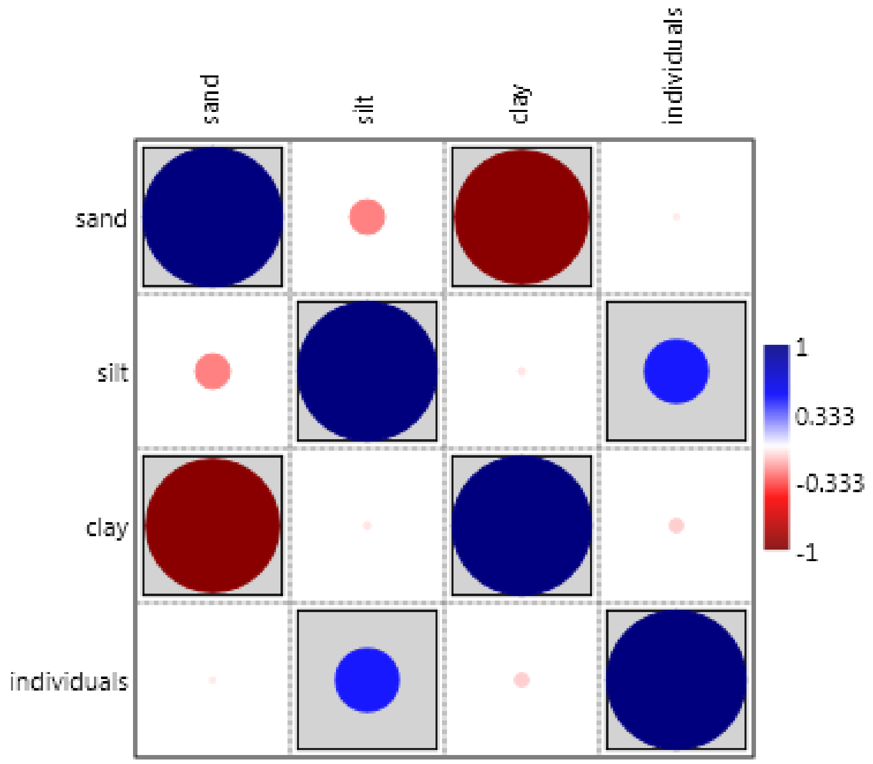


Fig. 20. Correlation plot showing the relation between soil characters and Myrmeleon larvae.

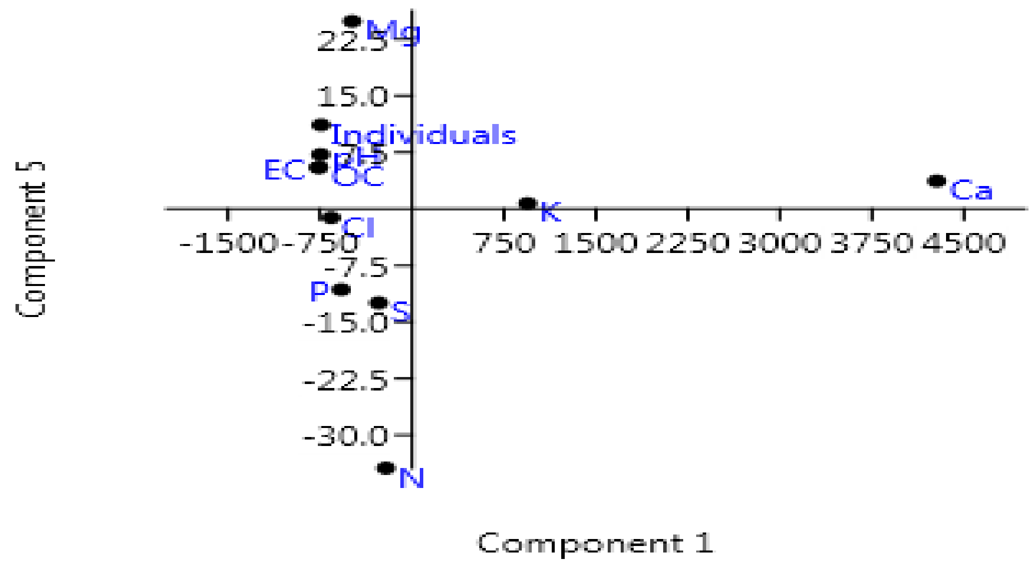


Fig. 21. PCA map showing negative correlation between K & Ca and number of individuals.

Table 22. Time taken for pit rebuilding and average temperature after the water spray

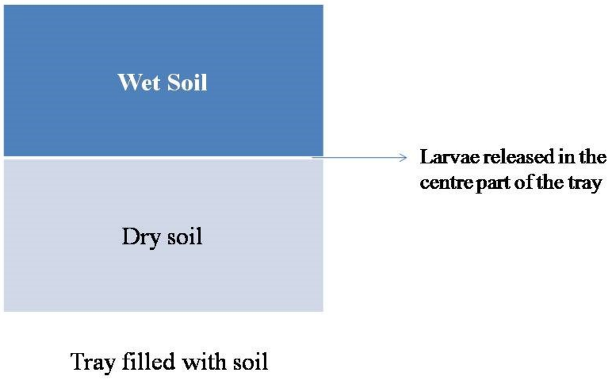
Experiment	Average time taken for pit rebuilding	Mean Temperature
Two spray	23 days	28-32^{o}C
Four spray	32 days	28-32^{o}C
Six spray	57 days	28-32^{o}C

Table 23. Comparison of pit building behaviour in natural and laboratory conditions

	Natural condition	**Laboratory condition**
Pit building in dry soil	Within 24 hours	Within 24 hours
Pit rebuilding in rainy condition	Up to 84 days	Up to 57 days
Pit depth	Same	Same
Pit diameter	Same	Same
Temperature of soil	28-32	28-32
Behaviour of larvae	In rainy season they move deep in to the soil and wait for the favourable condition (dry soil/soil temperature) to make its pits in the soil surface for predation	

Habitat choice

All larvae made pits in the dry soil area. The minimum distance between the pit and the centre portion of the tray was 1.8 cm and the maximum distance was noted as 12.1cm (Fig. 22). From this study it is inferred that *M. pseudohyalinus* larvae prefer dry soil for pit building.



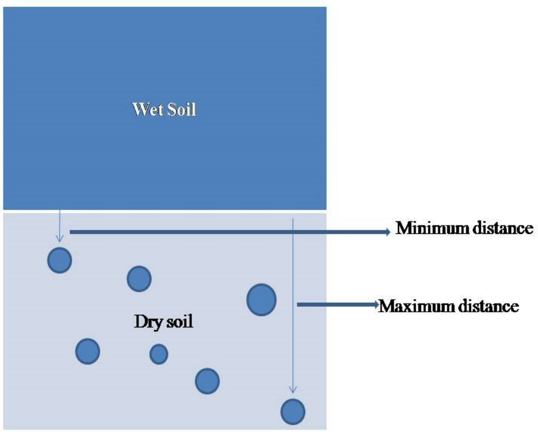


Fig. 22. Representation of pit building in wet and dry soil

2.5. DISCUSSION

This is the first study that documents the antlions and their ecology of southern India. *Myrmeleon pseudohyalinus*, a pit building species of genus Myrmeleon is a first report from India. Antlion larvae were collected from fifty study areas (Table 16) and according to the presence of pit building antlion; the sites were classified into four, namely abandoned areas (without any disturbances), human dwelling areas, forest boundaries and river banks. The most observed habitat was human dwelling area than other three habitats. It may be because of the presence of more protected areas from direct sunlight, rainfall and wind. Also there are lots of insects and small arthropods were plenty in human dwelling areas when compared to other areas. The human dwelling area comprises 54% of the total study area followed by abandoned areas (18%). The percentage of forest boundaries and river bank habitats in which the pit building antlion larvae observed was 14%. From this study it is also observed that the larvae prefer sand for making its pits and the percentage of sand in soil samples were 89.5%, 86%, 90% and 87% in abandoned areas, human dwelling areas, forest boundary areas and river banks respectively.

They are not only seen in these common habitats, but previous studies show the presence of larvae in sand dunes (*Myrmeleon hyalinus*) in Balkan Peninsula (Devetak *et al*., 2013). Here also soil and sand substrate were commonly found as pit building substrate, it may be because of the loose structure of the sand and dry soil which helps to make the steep conical pits and thereby enhances the predatory efficiency of this sit and wait predator. The study of Bozdogan and Satar (2017) show that the banks of wet lands are one of the most noted habitats of *Myrmeleon formicarius* larvae. This is true in the case of genus *Myrmeleon* which was collected from river banks also. Study by Pantaleoni *et al*., (2010) reported *M. mariaemathildae* were seen in dunes colonized by grassy vegetation which was a new habitat of pit building antlion larvae

From the field study, it was understood that the larvae made its pit in heap of sands, floor of abandoned buildings, shades of buildings (absence of direct sunlight), and shades of large trees like teak and coconut and also above ground level surface like terrace. The rainy season in the study area are prominent from June to September, a high number of adult antlions emerged before monsoon and the presence of adult antlions were more in early morning and evening. The immature larval stages were present in both protected and unprotected habitats. Although antlions were considered as bioindicators of global warming and deserts, some small variations also seen in different species. Previous studies shows that *Cueta* species prefer warm, dry and lighted habitats where as *M. quinquemaculatus* prefers humid, cloudy areas (Bakoidi *et al.*, 2020), *M. caliginosus* seen in desert habitat, and *M. obscurus* was seen in proximity of buildings and sheltered conditions. *M. obscurus* and *quinqemaculatus* co- existing in the same habitat too (Badano, 2020). Comparison of pit building in protected and unprotected microhabitat of *M. brasiliensis* shows no difference in abundance, but density is increased in protected habitats and also positively correlated the pit size and larval size in both habitats (Lima, 2020).

Morphometric analysis helps to identify the size and shape variation of different species of a genus or same species in different geographical areas. The body measurements of larvae, cocoon and adult of genus *Myrmeleon* were carried out. The larvae of *M. pseudohyalinus* and *M. hyalinus* are the two species of antlion larvae morphometrically analysed and compared. The larvae of *M. pseudohyalinus* collected from seven study areas (Fig. 3) of four districts show similar body size (both second and third instar). The mean LBL, LBW, LHL, LHW and ML were 0.64 cm, 0.28 cm, 0.24 cm, 0.14 cm and 0.14 cm respectively in second instar larvae. In third instar larvae, the mean LBL, LBW, LHL, LHW and ML were 0.89 cm, 0.37 cm, 0.34 cm, 0.2 cm and 0.16 cm respectively.

Previous studies show that the size of same species in different places or different habitats may vary. The comparison of morphological variations of antlion larvae (*Myrmeleon hyalinus*) in Mediterranean and desert populations shows that the body size is larger in Mediterranean populations when compared with desert populations. It is also studied that the pit size is considered as a reliable feature for identifying particular instars in larval stage (Lewandowski *et al.*, 2004; Bozdogan *et al.*, 2013). In this study the larval instars were predicted by correlating the size of pit and inside dwelling larva, and found that the pit diameter and larval body width are positively correlated (Krishnan and Kakkassery, 2016).

The body measurements or size in various species of genus *Myrmeleon* was slightly different in various countries. Third instar larva of *M. caliginosus* has body length- 8.75mm, head length- 1.66 mm, head width- 1.27 mm, mandible length- 1.7mm, in desert habitat but in the case of *M.obscurus* the measurements as follows, body length- 8.75mm, head length- 1.64 mm, head width- 1.2 mm, mandible length- 1.77mm and it is present in proximity of buildings and sheltered conditions. *M. quinqemaculatus* with a body measurements, body length- 15mm, head length- 3.32 mm, head width- 2.6 mm, mandible length- 3.43mm and has the same habitat seen in *M. obscurus* (Badano, 2020). Here, from this study it is understood that the third instar larvae of *M. pseudohyalinus* measures LBL- 9.9 mm; LBW- 4.6 mm; LHL- 3.2 mm; LHW- 1.9 mm; ML- 1.8 mm. For more accurate results more characters like distance between mandibles, mandible length, curved mandible length, mandible width, head length, head width, distance between mandible first and third tooth, distance between mandible second and third tooth, thorax length, thorax width, abdomen length and abdomen width (Scharf *et al.*, 2008) can be measured.

The body size has some relationship with its funnel shaped pit also. *Cueta sauteri* of Taiwan shows a positive correlation between larval body length and pit diameter in both field and laboratory conditions (Liang, 2010; Kross and Pilgrim, 2012). But from this study, the result shows a negative correlation between larval head length and pit depth and a postitive correlation between larval body width and diameter of its pits (Fig.6 and Fig. 7). Thus, an inference can be made from this correlation result that, the larval body width and length were the characters which decide the diameter of its pits**.** The pit diameter and depth are positively correlated (Krishnan and Kakkassery, 2016 and Liang, 2010), and helps the antlion by delaying the escape of prey which agree with the study of Kross and Pilgrim (2012). The study of Kitching (1984) shows a linear relationship between length of the *M. pictifrons* larvae and its pit diameter.

The knowledge about the size of cocoon (genus *Myrmeleon*) is a first study and regarding the size no references were available. However, this result gives a baseline data for the myrmeleontid fauna of Kerala. The mean circumference, diameter and width of the cocoon of genus *Myrmleon* are 2.6 cm, 0.8 cm and 0.1g respectively (Fig.12). The result of measurements of *M. pseudohyalinus* adult (Fig. 14) does not show much variation in the morphometry of different species of a genus. Here, the body measurements of *M. pseudohyalinus* were as follows, HTA- 24mm, FWL- 24mm, FWB- 6mm, HWL- 24mm, HWB- 5mm and AN- 4mm. However, the study of *Myrmeleon acerbus*, an Indian species (Kaur *et al.*, 2019), a member in the same genus showed slight difference and as follows, forewing length 24.59- 24.76 mm, Forewing breadth 7.51- 7.62 mm, Hindwing length 21.86- 21.92 mm, Hind wing breadth 6.02- 6.08mm.

The various abiotic parameters of the habitat of genus *Myrmeleon* were compared and correlated in this study (Table 19). Temperature, humidity, dew point, pressure, uv index, visibility and wind speed of study areas were checked and it was plotted against presence or absence of larvae, number of larvae and cocoon. The larvae show a significant negative correlation to dew point and a non significant negative correlation to humidity and uv index. Here, the mean dew point was 21ºC, mean humidity was 60.34% and mean uv index was 5.73. Dew point is the temperature at which water vapour content in air reaches the maximum point and the places with highest water content in air show a decrease in the larvae because the antlions prefer dry places. The uv index is moderate (5-6) in study areas (Mean UV index 5.73) irrespective of the different habitat. The mean temperature, humidity, dew point, pressure, UV index, visibility and wind speed observed were 30.60ºC, 60.34 %, 21.25ºC, 1010.45 millibars, 5.73, 11.56 and 10.07 km/h respectively.

Here the optimum temperature of *M. pseudohyalinus* lies between 30-32ºC, though antlions are shade loving and prefer dry conditions as well as low humid areas. The result of previous studies depicts that the *Myrmeleon immaculatus* larvae prefer high temperature than low temperature for frequent pit building (Arnett and Gotelli, 2001), not only the temperature influences the pit building of larvae, but also the optimum temperature (35ºC) helps to maintain the lowest level of mortality and shortest life cycle in *Myrmeleon obscures* (Bakoidi *et al.*, 2020). The study of Ngamo *et al.*, (2015) show that, the larvae prefer hot seasons than cool climate, the highest number of antlion were found at a temperature of 32ºC.

In the present climatic condition of Palakkad district, the highest number of pit and larvae (*M. pseudohyalinus*) were noted from January to March and April to June, the result agree with the study of Bozdogan and Satar, 2017. They studied the seasonal abundance of antlion larvae in Amanos Mountains, Turkey and they observed the maximum larvae in the month of May and June. From the field study it is understood that there is no relationship between pit size and soil temperature (Bozdogan and satar, 2017). Soil temperature has an important role in the pit making of *M. immaculatus* but, soil illumination has no significant effect on construction of pit. Studies by Klein (1982) inferred that the preference of shaded areas by antlion larvae was not because of light, but in response to temperature. The pit size of *M. formicarius* increased with the increase in sand particle size, but altitude have no impact on pit diameter and sand particle size (Bozdogan *et al.*, 2013). *M.crudelis* larvae cease feeding below 20ºC (Lambert *et al.*, 2011). Also adults lost their mass very quickly when exposed to desert conditions (Scharf *et al.*, 2009b). Previous study also shows a negative correlation between rainfall and number of larvae in *Myrmeleon brasiliensis* (Freire and Lima, 2019). Scharf *et al.,* (2009b) shows a negative correlation between body size and habitat temperature and the higher temperature accelerates the duration of pupal stage. Annual rainfall and humidity were positively correlated with body size, these parameters increases the quantity of insect prey. Rainfall influenced the B*rachynemurus* larval behaviour, if heavy rain persists; they remain under the sand for 2-3 days (Cain, 1987).

The antlion larvae inhabited soil composed of sand, silt and clay particles. According to the International Union of Soil Sciences (IUSS), the soil particles were classified as follows and this was used to interpret the soil texture in this study. Sand particles has low water and nutrient holding capacity, loose when dry and very low stickiness when wet. The silt components has low to medium water and nutrient holding capacity and shows some stickiness when wet. The clay component has high water and nutrient holding capacity, hard when dry and high degree of stickiness when wet.

From the 27 soil samples collected from study areas, 21 samples were classified under texture class sand and 6 samples were coming under fine sand. The sand texture was analyzed by evaluating the sand, silt and clay content of the soil samples. The correlation between the components of soil and number of individuals were performed and the result shows a positive correlation between the numbers of larvae with sand having highest silt content. One reason behind the preference of sandy soil for pit building substrate was the nature of sand and silt with low water holding capacity and easily dry when wet compared to clay soil.

The study of Phogat *et al*., (2015) described that the larvae build its pits in sand particle with low water holding capacity and low stickiness when wet and the present study agrees with this result. For the survival of antlion larvae they choose the sand particle for making its pit. If the rainy season or wet condition occurs, the larvae took a dormancy period by burying deep into the sand until the sand become dry. It is a survival mechanism of pit building antlion larvae.

Chemical composition of the media also has an important role in the pit building of antlion larvae. From this study, a negative correlation between calcium and potassium with the number of individuals were identified. The relationship between the chemical component of antlion inhabiting soil and its influence on larvae are not studied earlier and it can be studied in future. In general, higher the concentration of acidity, salinity, calcium and magnesium are suppressive to larval development. But potassium, sulphate and chlorides are good for larval development. Soils those are more favorable for the development of larvae are rich in potassium, magnesium and sulphates. The element that adversely affects the development of antlion is nitrogen (Maoge *et al.*, 2014).

CHAPTER- 3

LARVAL BEHAVIOUR PATTERNS

CHAPTER 3

LARVAL BEHAVIOUR PATTERNS

3.1. INTRODUCTION

Insect behaviour includes various activities such as predation, feeding, migration and courtship. The various behaviour patterns help the organism to survive better in different biotic and abiotic conditions. Both larvae and adults shows different patterns of specific behaviour which may be a strategy to develop its survival rate. Spider, honey bee and antlions are some of the organisms which make a special behaviour or architecture for building their shelter as well as improving predation. The spider makes its web for predation purpose, but honey bee makes its comb for shelter. In the case of antlion, it makes its pit for both shelter and predation.

For the successful survival, organisms exhibit different behaviour patterns which help to live in the present environment. The individuals adapt some behaviour for the smooth survival in that particular environment. The common behaviour patterns of insects include feeding, mating and predation. But in the case of Myrmeleontids, they are the good predators which use a planned strategy for feeding, pit building and predation. They made conical pits in the substrate and wait for the prey to fall down in to it. By making the conical pits, they got its shelter from enemies as well as food. But there are exceptions also. The Mediterranean antlion species *Neuroleon microstenus* do not build pit fall traps, they dug in sand backwards and wait for the prey (Devetak *et al*., 2010a)

Antlion larvae made its pit by using the substrate usually sand or dry soil through a series of backward movements. After making the pit, they wait for the prey and once the larvae encounter a prey, the different predatory behaviour pattern starts followed by feeding. Napotilano (1998) investigated about the predatory behaviour patterns of antlion larvae, from this he made a conclusion that a total of twelve discrete behaviour pattern where listed which is as follows.

1. Attack

   The head is moved rapidly forward while closing the mandibles and is often flicked rapidly back, expelling sand from the pit.

2. Holding

   The prey is gripped securely in the mandibles.

3. Submergence

   Holding prey, the larva moves down and back into the substrate until the entire larva and at least part of the prey are not visible.

4. Emergence

   Holding prey, the larva moves up and forward until the entire prey and at least part of the larva's head /mandible is visible.

5. Prey beating

   Holding prey, the larva rapidly flicks its head up and down (4-5 beats per bout), often drumming the prey on the substrate.

6. Feeding

   While at least one mandible tip is inserted, fluids are extracted from the prey, often alternating with mandibular probing and manipulation of the prey.

7. Pit clearing

   The head is moved laterally, accumulating sediment on the dorsal surface, they flicked rapidly back, expelling sediment.

8. Head roll

   The head is raised and swept in a circular motion along the pit wall, accumulating sediment in the pit center.

9. Prey clearing

   The mandibles are used to position prey on the dorsal head surface, then the head is flicked rapidly back, expelling prey.

10. Grooming

    The tip of one mandible is moved along the groove on the inside edge of the opposing mandible.

11. Quiescence

    Larva remains motionless, without prey, for 7 + seconds

12. Jaw set

    The larva pulls beneath the sand, while fully opening the mandibles. The eyes, antennae and mandible tips remain visible. (Napotilano, 1998)

Predation helps to maintain the balance of animal populations and the predators selectively remove the young, old and diseased or injured individuals from prey populations (Southwick, 1976). The Neuropterans are mainly predators in both larval and adult stages, but in the case of Family Myrmeleontidae, the larval stages are more predatory than adults. Adult antlions survival period is below one month when compared with the lengthy larval period of upto two years.

Both pit building and non pit building antlions were present and the species are given in Table 24. The pit builders have seven stemmata or larval eyes which are situated on eye tubercles. Non pit builders have more prominent eye tubercles as an adaptation to non pit building condition (Delakorda *et al*., 2009). The non pit building antlion species of genus *Brachynemurus* has sit and wait prey capture behaviour and they burrow backwards through the sand leaving a narrow furrow (Cain, 1987).

Table 24. Pit building and non pit building antlion species

Sl No.	Pit building antlion	Non pit building antlion
1.	*Euroleon nostras*	Neuroleon microstenus
2.	*Myrmeleon pictifrons* (Australian sp.)	Brachynemurus
3.	*Myrmeleon acer*	
4.	*Myrmeleon brasiliensis*	
5.	*Macroleon quinquemaculatus*	
6.	*Morter obscurus*	
7.	*Cueta sp*	
8.	*Hagenomyia sp*	
9.	*Myrmeleon inconspicuus*	

3.2. REVIEW OF LITERATURE

3.2.1. Pit building behaviour

The factors influencing pit building of antlion larvae include starvation or hunger level and density of larvae in that particular area.The spatial distribution of pits are regular and uniform in density 5 larvae per 100cm^2 in Myrmeleon larvae. If the density increased in an area, *Myrmeleon acer* Walker builds fewer and smaller pits. It is also found that the cannibalism behavior shows above the density of 5 individuals per 100cm^2 (Day and Zalucki, 2000) and there is no relation between antlion prey abundance and larval pit relocation (Scharf and Ovadia 2006). There are 38% of the pits did not contain antlions in the study area in Florida. The spreading strategy of all pits is random but in the case of live antlion it is clumped in nature (Boake *et al*., 1984).

Myrmeleon immaculatus without food for a month move once in every 10 days and build smaller pits (Heinrich and Heinrich, 1984). Griffiths (1986) studied Pit construction of *Macroleon quinquemaculatus* larvae (First, second and third instar) and concluded that starved, disturbed larvae move their pits from one place to another than well fed ones. Periodically the pits are enlarged and it was mostly during at night. *Myrmeleon bore* larvae never relocates its pits under starvation, whereas *Hagenomyia micans* relocated more frequently than *Myrmeleon formicarius* (Matsura and Murao, 1994). The reason behind the pit aggregation was the antlions reduced relocation tendency, this strategy known to be the evolutionary stable strategy (Tsao and Okuyama, 2012).

In the case of *Myrmeleon pictifrons*, no pits were built by larvae in sand with moisture content greater than 4%. Also no pits were built in substrate of grain size greater than 1mm (Kitching, 1984). Studies were found about the pit character of *Euroleon nostras* in different particle size. They prefer substrate as fine sand (≤ 0.23mm and 0.23-0.54mm). In particle size >1.54mm, the second instar larvae didn't make any pits (Devetak, 2005). Some experiments were performed for understanding the behaviour of *Myrmeleon formicarius* larvae in forest and non-forest areas of kahramanmaras province, Turkey. They conclude that sand particle size and locality features have an effect on pit size whereas soil temperature, altitude, weather had no effect (Bozdogan *et al*., 2013). Also studies were there about the pit bulding of *Euroleon nostras* and identified the pits lined with small slippery grains to maximise powerful avalanches to capture the prey quickly and they also preferred to eject larger grains from pit (Franks *et al*., 2019).

A computer model was also made to analyse shadow competition (the interception of prey by sit-and-wait predators closest to the source of prey arrival) in antlion larvae and the experiment was conducted in the species *Myrmeleon immaculatus*. The shadowing incorporated treatments had a tendency to make pits in the periphery (Linton, 1991). The experiments results that *Euroleon nostras* larvae of France produce efficient traps for easy predation of preys which direct the prey to the mouth of larvae without any attack. So they make steep sloped pits (Fertin and Casas, 2006). The *Euroleon nostras* larva has the sand tossing angle (the slopes created before and after sand throwing) positively correlated with the prey angle (movements of prey) (Vracko and Devetak, 2007). Maoge (2014) studied about the chetae of antlion larvae and how it helps to make pits in *Hagenomyia tristis* and *Myrmeleon obscures.* The pit construction strategy in thermal conditions, sand depth and soil type were studied, from this study, observed a positive correlation between sand depth and soil type (Alcalay *et al.,* 2014).

When the vibrational signals from the media are close to the antlion (*Myrmeleon crudelis*), they act frequently to get that prey. Also, from this study the larval pit size is highly correlated with larval size (Guillette *et al.*, 2009). Pit diameter is bigger with larval body mass such as they extend its trap by knowing the plenty of prey provided by the habitat. Also depicts that there is a positive correlation between pit depth and pit diameter (Scharf *et al*., 2009a). *Myrmeleon immaculatus* given supplemental heat increased pit size at a faster rate and the larvae fed once every three days build larger pits than fed daily (Niemisto, 2013). In the case of *Myrmeleon bore*, the number of pits and size was influenced by the soil temperature.

3.2.2. Predatory behaviour

The starvation experiments depicts that the *Myrmeleon hyalinus* larvae growing faster during the feeding phase and lost mass during starvation period (Scharf *et al.,* 2009a). The major defensive attack by ants (Fire ants- *Solenopsis invicta*) results in the death of antlion through starvation. The ants bite antlions immediately after they have been caught and sometimes grasps the antlions mandible and dies without releasing the hold causing the the antlion unable to make its pit (Lucas and Brockmann, 1981). Eisner *et al.,* (1993) illustrates the predation of *Myrmeleon carolinus* larvae against the formic acid spraying ants (*Camponotus floridanus*), and the larvae suck the body contents without puncturing the acid sac of ant.

In the case of *Myrmeleon immaculatus*, larger pits trapped larger prey and also the prey observed includes ants, spiders, beetle, midges, red mites and wasps (Heinrich and Heinrich, 1984). *Myrmeleon bore* captured 1.25 prey per day on average during spring to autumn, and in the rainy season the average prey per day decreased to 1.03 due to destruction of pits by rain (Matsura, 1986). Miler *et al.,* (2017) studied the cognitive ability of antlion larvae to differentiate between different sized preys by the help of vibrational cues of the substrate and prefer larger prey and ignore the smaller prey if they came together. Lima (2016) used *Drosophila melenogaster* (fruit fly larvae) as prey. Turza *et al*., (2020) explained as the *Formica cinerea* worker ants are the most found prey item in the pit of *Myrmeleon bore* and *Euroleon nostras*. Bakoidi *et al*., (2019) gave *Tribolium castaneum* (Coleoptera, Tenebrionidae) as prey for *Myrmeleon obscures* in rearing purpose. Cain (1987) studied the prey capture behaviour of *Brachynemurus* larvae of Florida. *Brachynemurus* larvae lying in the pit by exposing only its mandibles above sand. It took 15-50 minutes to feed and throw away the prey in laboratory conditions. If the surface temperature above 55ºC, the larvae burrow deeply. If it began to rain it will remain under the sand surface till the sand become dry. Napotilano (1998) identified twelve descrete behaviour patterns of *Myrmeleon mobilis*, which include attack, holding, submergence, emergence, prey beating, feeding, pit clearing, head roll, prey clearing, grooming, quiescence, and jaw set with description. Lambert *et al*., (2011) examined the feeding kinematics of antlion larvae *Myrmeleon crudelis*. The mean duration of prey capture strike was 17.60±2.82 msec. Kross and Pilgrim (2012) studied the predation rate of *Myrmeleon brasiliensis* larvae were analysed by offering leaf cut ant and the third instar larvae with a predation rate of 96.96%, second instar larvae with 69.7% and first instar larvae with 14.28%.

3.2.3. Intraspecific and interspecific interactions

Griffiths (1993) studied the intraspecific competition of antlion larvae (*Macroleon quinquemaculatus*) in Tanzania. They studied the three instars of larvae regarding food availability, hunger level and competition among *Macroleon* larvae. He inferred that, if the food availability was low, large larvae were not affected too much, but small larvae were found hungry because of interference competition of fed larvae. Prado (1993) investigated the asymmetric competition among *Myrmeleon uniformis* larvae of Southeast Brazil. Under laboratory conditions the larvae didn't move anywhere to make pits in three months food scarcity. Gotelli (1997) studied the competition and coexistence of two species of antlion larvae such as *Myrmeleon crudelis* and *M. immaculatus* in central Oklahoma.From their study it is clear that competition and predation are severe between similar sized larvae. The presence of overlapping generations co exist in the case of antlion larvae. Devetak (2000) studied the competition in two European antlion species *Euroleon nostras* and *Myrmeleon formicarius*. A negative correlation between pit building and larval density was noted. Barkae *et al*., (2014) studied about the factors influencing cannibalism in *Myrmeleon hyalinus*. Lima (2011) depicts that, food availability is the main cause of cannibalism. The study compared the cannibalism of *Myrmeleon brasiliensis* larvae in four different conditions to compare the density and food availability in the context of cannibalism.

3.3. RESEARCH METHODOLOGY

Pit building behaviour, trailing of antlion larvae, predatory/feeding behaviour and intraspecific and interspecific relationship of antlion larvae were carefully analysed in both natural and laboratory conditions. The observations from natural conditions were applied to imitate the natural condition in the laboratory. Before doing each behaviour experiment, standardized substrate, prey species, antlion instars, antlion species, tray size, texture of medium, abiotic factors (atmospheric temperature and humidity), number of antlion larvae in each experiment, and starvation period.

a. Substrate- The sand and dry soil collected from the field in which the presence of larvae were noted and dried in sunlight.

b. Prey species- Mostly ants (Order Hymenoptera) are considered as the common diet of antlions. From the field observation, the most preferred prey of antlion larvae was the ant species *Anoplolepis gracilipes*.

c. Antlion larval instar- Usually three instars (two moulting) are present and the larval instar was analyzed before the experiment from the body size of larvae (Krishnan and kakkssery, 2016).

d. Antlion species- The species of antlion which is used for the experiment was noted by using available literature (Lucas and Stange, 1981). Here, the larva of *Myrmeleon pseudohyalinus* was used as experimental organism.

e. Tray size- The dimension of tray used for the experiment was noted before the start of each experiment with special care to maintain the larval density.

f. Substrate particle texture- Dry soil and sand were used for behaviour experiments. Sand should be dried, the moisture content less than 1%-90% for 4 hours and sieved to get the exact particle fraction or used as such.

g. Abiotic factors- Atmospheric temperature and humidity were noted before the start of each experiment.

h. Number of larvae – The number of larvae must be same in each experiment, it was standardised from previous studies.

i. Starvation period standardization- The fed larvae was fed with 1 prey per day and the starved larvae was fed after 3 days of starvation.

3.3.1. Pit Building Behaviour

3.3.1.1 Pit buiding behavior in different medium, hunger level and instar

Most of the organisms use self secreted silk to form the traps, but larval antlion use materials from environment to make its trap (Franks *et al*., 2019). Pit building behaviour was analyzed in second and third instar antlion larvae of *Myrmeleon pseudohyalinus*. Pit building has two complementary frequently observed steps, the digging of soil and removal of dust from the pit. For this purpose the thick setae present on the IX abdominal segments are used (Ngamo, 2014).

Pit building behaviour of the fed and starved larvae was also noted. Continuous observation was done in the lab condition and natural conditions for studying the pit building nature of antlion larvae *Myrmeleon pseudohyalinus*. The behaviour observations were repeated in order to decrease the bias.

Table 25. Experimental design to analyse the pit building behaviour of *M. pseudohyalinus* larvae

Experiment	Medium (Sand/soil)	Larval Instar (Second/third)	Fed/Starved
1	Sand	Second	Fed
2	Sand	Third	Fed
3	Sand	Second	Starved
4	Sand	Third	Starved
5	Dry soil	Second	Fed
6	Dry soil	Third	Fed
7	Dry soil	Second	Starved
8	Dry soil	Third	Starved

The experiment was done in a plastic tray with a dimension of 23cm X 23 cm, filled with 2 cup (one cup is one litter each) of sand/soil which is collected from antlion inhabited area. The sand or soil depth was maintained as 5 cm (Vracko and Devetak, 2007). The second/third instar larvae fed daily by manually placing one ant (*Anoplolepis gracilipes*) with mean size 0.45±0.05cm (sample size- 137 individuals) as prey. In the case of starved larvae, they were starved for 3 days prior to the experiment. Single larvae were allowed to make its pit in each tray and it was kept in room temperature. The larvae kept 3 days prior to experiment to get acclimatised in that situation. Then the larvae released in the centre of the tray and allow it to make its pit for predation and shelter. From that time, the observations taken in every one hour (pit depth and pit diameter) to identify the progress of pit making process. A total of 12 hour observation was done in the day time, also noted the next morning observation for analysing the progress of pit building after a night.

A total of eight experiments were done for analysing the pit building behaviour of *M. pseudohyalinus* larvae (Table 25) and the experimental set up were given in Fig. 25. Different medium/substrate were given to second and third instar larvae for analysing whether any change in pit building behaviour in each conditions. Also checked whether any influence of hunger level in the pit buiding of *M. pseudoyalinus* larvae.

3.3.1.2. Pit characteristics in different types of substrates

Commonly antlion larvae are seen in dry soil, clay soil, sand and mixture of sand, soil and cement. This study is the comparison of the different soils/media which increase the efficiency of pit building behaviour of the antlions. For this purpose 48 larvae were collected from an abandoned house from which 20 larvae with same body length and body width (body length-1.1cm, body width-3mm) were selected for the experiment. Four trays (28X23cm) were taken and each tray was filled with different type of soils that is, dry soil, brick kiln/claysoil, sand and soil collected from partially completed building (mixture of cement, sand and soil). The soil depth was fixed to 5 cm in each tray. The larvae selected for the experiment was kept 24 hour starvation prior to introduction. Five larvae were introduced into each tray without disturbing them and observed their pit building behaviour. The average soil temperature was 33ºC and the relative humidity was 65 %. The experiment was repeated 6 times in order to decrease the error.

3.3.1.3. Trailing Behaviour

Trails are marks made by antlion larvae before deciding the apt place for making its pits and all the pit building antlion larvae make trails in soils before making its pits. The trailing behaviour of larvae was observed under laboratory condition. From this observation a short note was made. For this purpose, a single larva (*M. pseudohyalinus*) was placed on a single tray, and the trailing initiation and progress was analyzed prior to pit making. The dimension of the tray was 23cm X 23cm and soil depth was fixed to 3.2 cm in each experiment.

3.3.2 Predatory/Feeding Behaviour

According to Napotilano (1998), twelve discrete behaviour patterns were exhibited by antlion larva in its due course of time in predation. The various conditions, substrates are same as the above experiments and given in Table 26.

Table 26. The experiments designed for analyzing predatory behaviour of antlion larvae *M. pseudohyalinus*

Experiment	Medium (Sand/soil)	Larval Instar (Second/third)	Feed/Starved
1	Sand	Second	Fed
2	Sand	Third	Fed
3	Dry soil	Second	Fed
4	Dry soil	Third	Fed
5	Sand	Second	Starved
6	Sand	Third	Starved
7	Dry soil	Second	Starved
8	Dry soil	Third	Starved

Here, the plastic trays (23cmX23cm) were filled with sand or soil to a thickness of 5 cm according to the experiment. In the case of starved larvae, they were starved for 3 days prior to the experiment. Single larvae were allowed to make its pit in each tray and it was kept in room temperature. The larvae kept 3 days prior to experiment to get acclimatised in that situation. The larvae were then released in the centre of the tray and allowed to make its pit for predation and shelter.

The common ant *Anoplolepis gracilipes* (mean size 0.45±0.05 cm) was used as prey for larvae and it was manually placed in the centre of the pit and the behavior patterns were carefully noted in every second by using a hand lens. From the observation data the capture success of the larvae were analysed and the prey escape were calculated.

3.3.3. Types of Interactions- Intraspecific relationships

Intraspecific relationship of *M. pseudohyalinus* larvae were studied by examining cannibalism among the species. The collected *M. pseudohyalinus* larvae were fed eight days and kept in one week starvation (Barkae *et al*., 2014) for standardizing the hunger level. The antlion larvae were separated into two trays according to the instar. Third instar larvae were used for the experiment. The third instar larve were kept in three separate trays for making three hunger levels for cannibalism experiment. The trays filled with sand were labeled as well fed, fed and starved larvae (Table 27). The feeding of *M. pseudohyalinus* larva prior to the experiment was as follows.

Table 27. The hunger level standardization of *M. pseudohyalinus* larvae

1.	Well-fed	2 ants/day
2.	Fed	1 ant/day
3.	Starved	No feeding

After 7 days (one week) of feeding/starvation the larvae were collected and the following experiments were conducted (Table 28).

Table 28. Experiments designed to analyse cannibalistic behavior of *M. pseudohyalinus* larvae

Sl. No.	Experiment
1.	Well-fed vs Well-fed
2.	Well-fed vs Fed
3.	Well-fed vs starved
4.	Fed vs Fed
5.	Fed vs Starved
6.	Starved vs Starved

Six pair wise combination of hunger levels were tested by releasing larvae in to the tray filled with sand. In each tray filled with two cup of sand and the larvae were marked without disturbing them as well fed, fed and starved (Plate 20). Like each experiment two larvae were released in to the tray filled with sand and allow them to make its pits for predation. After five days the soil was sieved and the occurrence of larvae noted as follows.

a. Pupated

b. Dead (With injuries cannibalised) (Devetak, 2000) (Plate 20)

The experiments were repeated 6 times and a total of 36 pairs were tested (Barkae *et al*., 2014)

3.3.4. Types of Interactions- Interspecific relationships

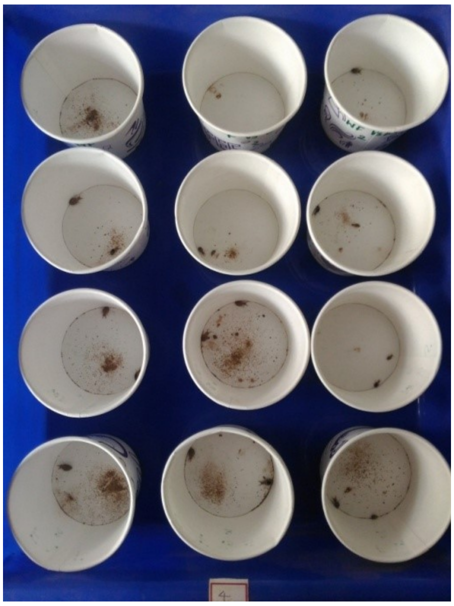
Interspecific relationships were studied by analyzing the predator and prey of antlion larvae Genus *Myrmeleon*. During the field study, observations were done to find out the presence of natural enemies of antlion larvae. For this purpose the natural habitat of antlion larvae like river side habitat, abandoned houses and heap of sand were carefully observed. Similarly observations were also done under laboratory conditions (rearing of antlion larvae) for identifying the natural enemy of this predator.

For understanding the type of prey or diet of antlion larvae Genus *Myrmeleon,* collected prey remnants from the antlion larval pits from study areas. The prey remnants analyzed under binocular microscope and photographs were taken for identification using available literature (Narendra and Kumar, 2006; Imms *et al*., 1977; Nayar *et al*., 1976; Borror *et al*., 1975).

3.3.5. Ethogram

Ethograms are consolidation of each behaviour of an organism and it gives an idea about the life style of that organism easily in a single graph. Here the ethogram of *M. pseudohyalinus* larvae was plotted in different condition, instar and substrate.

Plate 20




Marked Larvae for Cannibalism experiments

Dead antlion- Victim of competition

3.3 RESULTS

3.3.1. Pit Building Behaviour

3.3.1.1. Pit buiding behaviour in different medium, hunger level, and instar

The pit building behaviour of *Myrmeleon pseudohyalinus* larvae were observed under laboratory conditions. The antlion larvae build steep conical funnels which help to fall easily. The diameter and depth of the pit helps the easy falling of prey for this purpose. In each condition (fed and starved), instar (second and third) and medium (sand and soil), the pit building ability of *Myrmeleon pseudohyalinus* larvae was monitored and the interpretation was made according to the results made from Past 4.0 software.

In all the experiments, the diameter and depth were somewhat static up to 9 hours from the starting of pit making. From the 9^{th} hour onwards the larvae increases its depth and diameter suddenly for creating the steep conical shape. After 11 hours of pit building, the diameter was not increasing in a significant range.

The sample size in each experiment was 30 and the mean and standard deviation were shown in Fig 23 and 24. The progress of depth and diameter were plotted in Fig. 26 and 27 respectively. The mean pit diameter in second instar larvae ranges from 2.5cm to 3.6cm (Appendix 10) and that of third instar larvae was 3.2 to 3.5 cm (Appendix 11). The mean pit depth in second instar larvae ranges from 1.5 cm to 2.2 cm (Appendix 12) and that of third instar larvae was 2cm to 2.2 cm (Appendix 13).

Table 29. One way ANOVA-Test for equal means (Diameter)

	Sum of sqrs	df	Mean square	F	Critical value
Between groups:	20.1913	12	1.68261	15.05	1.86
Within groups:	10.1734	91	0.111796	Permutation p (n=99999)	
Total:	30.3647	103	1E-05		

Since the calculated F value 15.05 is greater than the critical f value 1.86 at 5% (Table 29). It is concluded that there is a significant difference between the pit diameter, size of larvae in different media (sand and soil) and different levels of feeding (fed and starved).

Table 30. One way ANOVA-Test for equal means (Depth)

	Sum of sqrs	df	Mean square	F	Critical value
Between groups:	7.17565	12	0.597971	14.53	1.86
Within groups:	3.74437	91	0.0411469	Permutation p (n=99999)	
Total:	10.92	103	1E-05		

Here, the calculated F value 14.53 is greater than the critical f value 1.86 at 5% (Table 30). It is concluded that there is a significant difference between the pit depth, size of larvae in different media (sand and soil) and different levels of feeding (fed and starved).

In the case of second instar larvae, the average diameters of the pit in the starting hour of pit building were 1.60 cm (sand medium, fed condition), 1.98 cm (sand medium, starved condition), 2.04 cm (soil medium, fed condition), and 2.12 cm (soil medium, starved condition). The final measurements of average diameters were 2.49 cm (sand medium, fed condition), 3.01 cm (sand medium, starved condition), 3.65 cm (soil medium, fed condition), and 3.13 cm (soil medium, starved condition). The fed larvae in soil medium have the highest average pit diameter in the final pit and the lowest value observed in fed larvae in sand medium. In the case of third instar larvae, the average diameters of the pit in the starting hour of pit building were 1.92 cm (sand medium, fed condition), 2.34 cm (sand medium, starved condition), 1.92 cm (soil medium, fed condition), and 2.06 cm (soil medium, starved condition). The final measurements of average diameters were 3.39 cm (sand medium, fed condition), 3.50 cm (sand medium, starved

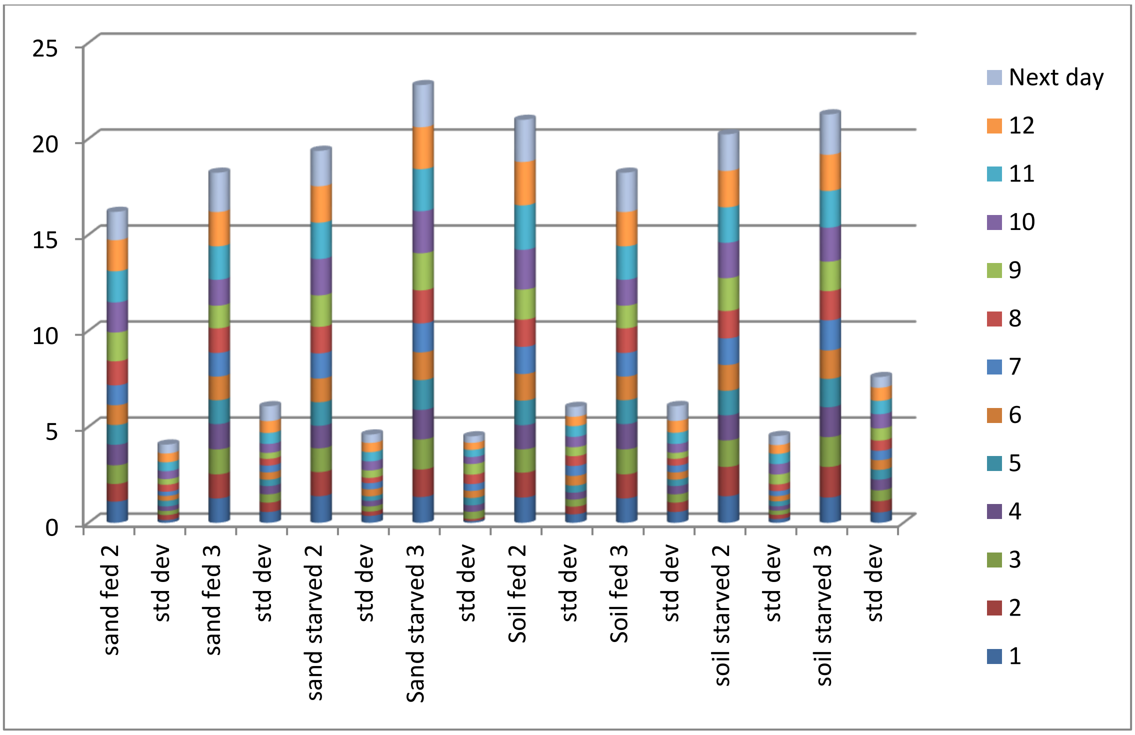


Fig 23. showing the progress of diameter (mean and standard deviation) while pit building of *M.pseudohyalinus* lrvae in different conditions, media and instar

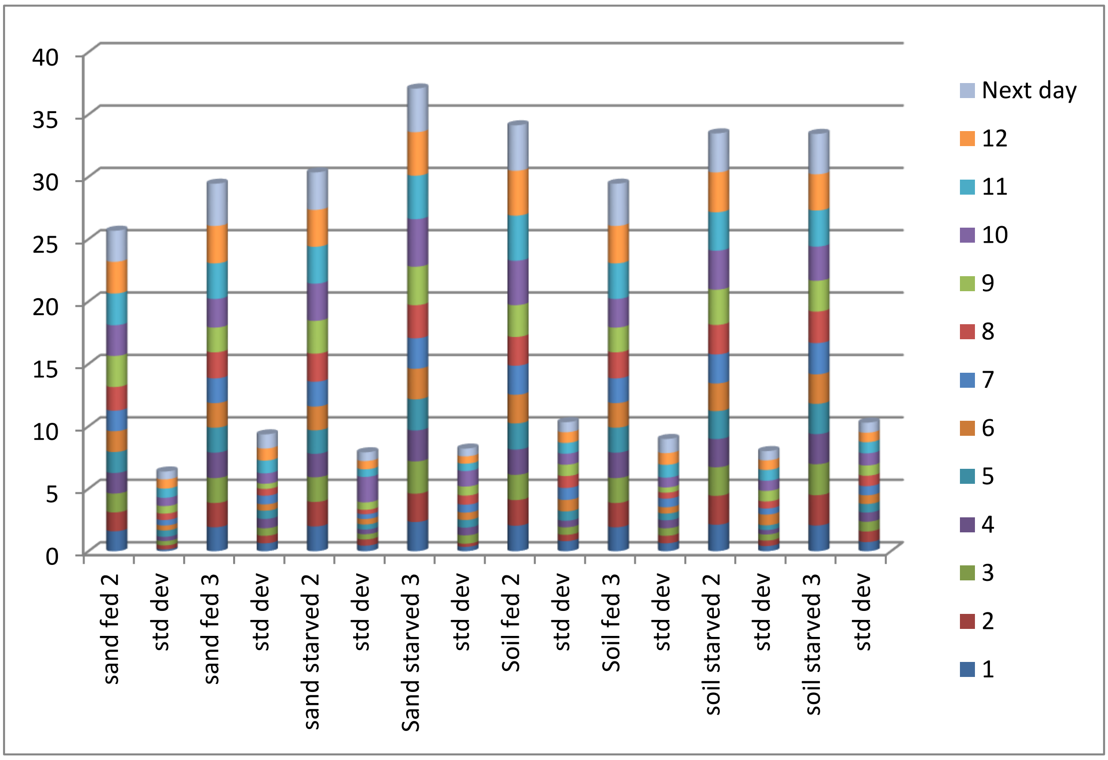


Fig 24. showing the progress of depth (mean and standard deviation) while pit building of *M.pseudohyalinus* lrvae in different conditions, media and instar

Experimental set up

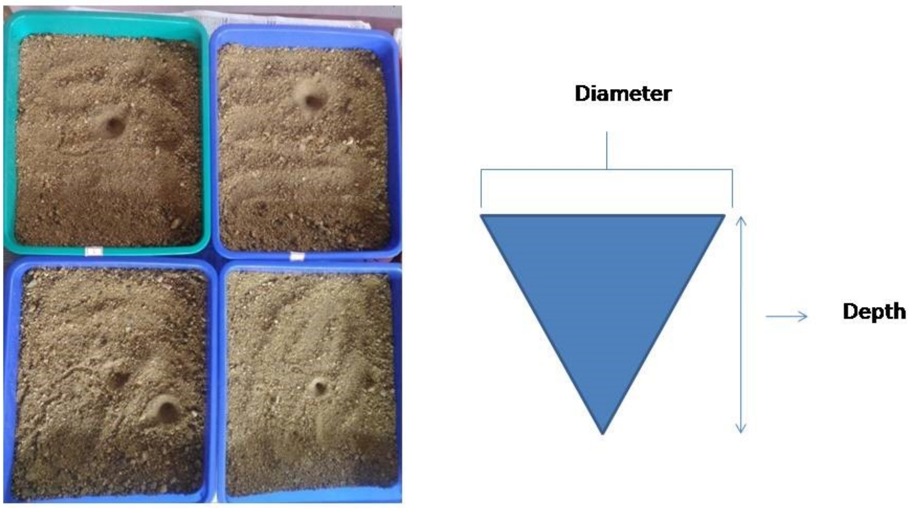


Fig. 25. The experimental set up for analyzing the pit building behaviour

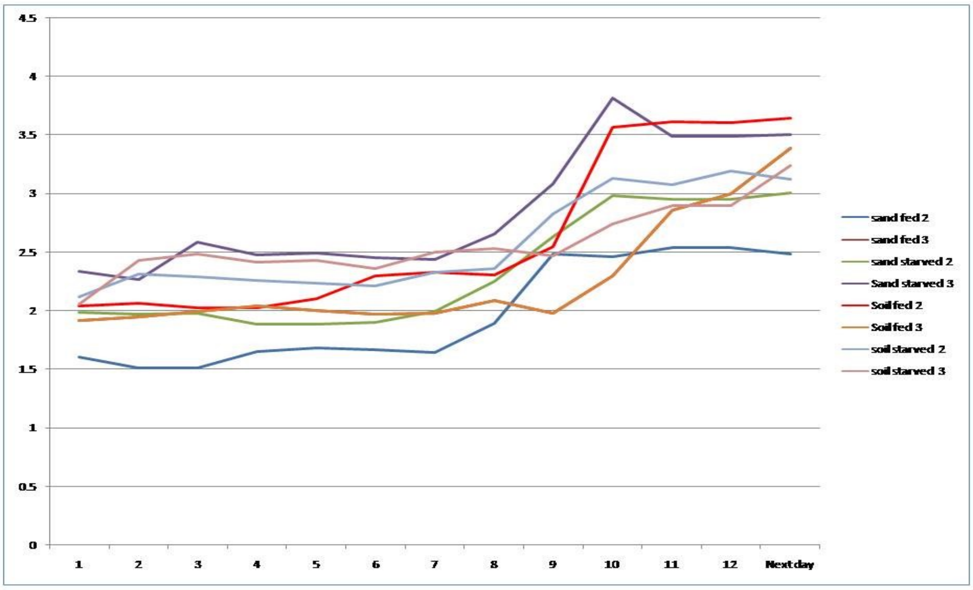


Fig. 26. Shows the progress in diameter while pit building of *M. pseudohyalinus* larvae in different conditions, media & instar

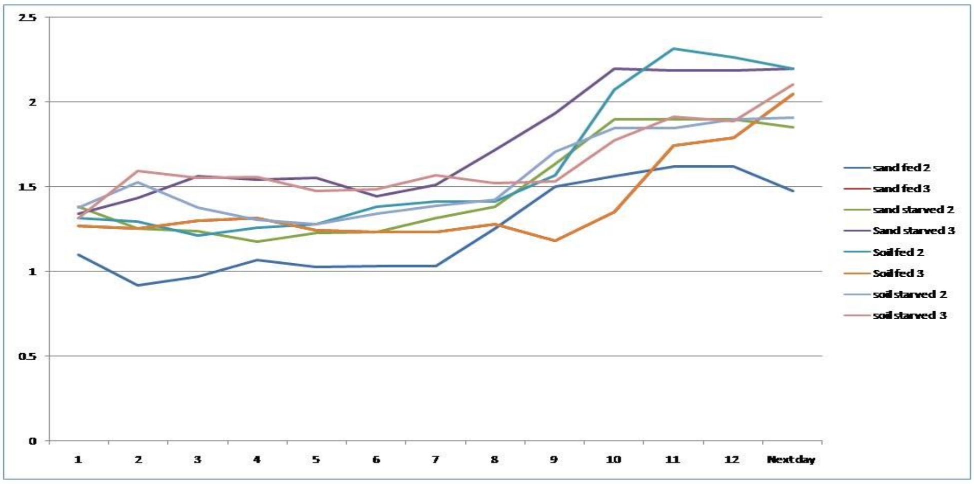


Fig. 27. shows the progress in depth while pit building of *M. pseudohyalinus* larvae in different conditions, media & instar

condition), 3.39 cm (soil medium, fed condition), and 3.24 cm (soil medium, starved condition). The starved larva in sand medium has the highest average pit diameter in the final measurement and the lowest value observed in starved larvae in soil medium.

In the case of second instar larvae, the average depth of the pit in the starting hour of pit building were 1.10 cm (sand medium, fed condition), 1.38 cm (sand medium, starved condition), 1.31 cm (soil medium, fed condition), and 1.38 cm (soil medium, starved condition). The final measurements of average depth were 1.48 cm (sand medium, fed condition), 1.85 cm (sand medium, starved condition), 2.20 cm (soil medium, fed condition), and 1.91 cm (soil medium, starved condition). The fed larvae in soil medium have the highest average pit depth in the final pit and the lowest value observed in fed larvae in sand medium. In the case of third instar larvae, the average depth of the pit in the starting hour of pit building were 1.27 cm (sand medium, fed condition), 1.34 cm (sand medium, starved condition), 1.27 cm (soil medium, fed condition), and 1.31 cm (soil medium, starved condition). The final measurements of average depths were 2.04 cm (sand medium, fed condition), 2.20 cm (sand medium, starved condition), 2.05 cm (soil medium, fed condition), and 2.10 cm (soil medium, starved condition). The starved larva in sand medium has the highest average pit depth in the final measurement and the lowest value observed in starved larvae in sand medium and fed larvae in soil medium.

3.4.1.2. Pit characteristics in different type of substrates

Within 3 hours, the introduced antlion larvae made their pits in the soil. Trailing behaviour was low in sand and the largest pits were built in the sand. The average pit diameter and pit depth was 3.80 cm and 2.85 cm respectively in sand. From this result we can inferred that Genus *Myrmeleon* makes their largest pits in the sand and that was the most preferred soil type. Also the size of the pit indicates the hunger level of the antlion larvae because it was kept 24 hr starvation before doing this experiment. The average depth and diameter in different substrates were given in Fig. 28.

In the case of second preference, the diameter was more in the brick kiln/clay soil than other two, it may be because of the clay soil is very fine and it is very difficult to build more deep pits. In the case of mixture (cement, sand and soil), they form pits with more depth by reducing its diameter. It is inferred that they do prefer the pit depth than diameter for pit building in order to reduce the prey escape. It is also assume that if the soil is more fine, antlion larvae build their pit with increased diameter.Trailing behaviour was low in sand and the largest pits were built in the sand.In the case of second preference, the diameter was more in the brick kiln/clay soil than other two, it may be because of the clay soil is very fine and it is very difficult to build with more deep pits. In the case of mixture (cement, sand and soil), they form pits with more depth by reducing its diameter. The different types of soil with its pit size were shown in Fig. 29.

3.4.1.3. Trailing Behaviour

- The trailing behaviour was observed between 3sec to 50 minutes from the introduction of larvae to the soil.
- In most cases, trailing started within 3seconds.
- From the centre portion of the tray the larvae first moved to the periphery of the tray.
- The larvae make its pits only after the calculation of the boundary of the habitat or environment by moving on to the periphery of the tray.
- Most of the time the trailing started from the centre to north of the tray and they moved near to the centre, which is south.
- After fixing the correct location for its pit building the backward rotation starts with the flipping of soil.

The trailing behaviour of larvae in natural and laboratory conditions were given in Plate 21.

3.4.2. Predatory/Feeding Behaviour

The twelve behaviour patterns were identified and from this data predatory efficiency (Table 31) of *Myrmeleon pseudohyalinus* larvae was explained.

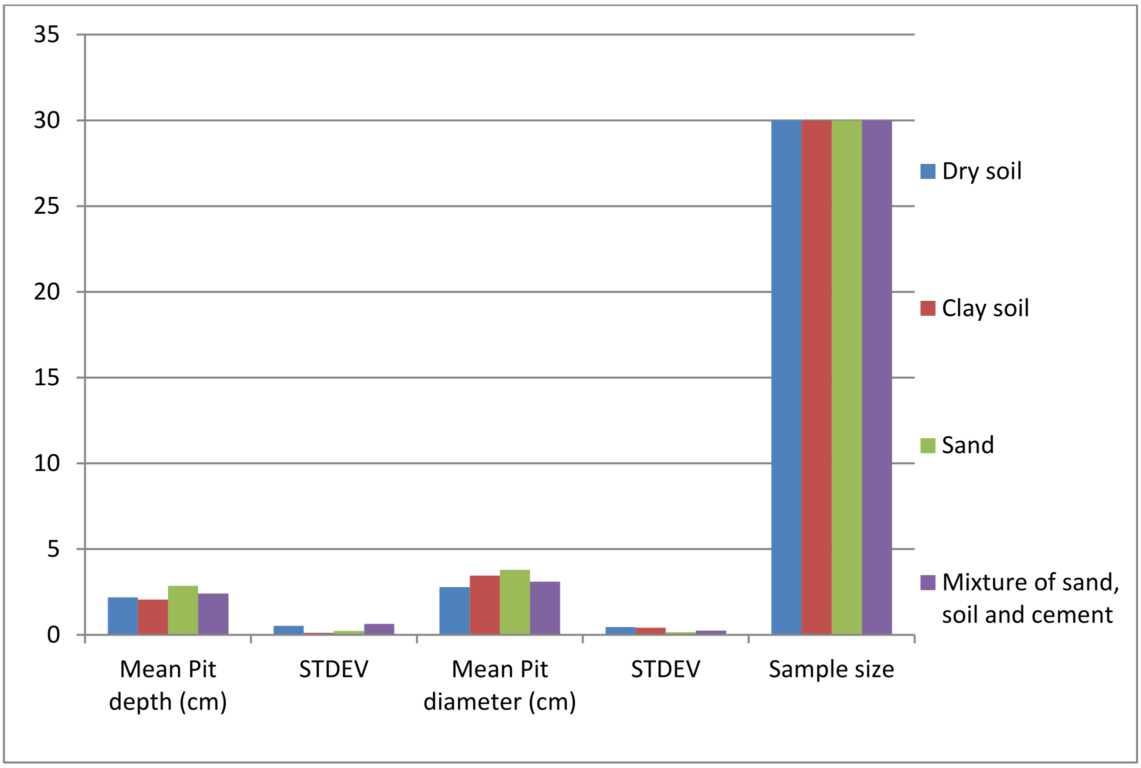


Fig. 28. Average depth and diameter in different substrates

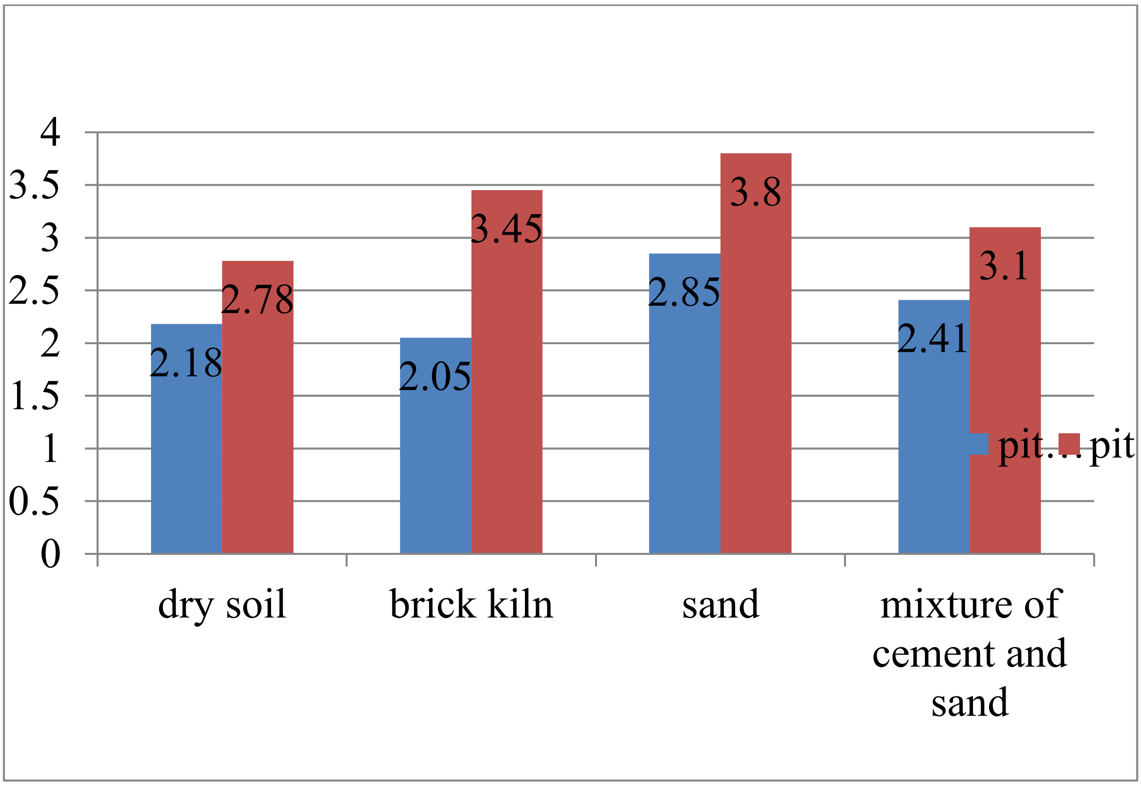
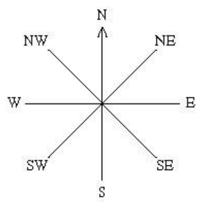


Fig. 29. Showing the different type of soil with its pit size

Plate 21



Turning from N to S is 180 ° clockwise or anticlockwise

Turning from NE to SE is 90 ° clockwise (or 270 ° anti-clockwise)

Turning clockwise from NE to E is 45 ° (or 315 ° anticlockwise)

Trails of antlion larvae both in natural and lab condition

75% of the larvae took 0-35 minutes for the completion of its feeding process (attack to jaw set) and the remaining 25% of the larvae took 0-45 minutes to complete its feeding irrespective of the conditions. Irrespective of the conditions, the second instar (95%) larvae were more successful than third instar (75 %) larvae.

Table 31. Showing the predatory efficiency of *Myrmeleon pseudohyalinus* larvae

Sl No.	Condition	Feeding time (Minutes)	Prey Escape (%)	Capture Success (%)
1	Sand, Fed, Second instar	0-45	20	80
2	Sand, fed, Thirdinstar	0-35	50	50
3	Sand, Starved, Second instar	0-45	0	100
4	Sand, Starved, Third instar	0-35	33	67
5	Soil, Fed, Second instar	0-35	0	100
6	Soil, Fed, Third instar	0-35	0	100
7	Soil, Starved, Second instar	0-35	0	100
8	Soil, Starved, Third instar	0-35	18	82

The Eigen values (Table 32) for Axis1 and 2 added upto 99.99% which indicates that 99% of the variance has been covered. Therefore robustness is very high and can be used for interpretation. The prey beating, emergence and submergence behaviours are influenced by the larval instar (second and third). While the quiescence, pit clearing and jaw set behaviour patterns are influenced by their condition that is whether it was fed or starved. Head roll behaviour is only related to the medium of the substrate in which the larvae inhabiting. Here sand or dry soil are the medium used for the study. The relationship between selected behaviour, instar, medium and condition were plotted in Fig.30.

Table 32. Eigen values

Axis	Eigen value	%
1	0.056716	87.61
2	0.008017	12.38
3	3.91E-06	0.006039

The Eigen values (Table 33) for Axis 1 and 2 added up to 90.8% which indicates that 90% of the variance has been covered. Therefore robustness is high and can be used for interpretation. Medium (sand and soil), condition (fed and starved) or instar (second and third) did not play much of a role in the behaviour but, time period seem to. While the behaviour in the first five and last five minutes of observation seemed very similar (mainly inactivity). Behaviour of 5-10 minutes also showed similarity to this group. Behaviour seemed 10-15 and 15-20 minutes were very unique and therefore lay in different quadrates. Rest of the five minute windows showed similar behaviour. The relationship between selected behaviour, instar, medium, condition and time period were given in Fig.31.

Table 33. The Eigen values

Axis	Eigen value	%
1	0.24361	69.45
2	0.074889	21.35
3	0.032186	9.175
4	0.000101	0.02891

The common ant (*Anoplolepis gracilipes* - worker) was used for the feeding purpose both in rearing and experiments, because it is the most abundant prey item in the antlion larval pit irrespective of species. In all the conditions, the larvae show similar behaviour patterns in first five minutes. Attack, honding, submergence, emergence, prey beating and feeding are the six behaviour patterns. In addition to this, head roll behaviour pattern is present in 5-10 minutes period of feeding except starved second instar larvae in sand medium. Starved second instar larvae in soil medium shows pit clearing behaviour in addition to this head roll.

The 10-15 minute period of fed second instar larvae, third instar larvae and starved third instar larvae shows similarity in behaviour patterns and the starved second instar larvae in sand media pit clearing and prey clearing were found in addition to the common behaviour (prey beating, feeding and head roll) in this time period.This indicate that the second instar starved larvae shows more hunger than third instar larvae and clear its pit and throw the prey from pit for making the pit again steep and wait for another prey. In soil media, the second instar fed larvae shows quiescence

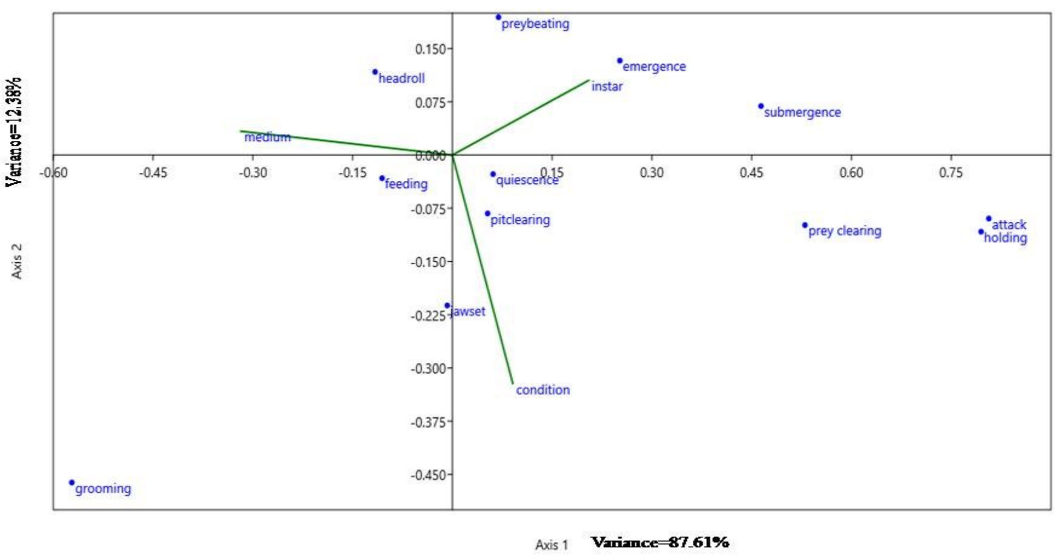


Fig. 30. CCA map showing relationships between selected behaviour and instar, medium and condition

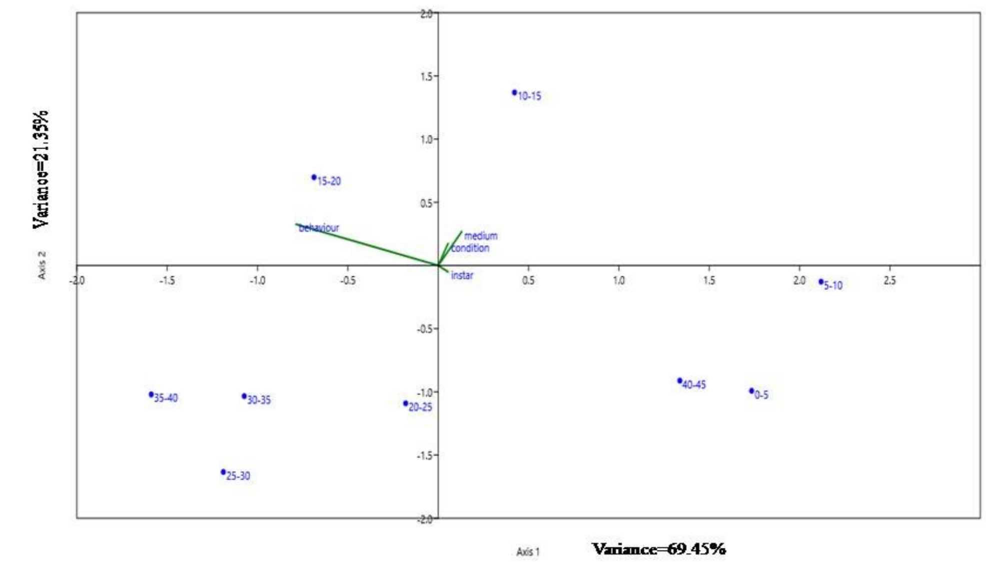


Fig. 31. CCA map showing relationships between selected behaviour and instar, medium and condition & time period

and jaw set behaviour pattern in this period and the third instar fed larvae shows similar behaviour pattern of second and third insat fed larvae in sand media.

15-20, 20-25 and 25-30 minutes are more important in feeding of *M. pseudohyalinus* larvae and the prey beating, feeding, pit clearing, head roll, prey clearing, grooming, quiescence and jaw set avtivities are at its peak. In 30-35 minutes, the jaw set and quiescence are the main patterns and considered as the end point of feeding behaviour.The twelve feeding behaviour patterns of *Myrmeleon pseudohyalinus* larvae in various conditions were plotted in Fig.32 to Fig. 39.

From the feeding behaviour study of *M. pseudohyalinus*, it was understood that the behaviour patterns of fed second instar larvae in sand medium (Fig. 32) and fed third instar larvae in soil medium (Fig. 37) shows a similar pattern with respect to the time period. Here, the maximum feeding activity was present in 15 to 30 minutes. Similarly, the fed third instar larvae in sand medium (Fig. 33) and starved third instar larvae in sand medium (Fig. 35) shows similar pattern with respect to time period. Here, two peaks were present in the activity patterns (0-15 minutes and 15-30 minutes). The remaining experiments such as starved second instar larvae in sand medium (Fig. 34), fed second instar larvae in soil medium (Fig. 36), starved second instar larvae in soil medium (Fig. 38) and starved third instar larvae in soil medium (Fig.39) didn't show a prominent pattern of feeding activity with respect to time period.

3.4.3. Intraspecific relationship-Cannibalism

Well fed vs Well Fed (two larvae in each experiment, six replications, sample size-12): In all the experiment one larva was dead and 50% of mortality was noted. The hunger level was same in each larva so that the competition resulted the mortality of 50% in each replication in both second and third instars.

Well fed vs Fed (two larvae in each experiment, six replications, sample size-12): Here 70% of well fed larvae and 30% of fed larvae were dead due to cannibalism in second instar larvae. No pupation was noted in second instar and the cannibalism noted was high in increased hunger levels in the experiment. In the case of third instar larvae, the mortality rate was same, but 20% of larvae were pupated (all are fed). Also 30% of larvae neither pupated nor dead.

Well fed vs Starved (two larvae in each experiment, six replications, sample size-12): In the case of second instar larvae 70% of starved larvae and 40% of well fed larvae were dead .20 % of larvae neither dead nor pupated. In the case of third instar larvae, only 10% of larvae were pupated. 50% starved larvae and 20% of well fed larvae were dead. Also 40% larvae neither dead nor pupated.

Fed vs Starved (two larvae in each experiment, six replications, sample size-12): 90% of fed larvae were dead in the case of second instar larvae. 10 % of starved larvae were pupated and neither dead nor pupated.70% of third instar fed and starved larvae were dead.

Fed vs Fed (two larvae in each experiment, six replications, sample size-12): 50% of larvae were dead and 10% was pupated. The remaining 40% neither pupated nor dead in second instar larvae. 40% dead 45% pupated and only 15 % has no change through out the period.

Starved vs Starved (two larvae in each experiment, six replications, sample size-12): Here both second and third instar, 50 % were dead, the remaining 50% has no change through out the experiment.

The detailed observed values were given in Appendix 14.

3.4.4. Interspecific relationships-Prey and Predation

Interspecific interactions are the relationship of an organism with other species or between species. Prey and predation are the two major interactions between species and here the interaction of pit building antlion larvae genus *Myrmeleon* with other organisms were illustrated.

The antlion larval pits were carefully observed for understanding the feeding behaviour in natural condition. The prey remnants were collected and observed under Leica Stereozoom research microscope attached with camera. The prey remnants which cleared adjacent to the pits after consuming the body fluid were collected and photographed. Due to predation, only some parts of the prey were available and these specimens were somewhat difficult for identification. The collected specimens included Insects, Arachnids and Millipedes. The prey items were identified using

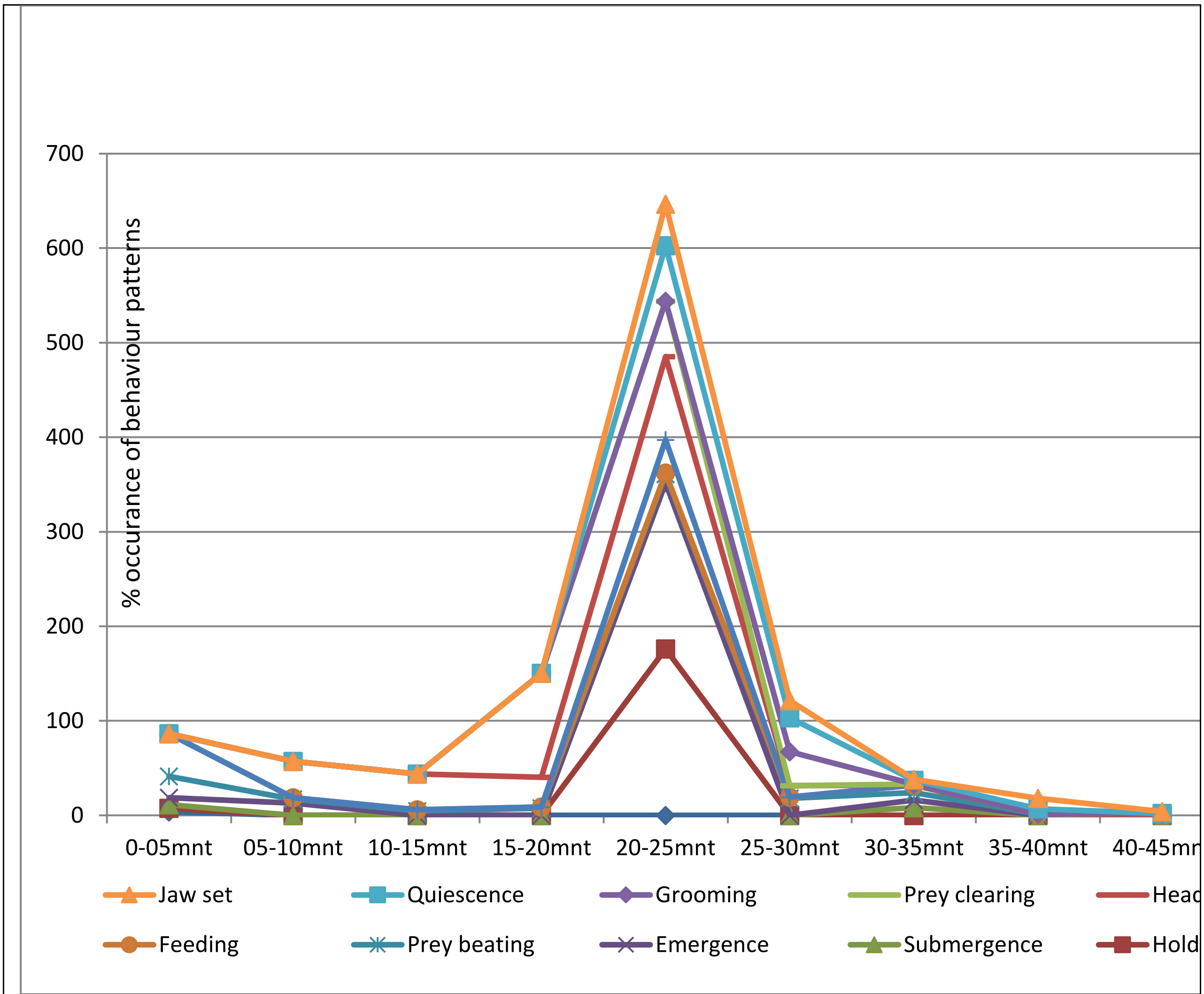


Fig 32. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed second instar larvae in each time intervals in sand medium.

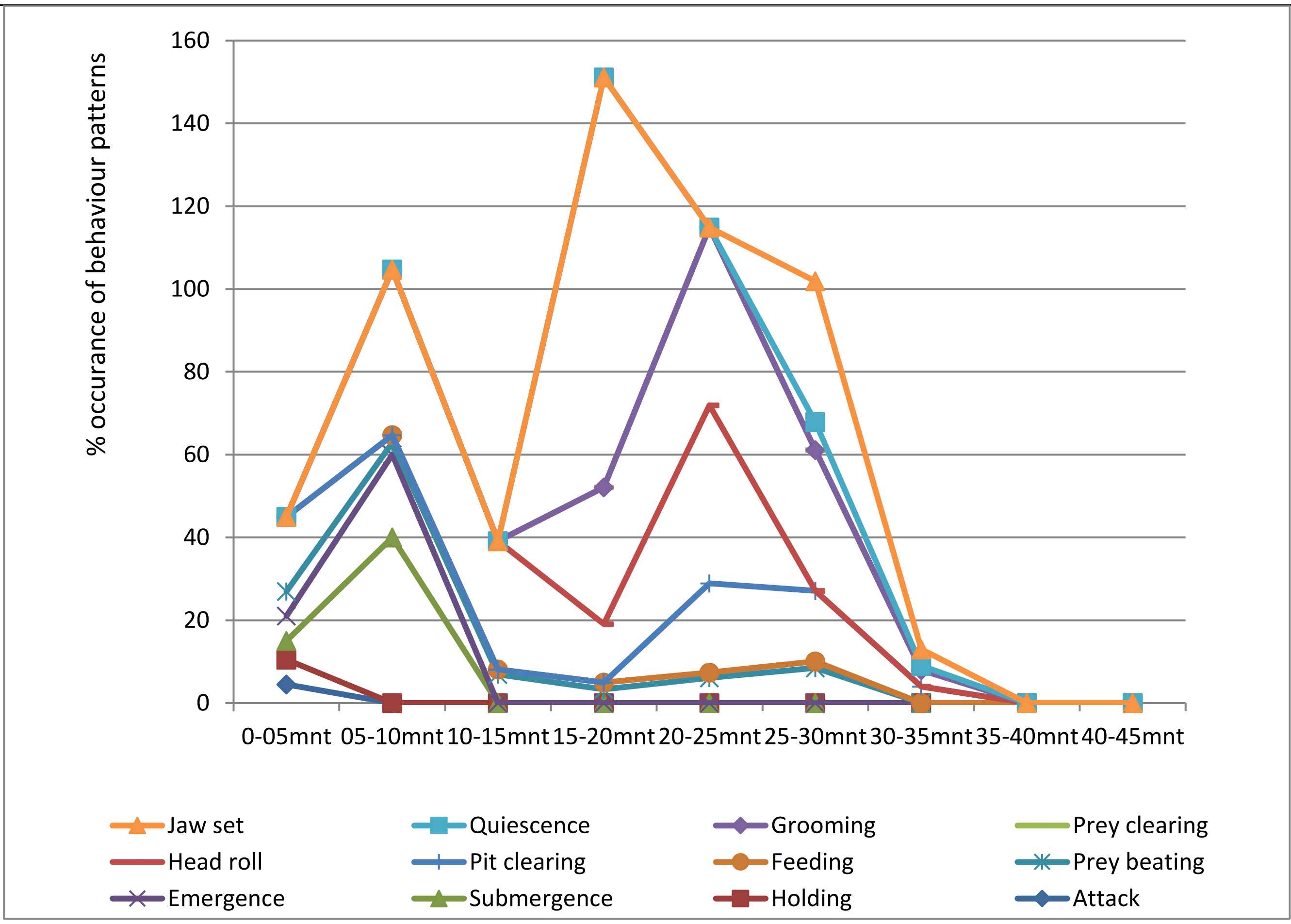


Fig 33. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed third instar larvae in each time intervals in sand medium

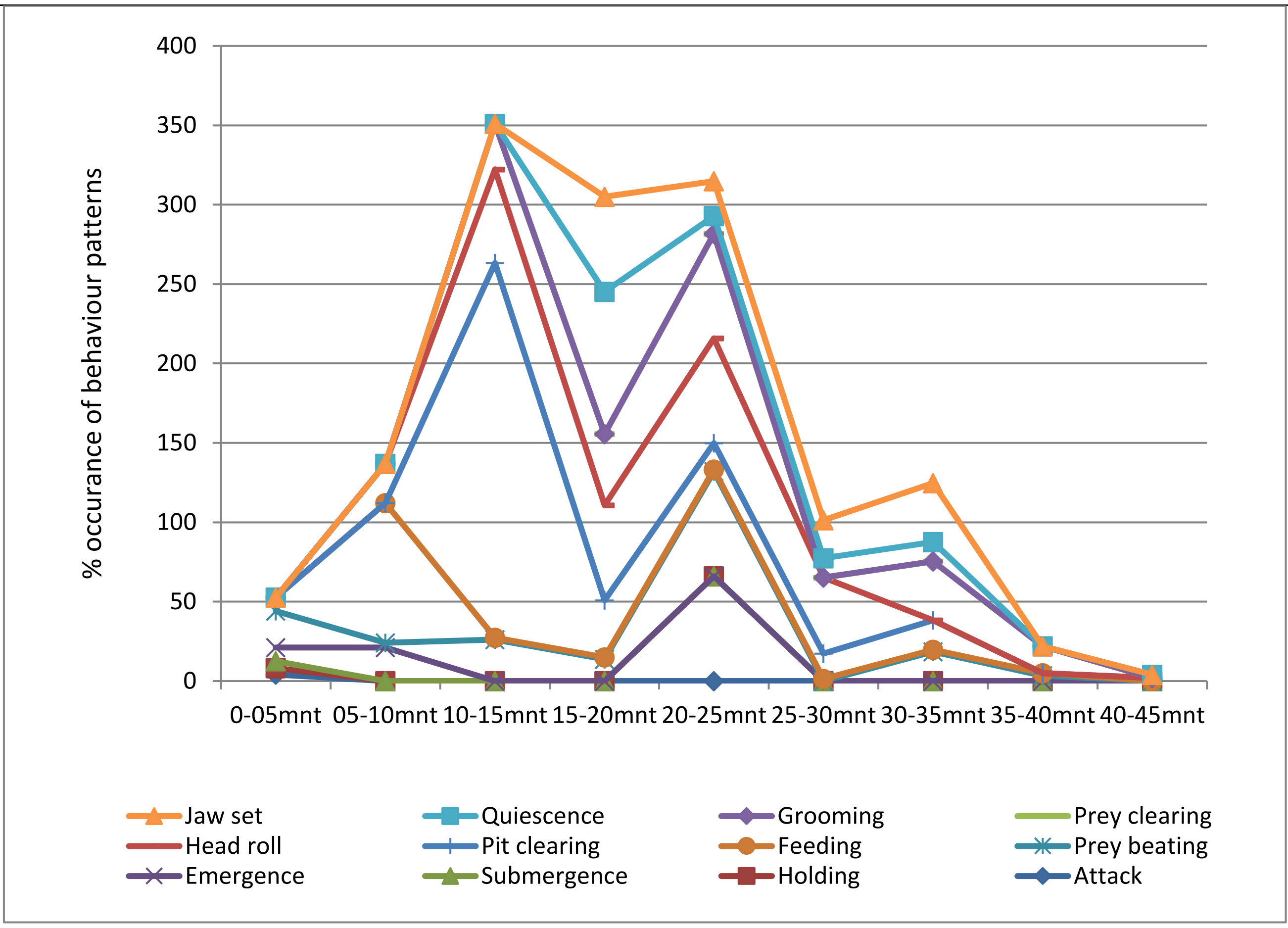


Fig 34. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* starved second instar larvae in each time intervals in sand medium

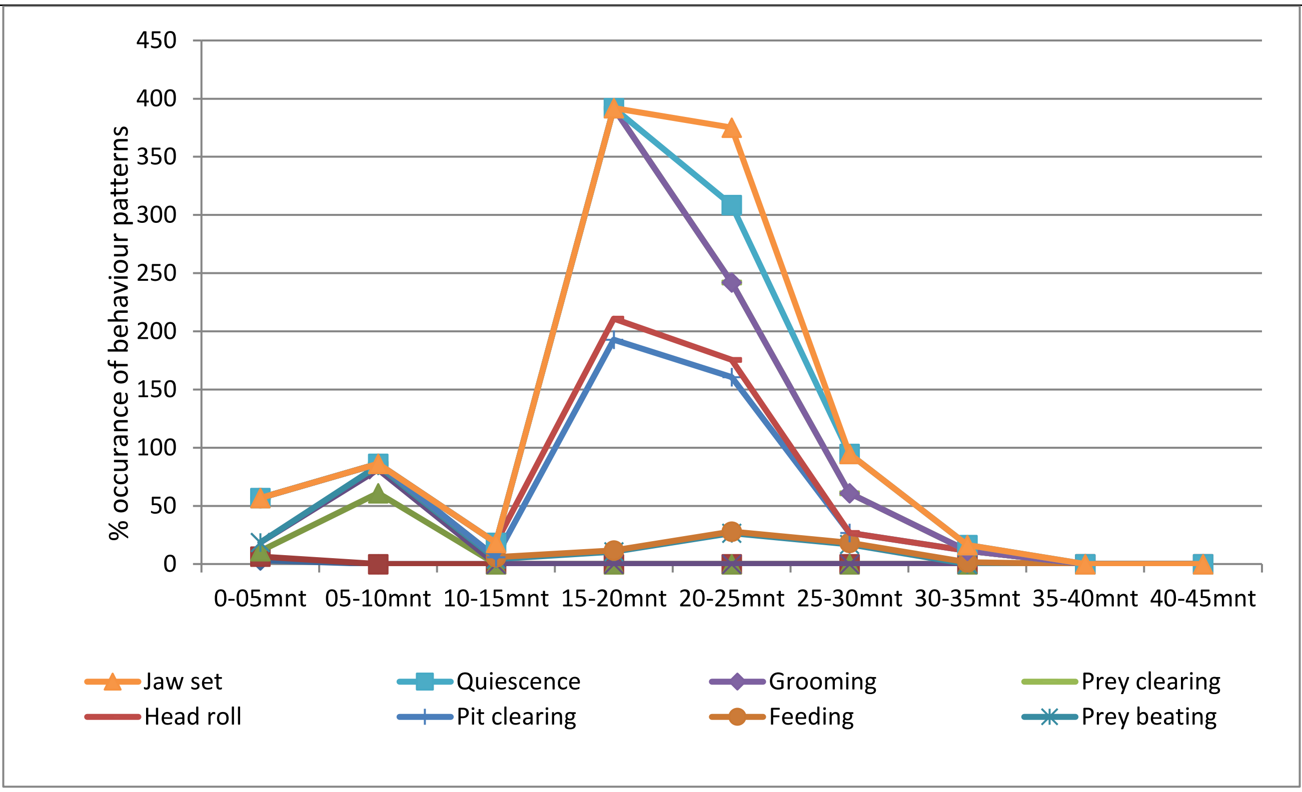


Fig 35. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* starved third instar larvae in each time intervals in sand medium

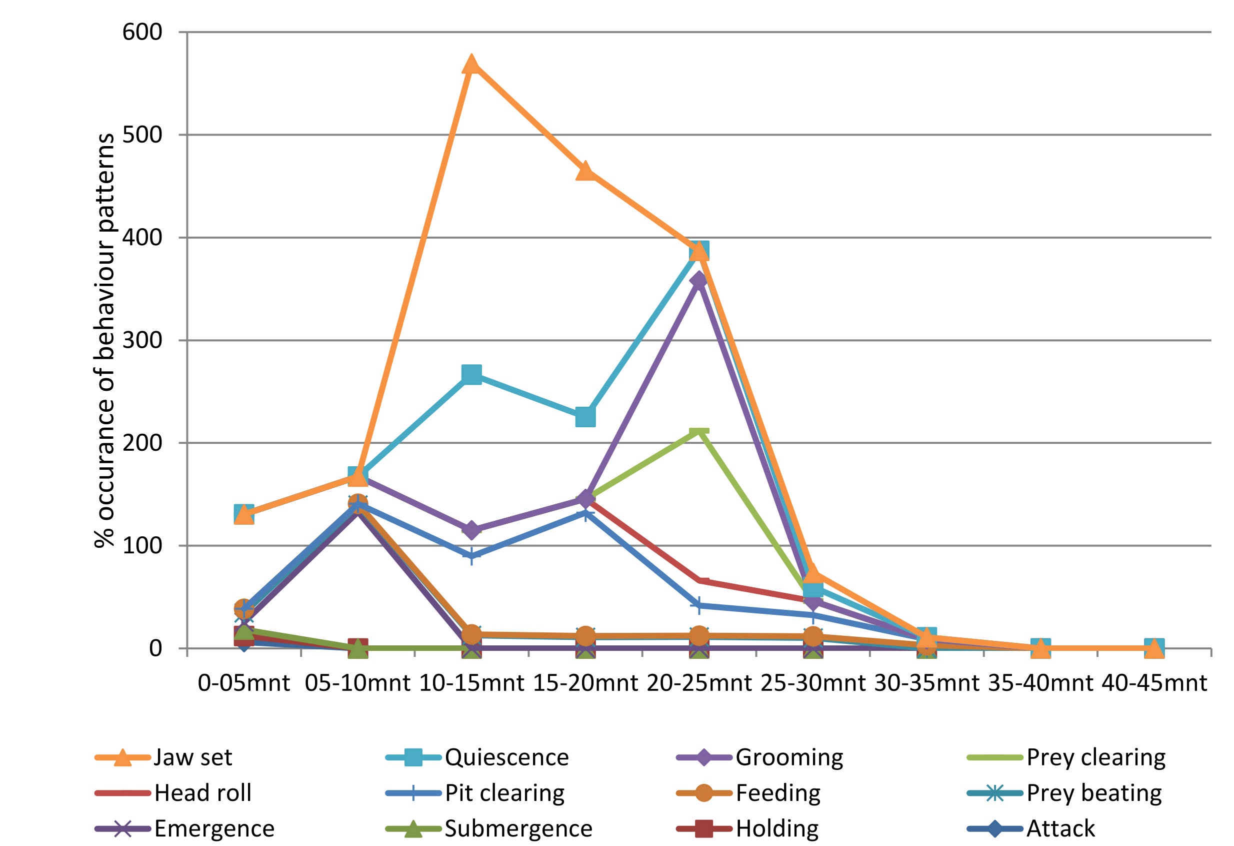


Fig 36. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed second instar larvae in each time intervals in soil medium

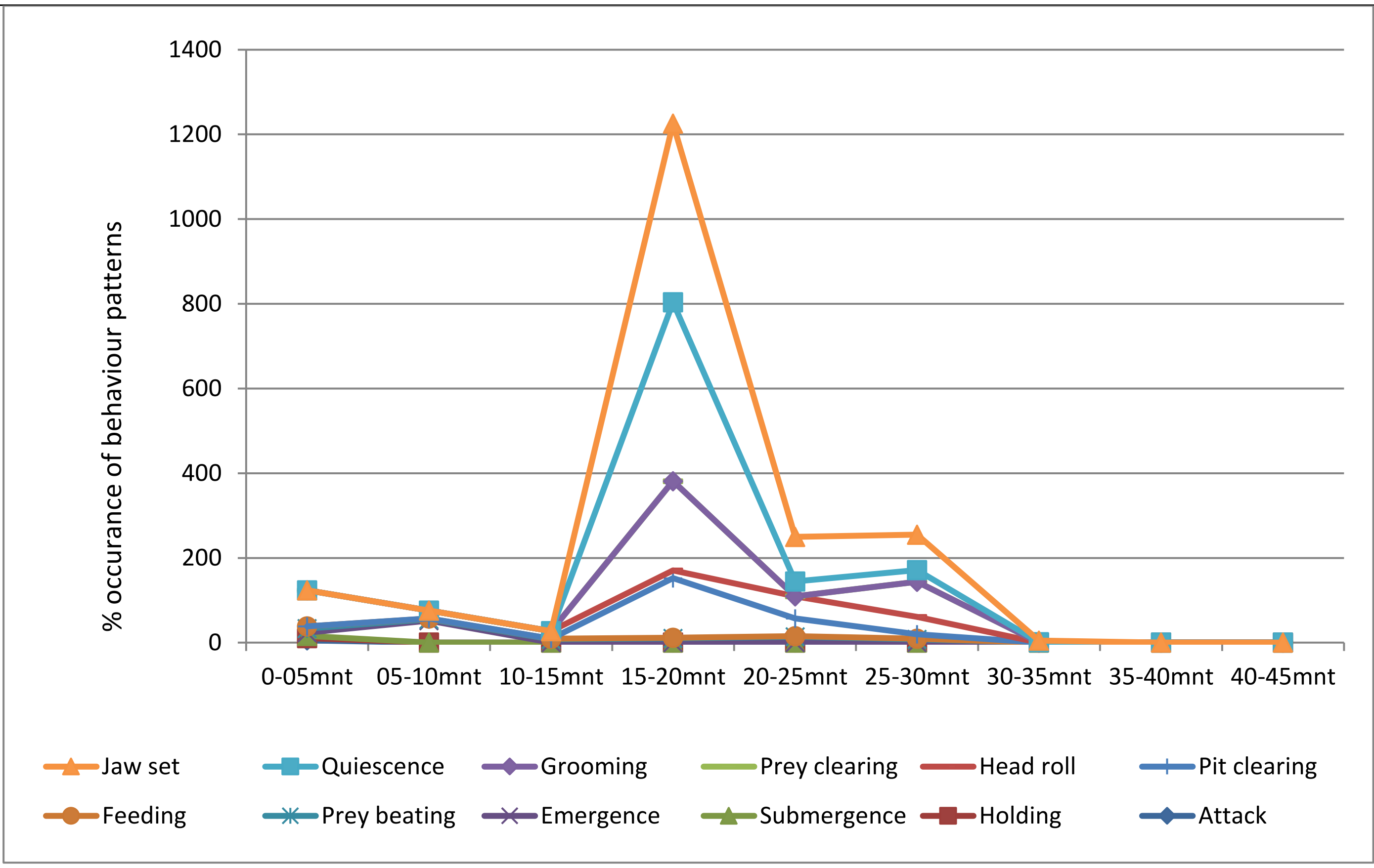


Fig 37. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* fed third instar larvae in each time intervals in soil medium

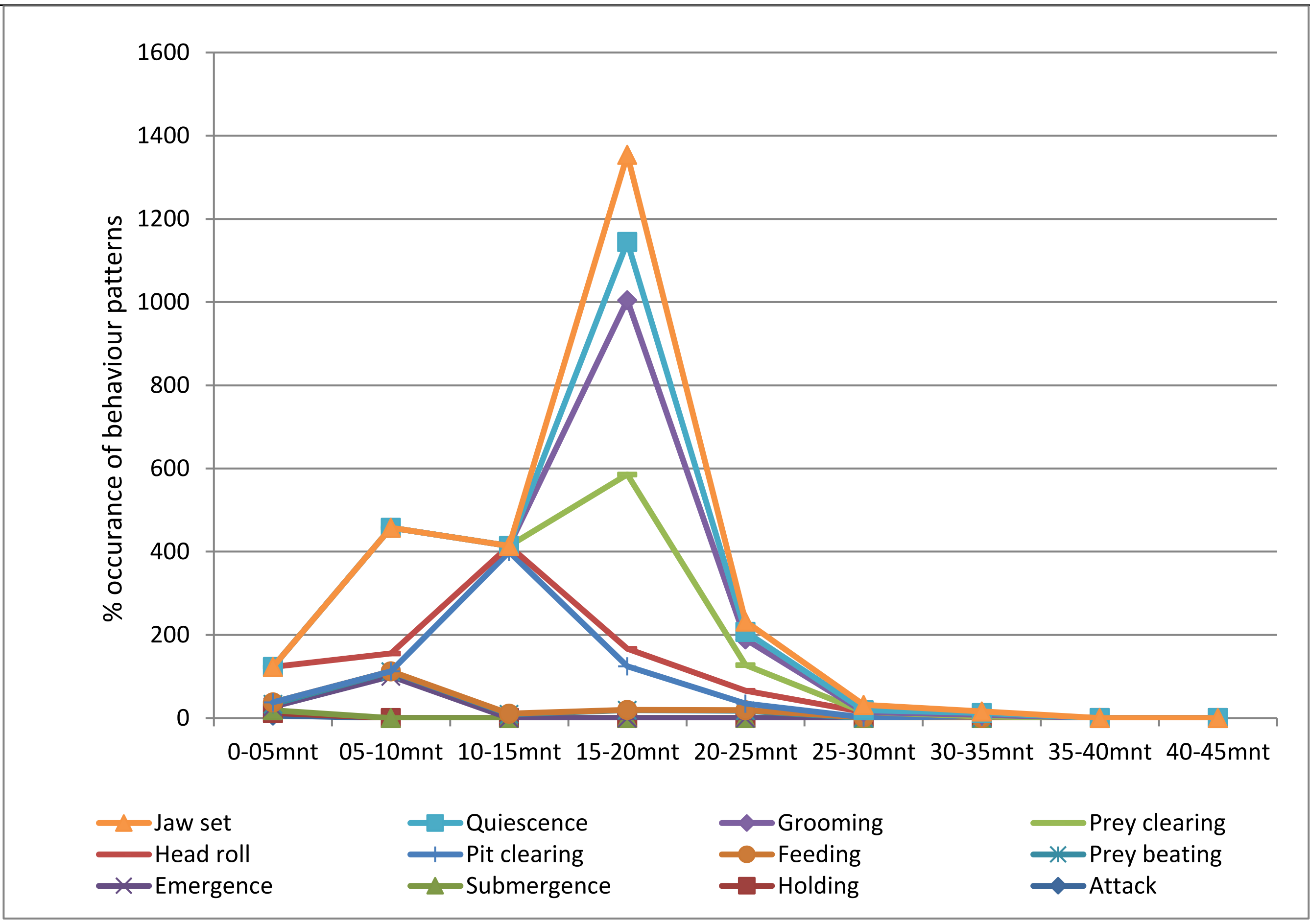


Fig 38. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* starved second instar larvae in each time intervals in soil medium

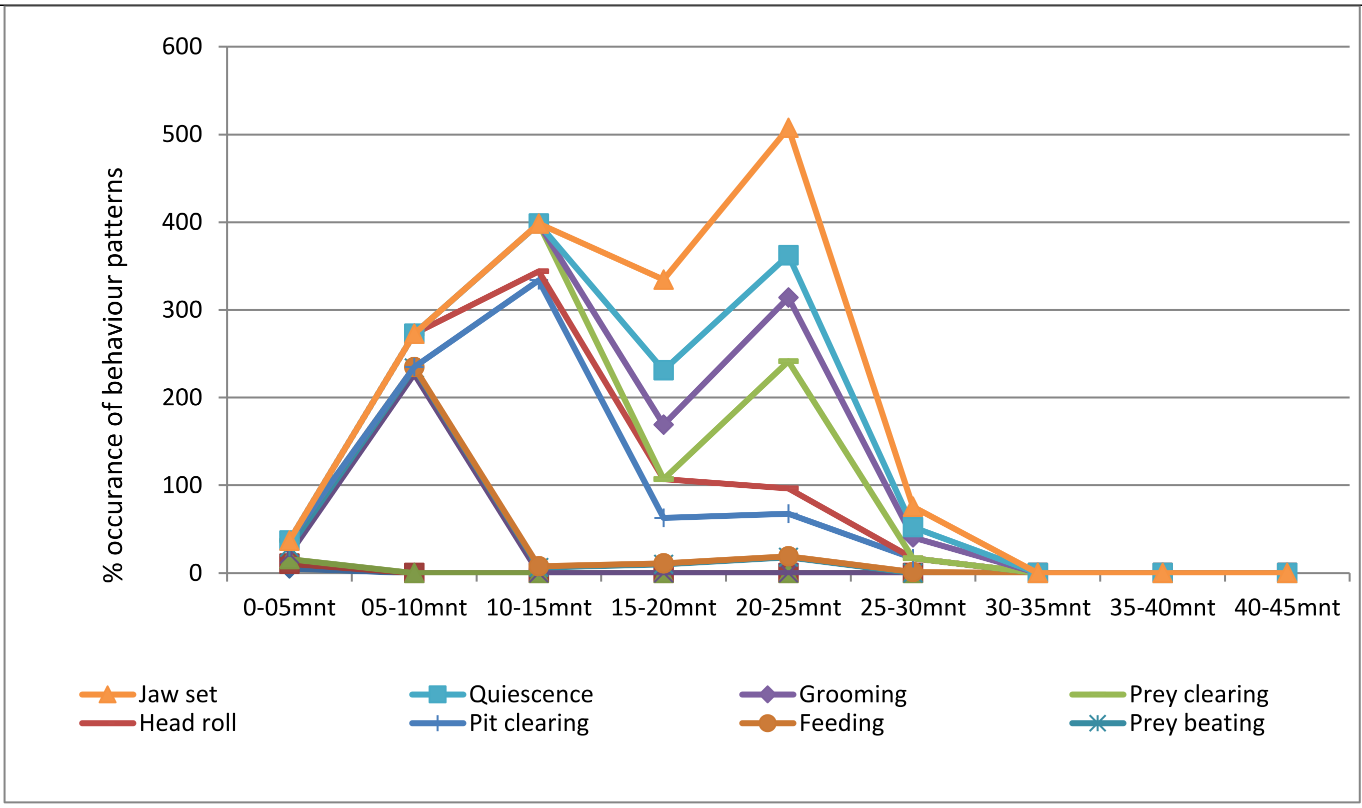


Fig 39. Feeding behaviour pattern of *Myrmeleon pseudohyalinus* starved third instar larvae in each time intervals in soil medium

available literature (Narendra and Kumar, 2006; Sebastian and Peter, 2012; Borror *et al*., 1975) and some specimens were identified by experts.

The prey species includes Order Hymenoptera, Coleoptera and Lepidoptera and very few numbers of spider and millipede. Given below the detailed prey species of antlion larvae (*Genus Myrmeleon*) collected from antlion larval pits.

The prey item was collected from Palakkad, Thrissur, Wayanad, Thiruvananthapuram, Pathanamthitta, Kannur, and Malappuram Districts of Kerala. The most preferred prey was Hymenopterans and it includes *Anoplolepis gracilipes* – worker, *Camponotus compressus*- worker, *Oecophylla smaragdina*- male, *Tetramorium smithi* and Genus *Crematogaster*. The small sized coleopterans like mupli beetle, cerambicids also collected from antlion pits. A spider species coming under Family: Gnaphosidae, *Scotopheu*s species was also collected.The members of the Family Gnaphosidae are commonly called mouse spiders and a total of ten species reported from India so far. The percentage occurrence of prey items were given in Fig. 40 to Fig. 46 and the photographs were given in Plate 22 and Plate 23.

During the study, the main predators in the laboratory rearing are spiders and lizards. One species of spider and one species of lizard were observed and the description is given below.

(1) *Heteropoda venatoria* (Family: Sparassidae)

H. venatoria (Plate 24) coming under family Sparassidae belongs to class Arachnida, are predators of the nature. The members of this family commonly called Giant crab spiders. They are the common house spiders and also seen in tree trunks in gardens. These are nocturnal spider and mainly a cockroach hunter. (Sebastian and Peter, 2012).

(2)Hemidactylus frenatus

These are lizard species coming under subfamily Gekkoninae with 607species and 63 genera (Plate 24). *Hemidactylus* is a large genus with well developed toe pads. Apart from houses, it is present in rocks, dry stone walls and trees (Maltison, 1992).

In the field condition some birds are also found to be eating antlion larvae.

3.4.5. Ethogram

The highest activity present in the predatory/feeding behaviour of the larvae was feeding followed by prey beating except the experiment with starved third instar larvae in sand medium. In this condition attack and holding behaviour patterns were observed more next to feeding pattern. In all the conditions the feeding pattern ranges from 47.8 to 77.8 % and that of prey beating 9.2 to 21 %. The detailed activity budgets of behaviours were given in Table 34.

Table 34. Feeding / predatory activity budget for each behaviour patterns in different conditions

Activity ↓ %→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	1.8	5	2.4	12.4	1.5	1.3	1.2	1.4
H	1.9	4.3	2.4	12.4	1.5	1.3	1.2	1.4
S	1.9	5.3	2.2	3.9	1.5	1.3	1.2	1.4
E	1.9	5	2.1	1.1	1.5	1.3	1.2	1.4
PB	16	21	10.4	9.2	11.6	15.2	9.4	12.1
F	70	47.8	71.4	54.1	73.9	71.9	77.8	71
PC	1	1.7	2.1	1.1	1.4	1.2	1.1	1.4
HR	1.8	4.8	2.2	3.7	4.7	4.4	4.1	6.3
PR	1.2	2.3	1.5	0.6	0.1	0.3	0.4	0.9
G	0.1	0	0	0	0.1	0	0.5	0.8
Q	1.4	2.4	2.2	1	1.6	1.3	1.2	1.4
JS	1	0.4	1.1	0.5	0.6	0.5	0.7	0.5
Total	100	100	100	100	100	100	100	100

1-Fed second instar larvae in sand medium, 2- Fed third instar larvae in sand medium, 3- Starved second instar larvae in sand medium, 4- Starved third instar larvae in sand medium, 5- Fed second instar larvae in soil medium, 6- Fed third instar larvae in soil medium, 7- Starved second instar larvae in soil medium, 8- Starved third instar larvae in soil medium.

The ethogram (feeding behaviour) of *M. pseudohyalinus* larvae were plotted as bar diagrams from Fig. 47 to Fig. 54.

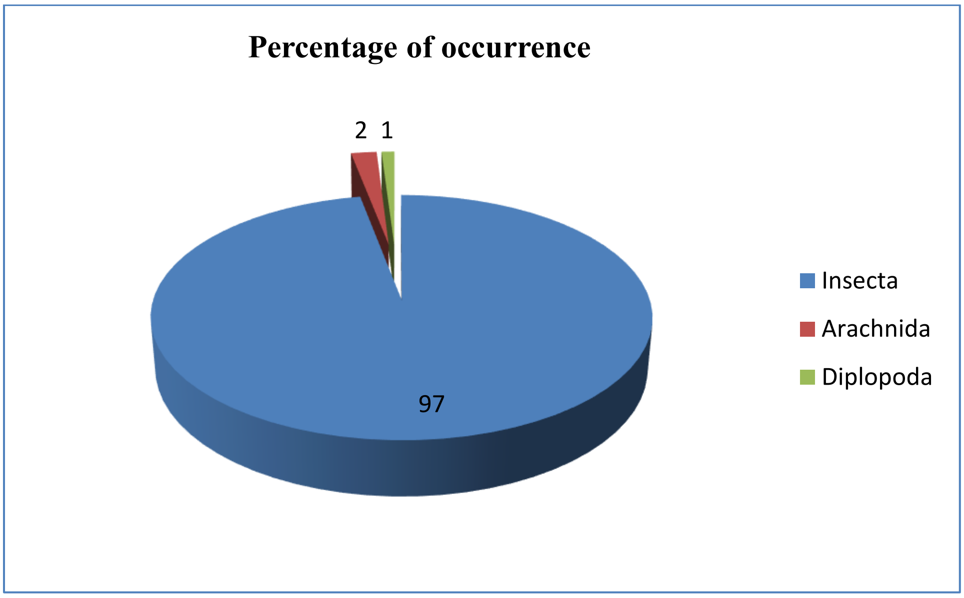


Fig. 40. Percentage occurrence of prey items collected from antlion larval pits (Genus *Myrmeleon)* in 2016

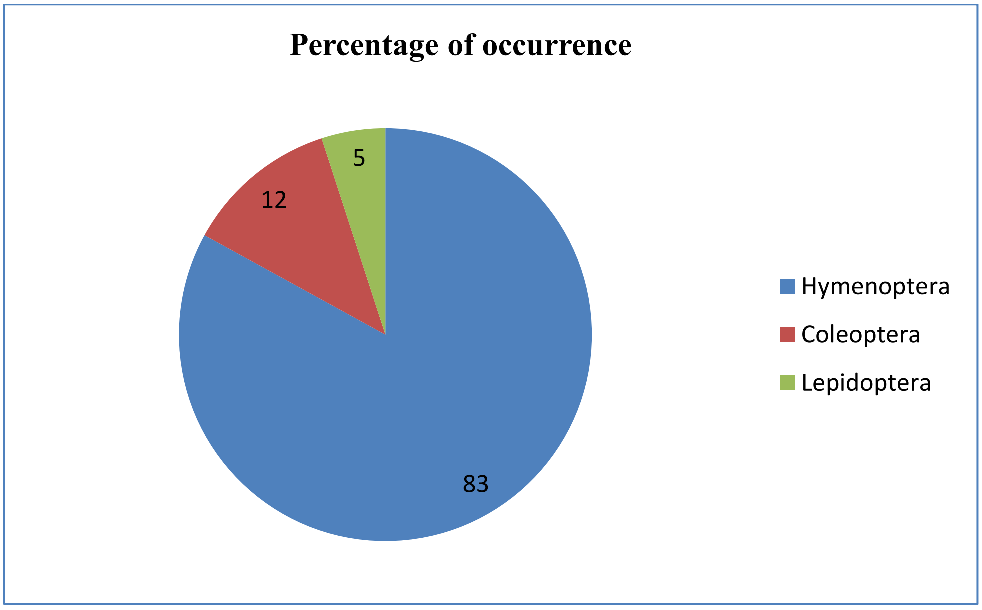


Fig. 41. Percentage occurrence of insect prey items collected from antlion larval pits (Genus *Myrmeleon)* in 2016

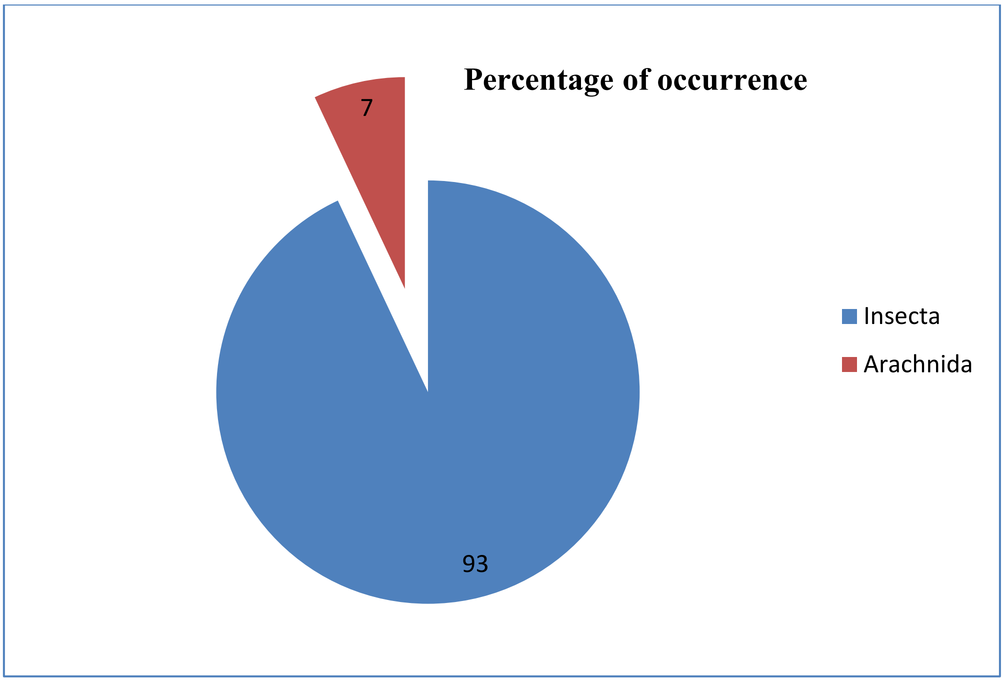


Fig. 42. Percentage occurrence of prey items collected from antlion larval pits (Genus *Myrmeleon)* in 2017

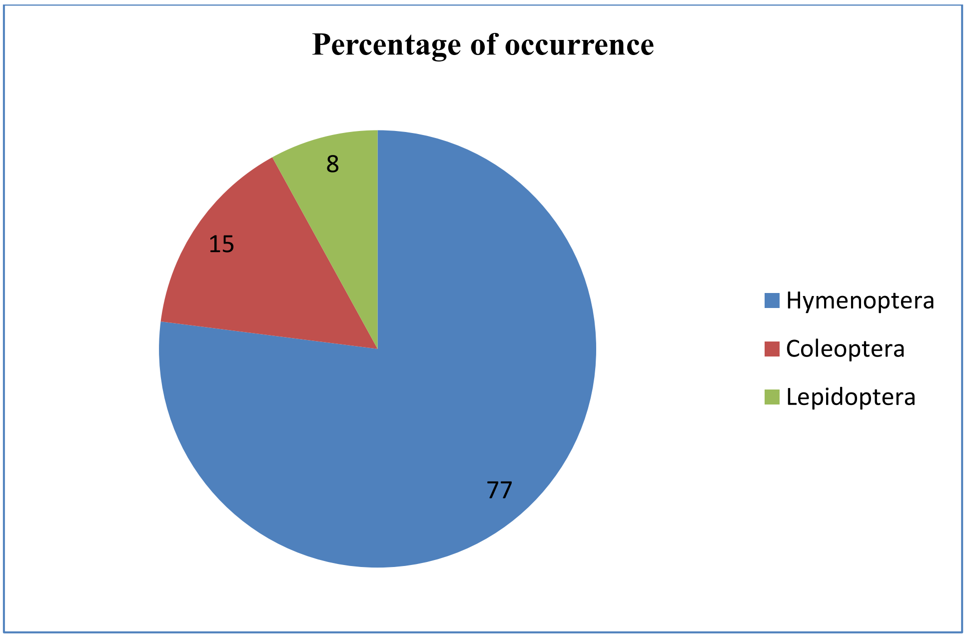


Fig. 43. Percentage occurrence of insect prey items collected from antlion larval pits (Genus *Myrmeleon)* in 2017

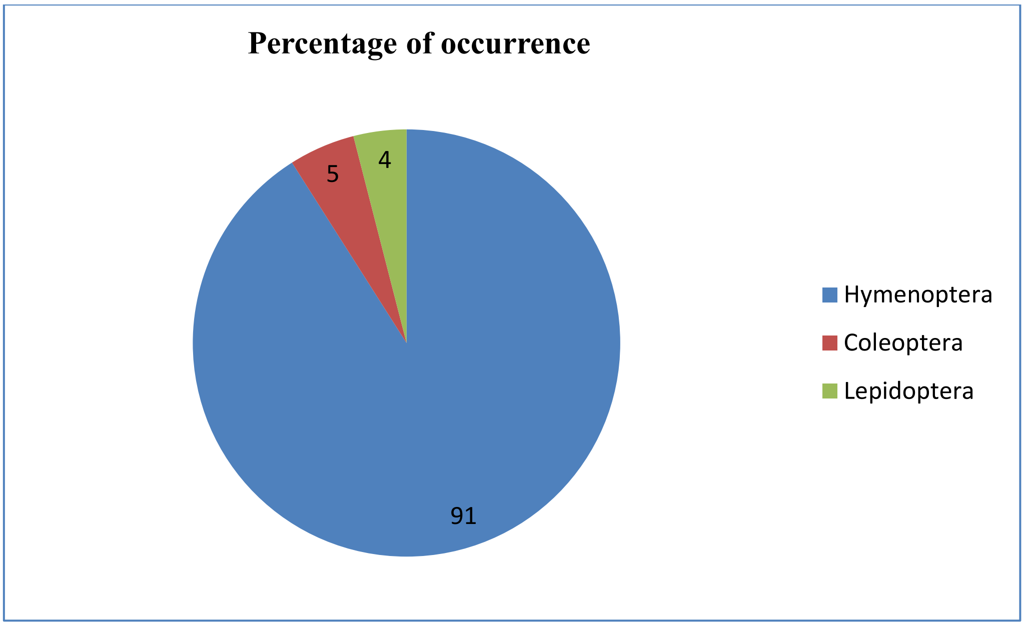


Fig. 44. Percentage occurrence of prey items collected from antlion larval pits (Genus *Myrmeleon)* in 2018

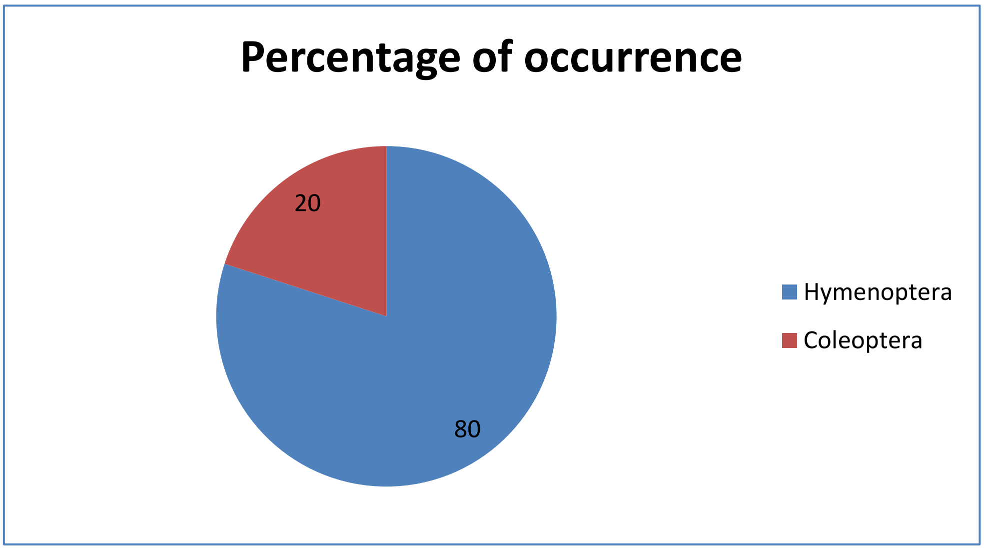


Fig. 45. Percentage occurrence of prey items collected from antlion larval pits (Genus *Myrmeleon)* in 2019

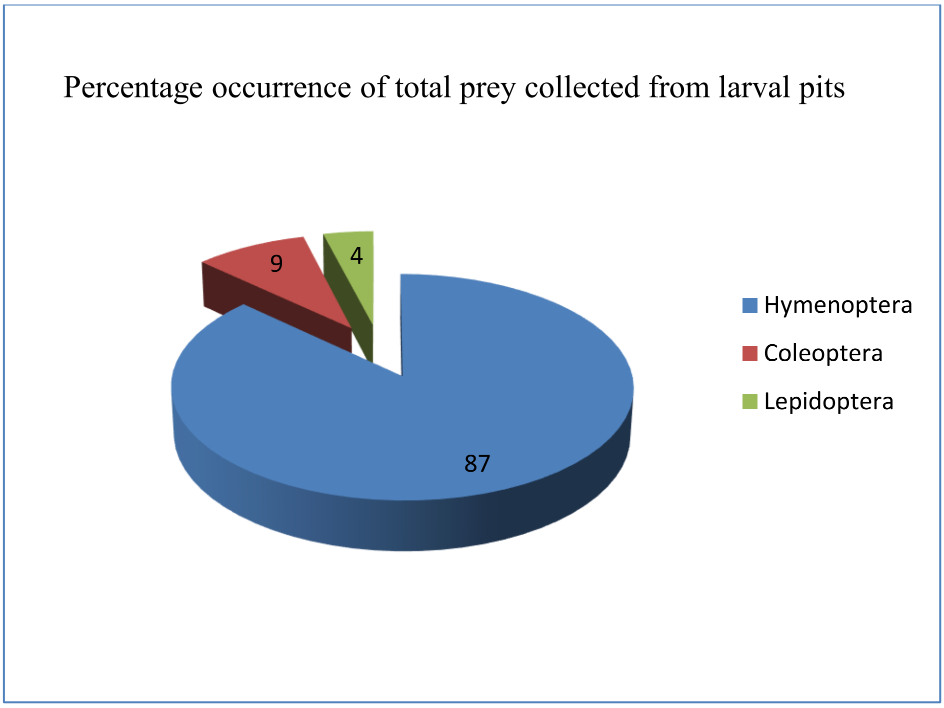


Fig 46. Consolidated data of prey preferred by antlion larvae Genus *Myrmelon*

Plate 22

Order Hymenoptera

Camponotus compressus- worker

Oecophylla smaragdina- male

Genus Crematogaster

Tetramorium smithi

Anoplolepis gracilipes

Plate 23

Order Coleoptera

Scotopheus Sp.

Diplopoda

Plate 24

Hetropoda venatoria

Hemidactylus frenatus

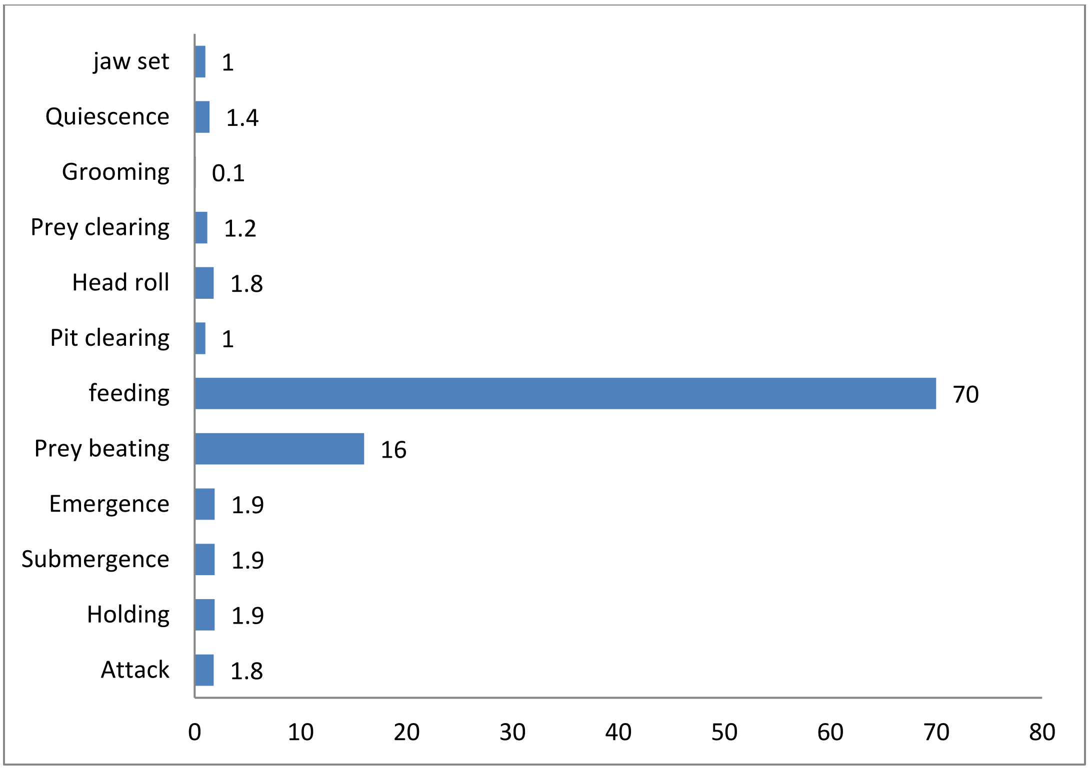


Fig. 47. Feeding / predatory activity budget for each behaviour patterns in sand medium, fed second instar larvae

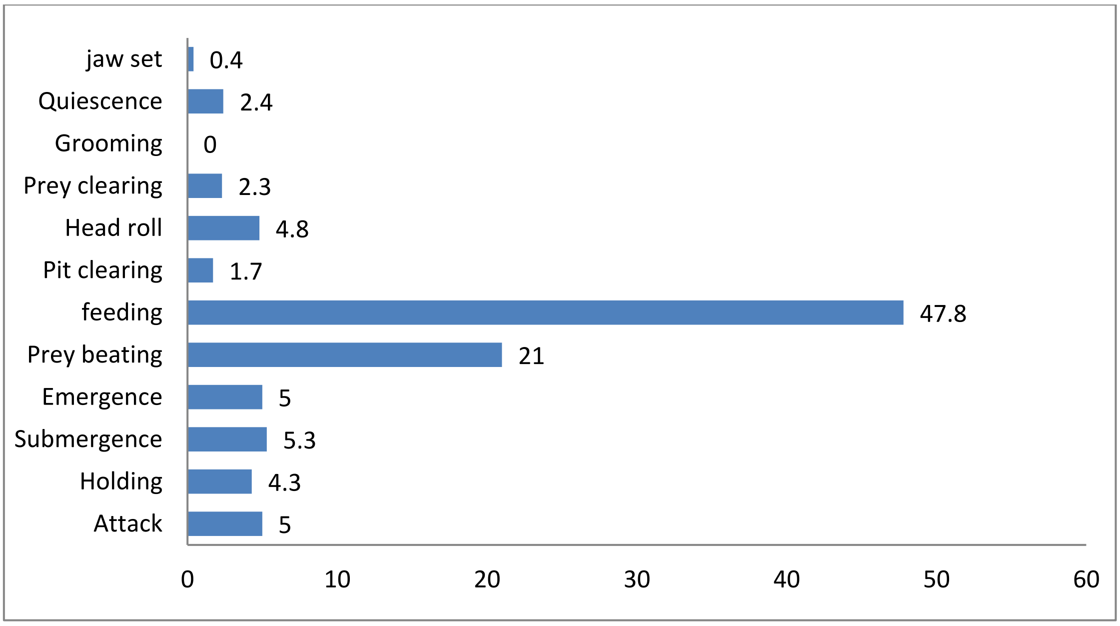


Fig. 48. Feeding / predatory activity budget for each behaviour patterns in sand medium, fed third instar larvae

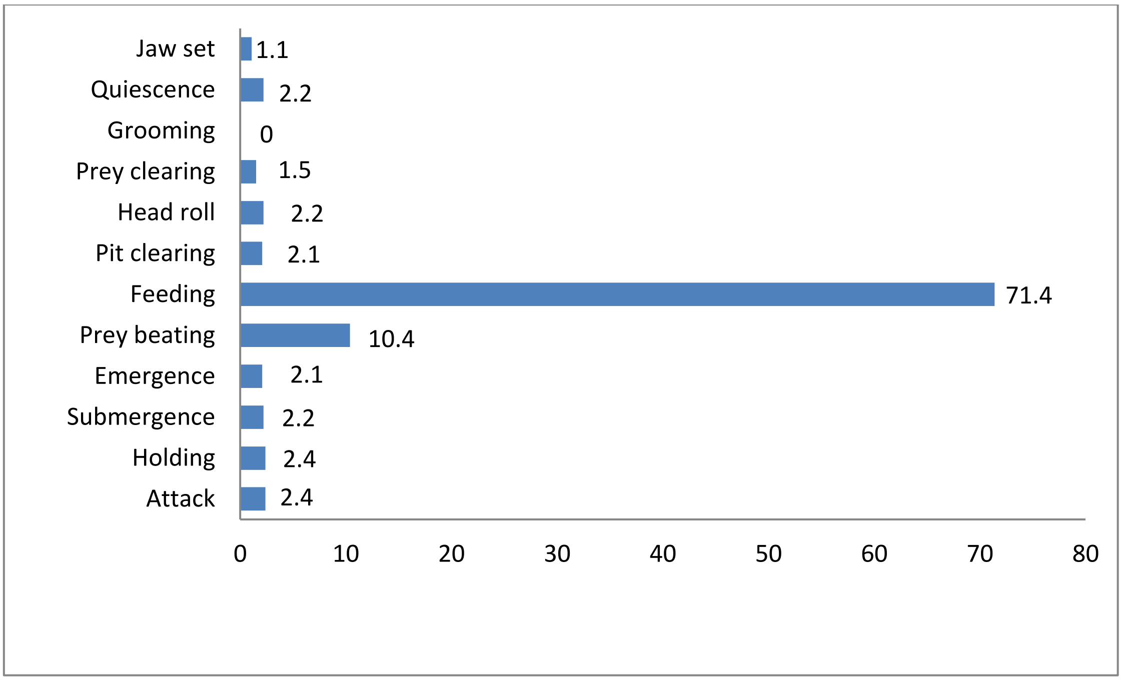


Fig. 49. Feeding / predatory activity budget for each behaviour patterns in sand medium, starved second instar larvae

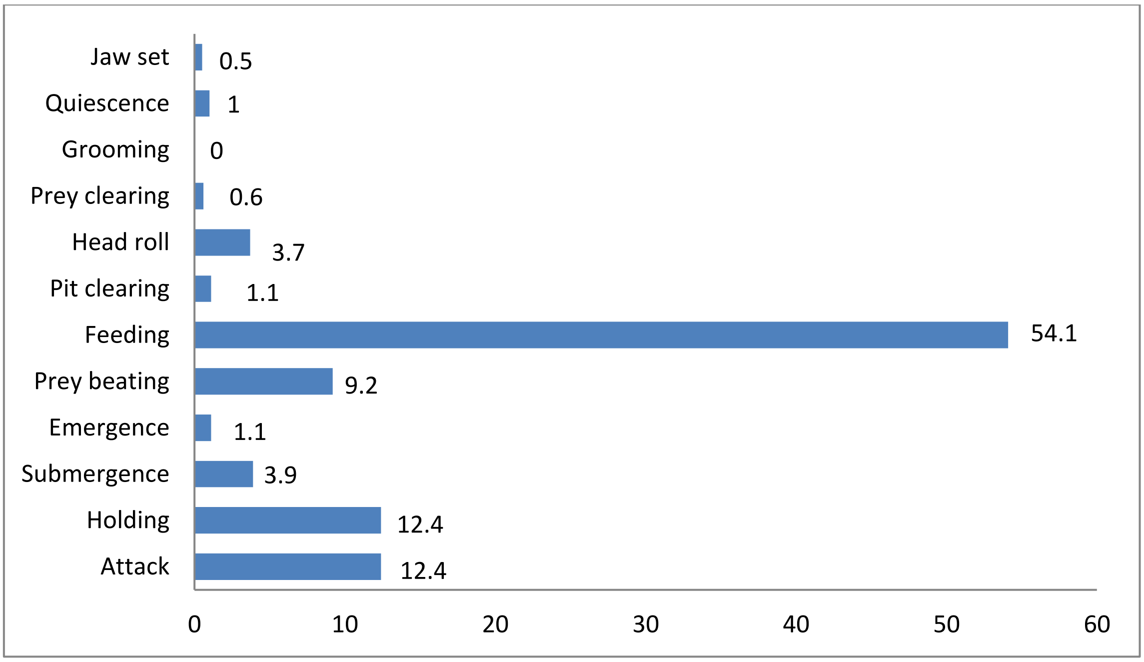


Fig. 50. Feeding / predatory activity budget for each behaviour patterns in sand medium, starved third instar larvae

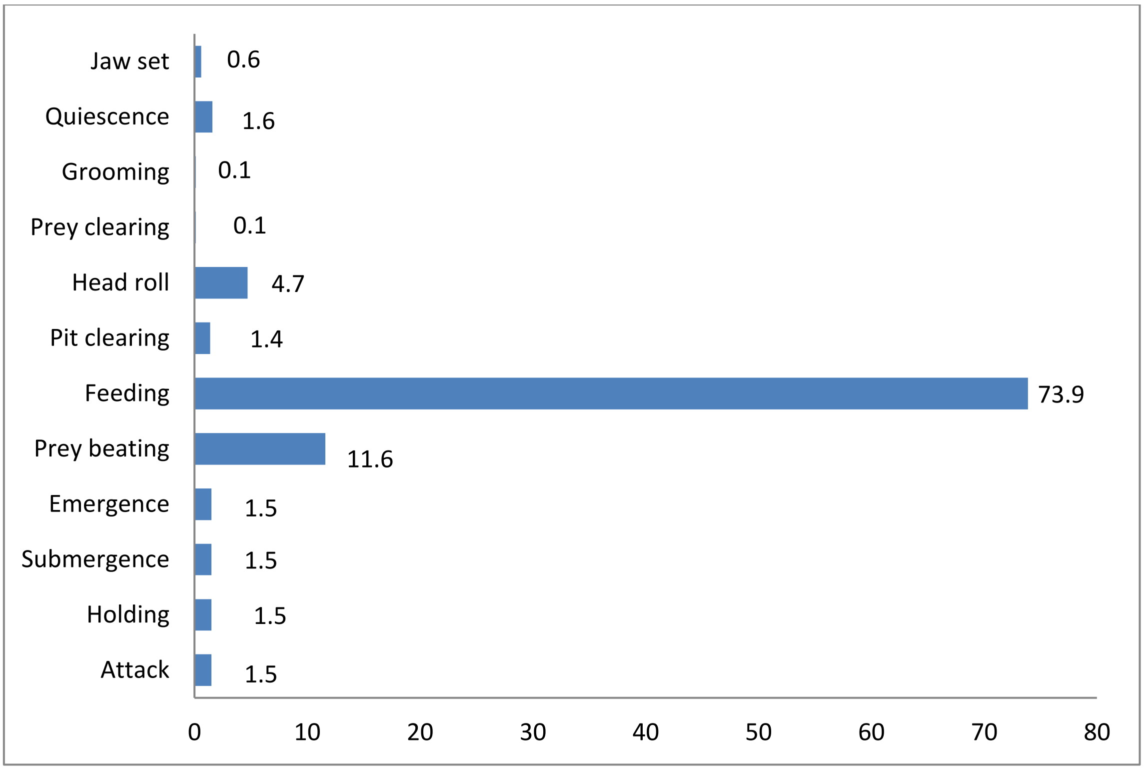


Fig. 51. Feeding / predatory activity budget for each behaviour patterns in soil medium, fed second instar larvae

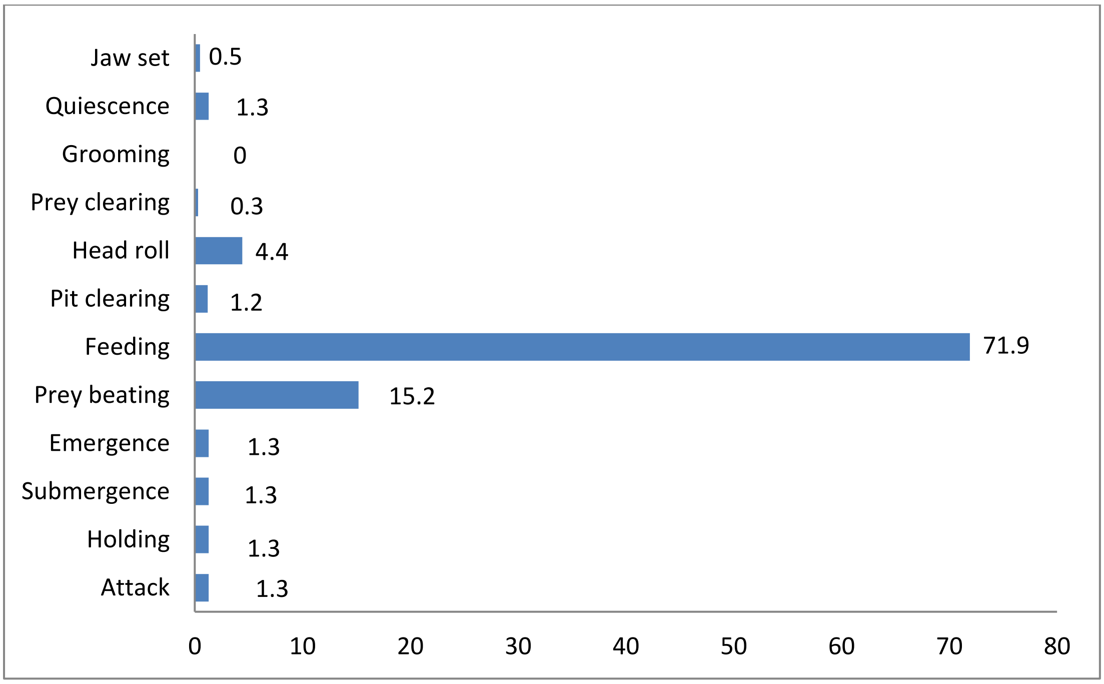


Fig. 52. Feeding / predatory activity budget for each behaviour patterns in soil medium, fed third instar larvae

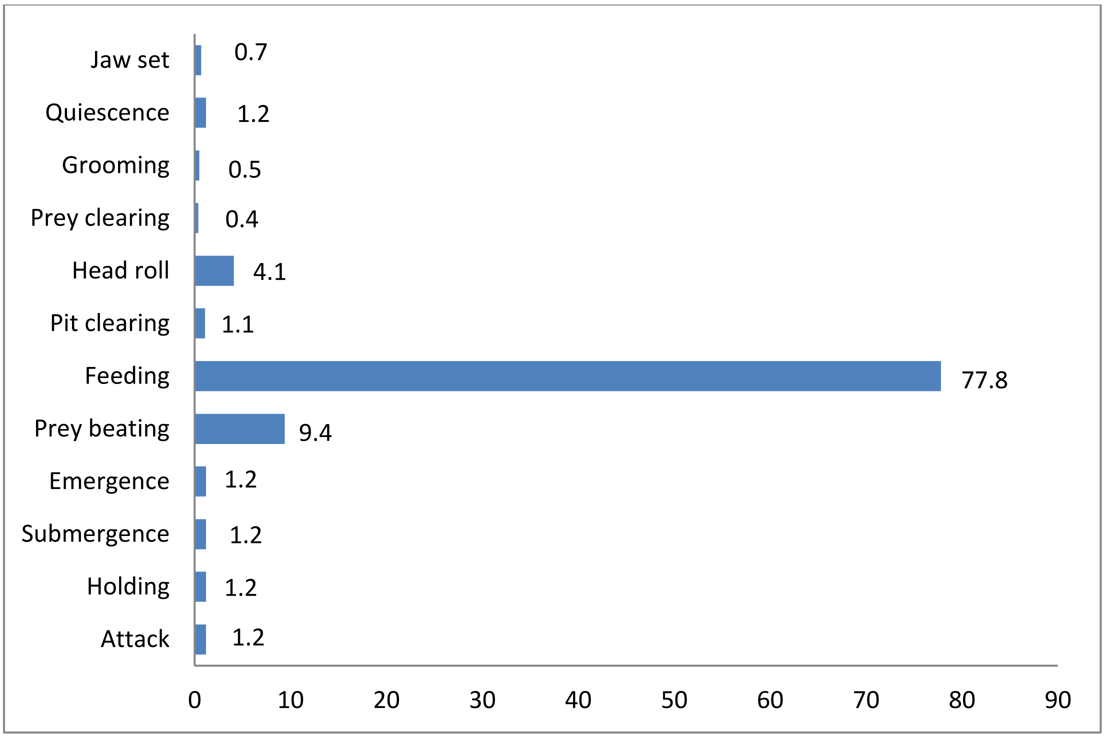


Fig. 53. Feeding / predatory activity budget for each behaviour patterns in soil medium, starved second instar larvae

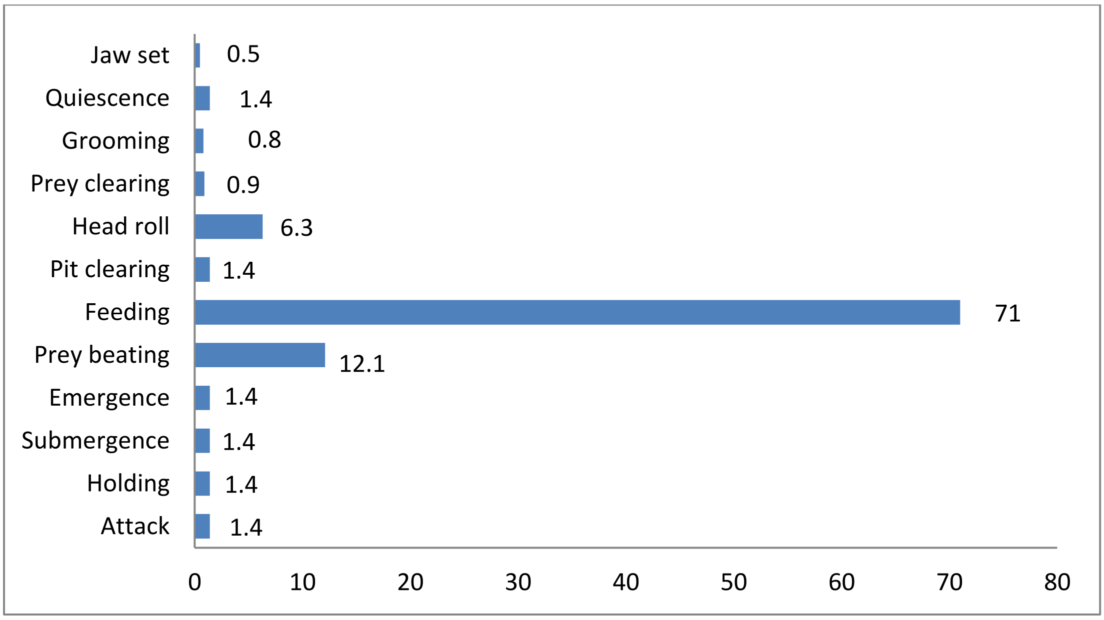


Fig. 54. Feeding / predatory activity budget for each behaviour patterns in soil medium, starved third instar larvae

3.4. DISCUSSION

Pit building antlion larvae made its conical pit by performing a series of concentric backward movements followed by flipping of sand or soil. The depth and diameter of pit increases with the spiral movement up to the mark when maximum prey capture success is reached. Pit building is seen in different species of antlions which are coming under family Myrmeleontidae. Here, *M. pseudohyalinus* larvae made its steep conical shaped pits for predation, it is very similar to the pit building of *M. hyalinus* which also make simple inverted cones (Pit diameter 30±0.5mm, min 25mm, max 34mm), but in *Cueta lineosa* they make two inverted truncated cones inserted on one another (Devetak *et al.*, 2020).

The pit building behaviour of *Myrmeleon pseudohyalinus* larvae in different media (sand and soil), hunger level (fed and starved) and instars (second and third) were studied. The diameter and depth are the two characteristics of funnel shaped pits. The increase in the diameter and depth helps the larvae to build its conical shaped pit. The depth and diameter remain similar up to 9^{th} hour of pit building and a sudden progress occurs in the next 3 hours. In second instar larvae, the fed larvae in soil medium has the highest pit size and fed larvae in sand medium has the lowest pit size in pit building experiments. But in the case of third instar larvae, the starved larvae in sand medium has the highest pit size and the lowest pit diameter observed in starved larvae in soil medium and that of depth in starved larvae in sand medium and fed larvae in soil medium.

The pit building behaviour shows similar in both laboratory and field conditions (Liang, 2010), so that the pit building antlion species can easily be studied under captivity. There are many factors which influence the pit building of antlion larvae, and it was compared in previous studies by using different species of *Myrmeleon*, *Cueta* and *Euroleon*. From the field observation data, it is reported that the maximum pit diameter of *M. formicarius* was 4 cm in non forested areas and 2.5cm in forest areas (Bozdogan *et al.*, 2013). But from the laboratory study, the maximum diameter is 3.65 cm in *M. pseudohyalinus* larvae.

The pit characters were studied in four different types of substrates which were collected from natural habitat of pit building antlion larvae. Dry soil, clay soil, sand and a mixture of sand, soil and cement collected from partially built house. The mean pit depth and diameter observed was high in sand medium (depth-2.85 cm and diameter- 3.80 cm). The mixture of sand, soil and cement has the second highest pit size with a depth of 2.41 cm and diameter 3.1 cm. Larvae made it pits in dry soil and clay soil with moderate size.

Antlion larvae build its pit for predation and shelter and the predatory efficiency was described by examining the predatory behaviour patterns. From this experiments, the prey escape and capture success were calculated and found 67-100% capture success of prey irrespective of different conditions, hunger level and instars. Prey escape was noticed in fed second instar larvae in sand medium, fed third instar larvae in sand medium, starved third instar larvae in sand medium and starved third instar larvae in soil medium (Table 31). From the result, it was understood that the prey escape was highest in sand medium and the highest capture success was observed in soil medium.

All the pit building antlion larvae has a similar pattern of feeding the prey and the behaviour patterns were influenced by the softness or hardness of the body of prey. The predatory behavior was analysed by Napotilano (1998) using termite (*Reticulitermes flavipes*), ant (*Prenolepis impairs*) and beetle (*Alphitobius diaperinus*) and all preys followed a core pattern of behaviours. The only difference was occurred in prey beating behaviour, 90% of beetles, 20% of ants and 10% of termite trials were shows this behaviour and it may be an adaptation to enhance the penetration of mandibles in beetles . The present study shows, *Myrmeleon pseudohyalinus* larvae took 35 to 45 minuts to feed its prey in laboratory conditions. The capture success noted from the study was 50-100% and the second instar larvae have the more capture success (95%) than third instar larvae (75%). But the previous study of Nonato and Lima (2011) differ from the present study in that, they noted that the third instar (96.96%) larvae are more successful than second instar larvae (69.70%).

The intraspecific interaction or cannibalism in *M. pseudohyalinus* larvae in different hunger levels were studied by conducting six experiments. The experiments were Well fed vs Well fed, Well fed vs Fed, Well fed vs Starved, Fed vs Starved, Fed vs Fed and Starved vs Starved (in second and third instar larvae). In the case of second instar larvae, the highest mortality was observed in the experiment Fed vs Starved (90% of fed larvae were dead), the starved larvae (higher hunger level) cannibalized the fed larvae. 70% of the mortality was observed in the experiments Well fed vs Fed and Well fed vs Starved. In the case of third instar larvae, 80% of mortality was observed in experiment with Fed vs Fed followed by Well fed vs Fed and Fed vs Starved.

The factors influencing cannibalism include developmental stage, sand depth, and conspecific density and hunger level. The cannibalism in antlion species studied by Lima (2016) and the food availability influences the antlion larvae *Myrmeleon brasiliensis* to do cannibalistic behavior, the lesser the food availability, the higher the cannibalism. The evaluation of both food availability and density were analysed and came to the conclusion that absence of food causes cannibalism in both high and low density of antlion larvae. Also records were present that the cannibalism occurs only at densities greater than 5 antlion larvae per 100 cm^2 in *Myrmeleon acer* larvae (Day and Zalucki, 2000). Cannibalism frequency was higher in the case of both individuals was in starved condition. 38 % of cannibalism occurred in *M. hyalinus* larvae (Barkae *et al*., 2014). But the present study disagree with the result, here a 50% of mortality was noticed both larvae in starved condition. Cannibalistic behaviour of *Myrmeleon brasiliensis* larve were studied by Lima (2016) in four treatments, low density/without food, low density with food, high density without food, high density with food. *Drosophila melanogaster* was used as prey and high density without food treatment found to be higher cannibalistic behaviour than other three treatments.

As a part of interspecific relationship study, analysed the prey items and observed the prey of antlion larvae which includes 87% of Hymenoptera, 9% of Coleoptera, and 4% of Lepidopterans. Mainly ants are the most preferred prey of antlion larvae according to this study in natural condition. There are many studies which quantified the prey of antlion larvae in different countries and this result shows the predatory capacity of antlion larvae in an ecosystem. Also the ecosystem services were found as a predator which influences the foodweb and controlling lots of small insect populations. The detailed prey items which is controlled by pit building antlion species helps to understand how diverse the predatory behaviour. The prey eaten by non pit building antlion species Brachynemurus includes worker ants, alate male ants, pygmy mole cricket, and beetles (Staphylinidae and Elateridae) and also there is a correlation between prey weight and feeding time (Cain, 1987). Out of 228 prey items 79 are ants , 36 spiders, 32 beetles, 27 midges, 21 red mites, 19 small wasps, 2 caterpillars, leaf hoppers, millipedes and hemipteran bugs were observed and 6 other miscellaneous winged insects (Heinrich & Heinrich, 1984). The prey experienced by

Szentkiralyi and Kazinczy (2002) from antlion larval pits are Formicoidea, Hymenoptera, Collembola, Coleoptera, Diptera, Homoptera, Heteroptea, Araneidea, Aphidina, Acariformes and Staphylinidae . Ngamo *et al*., (2015) observed the prey of antlion larvae includes Hymenoptera (Family- Formicidae and Pompilidae), Diptera (Drosophilidae, muscidae), Orthoptera (Gryllidae), Isoptera (Termitidae), Coleoptera (Carabidae), and Araneae (Araneidae). Ant species *Myrmicaria opaciventris* (Family Formicidae) comprises 40% of the total prey trapped. *Myrmeleon quinqemaculatus* prey consists of arachnids, crustaceans, insects and myriapods, 85.97 % include hymenopterans (Djibo *et al*., 2020).

Ants are the common prey of pit building antlion larvae and the minimum distance of antlion pit and ant nest were noted as 9 cm and maximum distance was 44 cm. Also shows that a minimum distance of 27 meter from the pit building antlion larvae and water body. There are not many studies on the predators of antlion larvae in the world. In Israel, hyper desrt area, antlion larvae predated by 4 species of scorpions (*Orthochirus scrobiculosus*, *Buthus Israelis, Buthacus yotvatensis, Buthacus* sp) (Segev *et al.*, 2019). Here the predators in the laboratory rearing are *Heteropoda venatoria* and *Hemidactylus frenatus*.

The study has given some insights on the ecology and behavior of antlion fauna in India with a new report. Provided data about habitat and its abiotic characters, morphometry of larvae, cocoon and adult, physical and chemical nature of soil, seasonal adaptation and habitat choice of antlion larvae in detail as a part of ecology. Observations in the study area are explained in the ecology part and it was validated by doing some experiments in the laboratory as a part of behavior study. The prey, predator, pit building behavior, feeding behavior and cannibalism were explained in the behaviour part and this is the first study of antlions in this region. Hopefully further study can take off from the data given in thi study. It may consider as a comprehensive study of pit building antlion especially *M. pseudohyalinus* species in this region.

SUMMARY

SUMMARY

Antlions are classified under Order Neuroptera and Family Myrmeleontidae. They are holometabolous insects having a long larval stage and the larval antlions build a conical pit for predation and shelter. Myrmeleontid family includes both pit -builders and non-pit builders and its behavior is somewhat exceptional and interesting like bee hive making of honey bee and web of spider etc. The study mainly emphasis on four objectives; to study the different habitat of antlion larvae, to analyze the physical and chemical structure of soil, to investigate the antlion larval behavior patterns, to examine the intraspecific and interspecific interactions of antlion larvae.

The habitat of pit building antlion larvae were studied by observing the natural habitat of pit building larvae. The pit building medium (soil and sand) were studied both texture and chemical components and the above aspects were studied under ecology of antlion larvae. Whereas the larval behavior patterns such as pit building, trailing, feeding and the type of interactions (Intra-specific and inter-specific) were studied under larvar behavior study.

From the study, the species *Myrmeleon pseudohyalinus* was observed as the dominant pit building antlion species present in kerala. So that, the ecology and behavior of this species was studied and the comparison was made with the studies of other countries due to the lack of research in this area in India.

A total of 68 study sites were visited which includes 14 districts of Kerala and the antlions were collected from 50 areas which include 12 districts of kerala. *Myrmeleon pseudohyalinus* was the species mostly found in Kerala, and it is a first report of the species from India and the sequence was deposited in NCBI with an accession number MN711710. The four habitats of pit building antlion larvae genus *Myrmeleon* include abandoned areas, human dwelling areas, forest areas and riparian. The distribution of genus *Myrmeleon* in each districts was plotted and the morphometry of larvae, cocoon and adult were studied.

As a part of ecology study, the abiotic characters of the habitat were noted, such as temperature, humidity, pressure, uv index, visibility, wind and dewpoint. The texture of soil in which the larvae used to make its pit was analysed and the

larvae prefer sand, it may be because of the low water holding capacity of sand which will dry easily when wet. Also the chemical nature of soil was evaluated in which the increase in the amount of Calcium and Potassium causes the decrease in number of antlion larvae. Also the adaptability in rainy seasons were studied by giving imitated rainy conditions, the larvae rebuild its pits in 23, 32 and 57 days in two spray, four spray and six spray conditions respectively and the temperature of soil was preferred as 28-32ºC.The pit depth and diameter of antlion larvae were same in both natural and laboratory condition.

As a part of behavior study of antlion larvae illustrated the mechanism of pit building, feeding and interaction between species and within species. These behaviors were studied by direct observation in both natural and laboratory conditions and various experiments were done according to the previous works of experts. The *Myrmeleon pseudohyalinus* larvae took almost twelve hour to complete its pit for predation in which the depth and diameter increase was somewhat static upto nine hours. A positive correlation of larval body width and pit diameter and a negative relation of head length and pit depth were noted. The feeding behavior patterns and condition, instar and medium were statistically analyzed. The instar of larvae influences the prey beating, emergence and submergence activities. The behavior patterns in first five minutes and last five minutes were very similar in each feeding experiments.

The types of interactions were studied in which cannibalism was studied under intraspecific interaction and prey and natural enemy were identified and included under interspecific interactions. Cannibalism is influenced by hunger level of larvae, hence this behavior was studied and compared between different hunger levels. The prey items of antlion larvae genus *Myrmeleon* which were collected from antlion larval pits include Order Hymenoptera, Order coleopteran and one species of spider (*Scotopheus sp*) and a Diplopoda from natural habitat. The natural enemies of Genus *Myrmeleon* larvae include *Heteropoda venatoria* (Spider) and *Hemidactylus frenatus* (Lizard).

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APPENDIX

Appendix 1- Correlation matrix of Genus *Myrmeleon* (Larvae and cocoon)

	LBL	LBW	LHL	LHW	ML	CD	CW
LBL		0.80473	0.60146	0.34574	-0.17687	0.12778	-0.11653
LBW	0.80473		0.69062	0.56905	0.15387	0.11844	0.051048
LHL	0.60146	0.69062		0.17983	0.005667	0.24225	0.020434
LHW	0.34574	0.56905	0.17983		0.4716	0.059899	0.042294
ML	-0.17687	0.15387	0.005667	0.4716		0.25671	0.081101
CD	0.12778	0.11844	0.24225	0.059899	0.25671		-0.13696
CW	-0.11653	0.051048	0.020434	0.042294	0.081101	-0.13696	

Appendix 2-. Correlation matrix of larval morphometry of *M Pseudohyalinus*

	Body length	Body width	Head length	Head width	Mandible
Body length		0.91568	0.73665	0.71957	0.72723
Body width	0.91568		0.8006	0.76198	0.69873
Head length	0.73665	0.8006		0.64598	0.72421
Head width	0.71957	0.76198	0.64598		0.66934
Mandible	0.72723	0.69873	0.72421	0.66934	

Appendix 3- Correlation matrix of larval morphometry of *M. hyalinus*

	Body length	Body width	Head length	Head width	Mandible
Body length		0.84912	0.76261	0.7287	0.62649
Body width	0.84912		0.66228	0.70823	0.66881
Head length	0.76261	0.66228		0.78247	0.65186
Head width	0.7287	0.70823	0.78247		0.812
Mandible	0.62649	0.66881	0.65186	0.812	

Appendix 4- One Way Anova Comparing means among species and the genus-LBL

Test for equal means					
	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	0.0175	2	0.00875	0.1739	0.8407
Within groups:	3.47125	69	0.050308	Permutation p (n=99999)	
Total:	3.48875	71	0.8478		

Appendix 5- One Way Anova Comparing means among species and the genus-LBW

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	0.071944	2	0.035972	3.006	0.05604
Within groups:	0.825833	69	0.011969	Permutation p (n=99999)	
Total:	0.897778	71	0.05772		

Appendix 6- One Way Anova Comparing means among species and the genus-**LHL**

Test for equal means					
	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	0.035833	2	0.017917	3.645	0.03127
Within groups:	0.339167	69	0.004915	Permutation p(n=99999)	
Total:	0.375	71	0.02611		

Appendix 7- One Way Anova Comparing means among species and the genus-LHW

Test for equal means					
	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	0.067778	2	0.033889	17.7	6.23E-07
Within groups:	0.132083	69	0.001914	Permutation p (n=99999)	
Total:	0.199861	71	1.00E-05		

Appendix 8- One Way Anova Comparing means among species and the genus-ML

Test for equal means					
	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	0.0325	2	0.01625	6.694	0.002203
Within groups:	0.1675	69	0.002428	Permutation p(n=99999)	
Total:	0.2	71	0.00269		

Appendix 9- Soil texture and components in different soil samples collected from different habitats of Kerala

Sl No	District		Study area	Sand (%)	Silt (%)	Clay (%)	Texture class
1	PKD	AA	Parli	91	6	3	Fine sand
2	PKD		Edathara	87.8	0	12.2	sand
3	PKD		Pezhumpara	81.8	4	14.2	sand
4	TCR		Murukkumpara	97	2	1	Fine sand
5	PKD	HDA	Parli Manamthody	85.8	2	12.2	sand
6	TCR		Wadakkancherry	92	5	3	Fine sand
7	TCR		Nedupuzha	97	2	1	Fine sand
8	TCR		Kodungallur	85.8	2	12.2	sand
9	TVRM		Vellayani	87.8	0	12.2	sand
10	KNNR		Thalassery	77.8	4	18.2	sand
11	WYND		Kattikulam	71.8	4	24.2	sand
12	MLPM		Nilambur Dippo	85.84	2	12.16	sand
13	MLPM		Irrigation office	85.84	2	12.16	sand
14	MLPM		Idimuzhikkal	89.84	2	12.16	sand
15	PTMA		Tiruvalla	89.84	0	10.16	sand
16	PKD		Moyan modal school	81.84	2	16.16	sand
17	TCR		vettikkattiri	81.84	4	14.16	sand
18	PKD		Kanniyampuram	89.84	0	10.16	sand
19	PKD		Kinavallur	89.84	0	10.16	sand
20	PKD	FB	Thiruvizhamkunnu	97	2	1	Fine sand
21	MLPM		Bengalow kunnu	85.84	0	14.16	sand
22	PKD		Dhoni temple	85.84	2	12.16	sand
23	PKD	RB	Parali Riverbank	97	2	1	Fine sand
24	TCR		Poomala	83.8	0	16.2	sand
25	TCR		Ezhattumugham	87.8	0	12.2	sand
26	WYND		Kuruva	87.8	0	12.2	sand
27	TCR		Thumboormuzhi	79.84	4	16.16	sand

Appendix 10 -Average diameter progress in each hour in different conditions of second instar larvae

Diameter	1	2	3	4	5	6	7	8	9	10	11	12	Next day
sand fed	1.6	1.51	1.51	1.65	1.68	1.67	1.64	1.89	2.49	2.46	2.54	2.54	2.49
sand starved	1.98	1.96	1.97	1.88	1.88	1.90	1.99	2.25	2.63	2.98	2.96	2.96	3.01
soil fed	2.04	2.06	2.02	2.02	2.09	2.29	2.33	2.30	2.54	3.56	3.61	3.60	3.65
soil starved	2.12	2.31	2.29	2.26	2.24	2.21	2.33	2.36	2.83	3.13	3.08	3.19	3.13
Average	1.94	1.96	1.95	1.95	1.97	2.02	2.07	2.20	2.62	3.03	3.05	3.07	3.07

Appendix 11- Average diameter progress in each hour in different conditions of third instar larvae

Diameter	1	2	3	4	5	6	7	8	9	10	11	12	Next day
sand fed	1.92	1.95	1.99	2.04	2	1.97	1.98	2.09	1.98	2.29	2.85	3	3.39
sand starved	2.34	2.27	2.59	2.48	2.49	2.45	2.44	2.65	3.08	3.81	3.49	3.49	3.50
soil fed	1.92	1.95	1.99	2.04	2	1.97	1.98	2.09	1.98	2.29	2.85	3	3.39
soil starved	2.06	2.43	2.48	2.41	2.43	2.36	2.5	2.53	2.47	2.74	2.9	2.9	3.24
Average	2.06	2.15	2.26	2.24	2.23	2.19	2.23	2.34	2.38	2.79	3.02	3.09	3.38

Appendix 12- Average depth progress in each hour in different conditions of second instar larvae

Depth	1	2	3	4	5	6	7	8	9	10	11	12	Next day
sand fed	1.1	0.92	0.97	1.07	1.03	1.03	1.03	1.25	1.5	1.57	1.62	1.62	1.48
sand starved	1.38	1.25	1.24	1.18	1.23	1.23	1.31	1.38	1.64	1.89	1.89	1.89	1.85
soil fed	1.31	1.29	1.21	1.26	1.28	1.38	1.41	1.41	1.57	2.07	2.31	2.26	2.19
soil starved	1.38	1.53	1.38	1.31	1.28	1.34	1.39	1.43	1.71	1.85	1.85	1.89	1.91
Average	1.29	1.25	1.19	1.20	1.20	1.25	1.29	1.37	1.60	1.85	1.92	1.92	1.86

Appendix 13- Average depth progress in each hour in different conditions of third instar larvae

Depth	1	2	3	4	5	6	7	8	9	10	11	12	Next day
sand fed	1.27	1.25	1.3	1.32	1.24	1.23	1.23	1.28	1.18	1.35	1.74	1.79	2.05
sand starved	1.34	1.43	1.56	1.54	1.55	1.44	1.51	1.72	1.93	2.19	2.19	2.19	2.19
soil fed	1.27	1.25	1.3	1.32	1.24	1.23	1.23	1.28	1.18	1.35	1.74	1.79	2.05
soil starved	1.31	1.59	1.55	1.56	1.47	1.48	1.56	1.52	1.53	1.77	1.91	1.89	2.10
Average	1.29	1.38	1.43	1.43	1.38	1.35	1.38	1.45	1.46	1.67	1.89	1.91	2.09

Appendix 14- Intraspecific interactions-Results of cannibalism experiments

		Second instar			Third instar		
		Dead %	Pupated%	No change%	Dead%	Pupated%	No change%
Well fed vs Well fed		50	-	50	50	-	50
Well fed vs Fed	Well fed	70	-	30	70	-	30
	fed	30	-	70		20	80
Well fed vs Starved	Well fed	40	-	60	20	10	70
	Starved	70	-	30	50	-	50
Fed vs Starved	Fed	90	-	10	70	-	30
	Starved	10	-	90		70	30
Fed vs Fed		50	20	30	80	10	10
Starved vs Starved		50		50	50		50

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Physico-chemical properties of pit soil of antlion larvae (Genus *Myrmeleon sp.*) with special reference to its population density

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Abstract
Antlion larvae made its conical pits in dry loose soil and predate small insects that fall down in to it. The Genus Myrmeleon coming under family Myrmeleontidae is a common pit building antlion larvae of Kerala, also a least studied group too. The larval stage is lengthy and may take up to two years to complete its larval stage. So the substrate should be more apt for the pit building behaviour as well as the high survival rate of the antlion larvae. Here, the antlion larvae Genus Myrmeleon inhabited substrate was collected and texture was noted by Hydrometer method. Out of the 27 soil samples collected from seven districts of Kerala, all the samples are coming under the texture class sand (six samples- fine sand, 21samples- sand). The four habitats were noted such as abandoned area, human dwelling area, forest boundaries and river banks. The habitat wise sand, silt & clay content were also examined and its chemical components were estimated. The highest number of larvae were collected from abandoned areas followed by human dwelling area and the textures in these two habitats were sand. The conclusion from this study depicts that, they prefer sand for making its pit and the population density of this medium is high. Ten chemical components of the media were correlated with population density and show a negative correlation with K and Ca.

Keywords: genus *Myrmeleon*, soil texture, hydrometer method, habitat, Kerala

Introduction

Antlion larvae Genus *Myrmeleon* is seen in loose soils and they make conical pits and wait for the prey that fall down in to it. The most common species coming under Genus *Myrmeleon* in Kerala are *Myrmeleon hyalinus* and *Myrmeleon pseudohyalinus*. In India published research works were not available in the larval behaviour studies and the special characters of their substrate wasn't analysed yet. The pit making of antlion larvae influenced by lots of parameters such as type of soil, soil illumination and vibrations. Though they are shade loving creatures it made its conical pits in abandoned areas and under the trees. The significance of the study relies on the life span of the antlion larvae. Antlion larvae took upto two years to emerge as adult from its larval stage. They are holometabolous insects and the larval stage is lengthy and they gave immense importance to lay its eggs in a safe substrate to maximise the survival rate.

Not many works were done about the soil type and preference of antlion larvae, but some works regarding soil stability and vibration were found that the larvae considered as the bioindicators of soil stability because they prefer fine grained, stable sands for pit building. According to state of environment report Kerala, the soil of the state has acidic in nature in general, low water holding capacity and high phosphate fixing capacity [2]. Soil formation is influenced by the climate, vegetation and hydrological conditions of the particular area and according to these lots of soil classification is present.

The main characteristic features of soil include soil temperature, soil moisture and soil texture. The different species of genus *Myrmeleon* was used for most of the previous studies and the pit building of *Myrmeleon pictifrons* in moisture condition and different grain size were studied by Kitching[5]. Sand preference study reveals the preference of find sand[6] for pit building in *Myrmeleon sp*. Instability of sandy soil was studied by Halloran *et al.*, [4] and the antlion larvae (*Myrmeleon crudelis)* considered as bioindicator of soil stability. The pit building strategy in different conditions like sand depth, soil type and thermal conditions were studied by Alcalay *et al.*, [1]. Maoge *et al*., [7] analysed the chemical composition of the media and the role in the pit building of antlion larvae.

Materials and Methods

Study area: Soil sample were collected from antlion larval pits and the larvae inhabited area. 100 grams of soil samples were used for the texture analysis. The soil samples were collected from 27 study areas coming under seven districts of Kerala. Physical Properties of Soil- Hydrometer method- Procedure:- Weighed 50 g fine textured soil or 100 g coarse textured soil (>75-80% sand) which was passed through a 2mm sieve based on oven dry condition in to a beaker. Added 50 ml of 6% Hydrogen peroxide (H_2O_2) and covered the beaker with a watch glass and place it on a water bath until oxidation of organic matter is completed (indicated by the presence of effervescence), removed the beaker and cool. After cessation of frothing transferred the contents in to a dispersing cup with about 400ml of distilled water. Added to it 100 ml of calgon solution. Stirred the suspension for10 minutes by an electric stirrer. Transferred the suspension into a litre graduated cylinder and makeup the suspension up to 1 litre mark with distilled water. Stopper the mouth of the cylinder and shaked vigorously upside down and back several times for about 1 minute. Placed the cylinder on a table and noted the time immediately. Dipped the Hydrometer in to the suspension and took the first reading after 4 minutes when particle >0.02mm have settled (Start inserting the hydrometer 10 seconds in advance of the reading time). Carefully removed the hydrometer and washed with distilled water and noted down the temperature of the suspension. Kept the suspension undisturbed and dipped the hydrometer again at the end of 2 hours after initial shaking was stopped. Now, the particles greater than 0.002mm (sand+silt) have settled. Recorded the hydrometer reading. Calculated the percentage of sand, silt and clay and determined the textural class using ISSS textural triangle [8].

Chemical properties of soil- The chemical components like Hydrogen ion concentration(PH), Electrical conductivity (EC), Organic carbon (OC), Nitrogen(N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S) , Boron (B) and Chlorine (Cl) were analysed in the labs of IRTC Mundur, Palakkad and KFRI, Peechi, Thrissur. PH and EC were determined by using PH meter and conductivity meter respectively. The principle behind the estimation procedure of OC was according to Schollenberger [9] and the methodology used for the estimation was Walkley-Black Wet Digestion Method [10]. The molybdenum blue method was used for the estimation of P and S by $CaCl_2$ extraction and the estimation of Ca, Mg, Fe, Mn, Zn and Cu by Atomic Absorption Spectrophotometry. Boron estimated by Hot-water soluble Boron method [3] and Nitrogen in the soil sample was determined by Alkaline permanganate method. Chloride determination is based on the formation of nearly insoluble silver salts. The relationship between chemical components and number of individuals were analysed in PAST software.

Population density of antlion larvae- The number of larvae were counted from the study area itself and the relationship between the population density and its chemical and physical properties of soil were done.

Results and Discussion

Table 1: Study areas in which the soil collections were made

Sl No.	Name of place	Habitat	Latitude	Longitude
1	Parli	Abandoned	10º47’36”N	76º33’52”E
2	Thiruvizhamkunnu	Forest	11º02’23”N	76º22’16”E
3	Parali Riverbank	Riparian	10º47’15”N	76º33’41”E
4	Parli Manamthody	Human dwelling	10º47’33”N	76º33’51”E
5	Edathara	Abandoned	10º47’29”N	76º33’58”E
6	Pezhumpara	Abandoned	10º34’41”N	76º36’13”E
7	Murukkumpara	Abandoned	10º49’62”N	76º32’94”E
8	Wadakkancherry	Human dwelling	10º39’52”N	16º15’05”E
9	Nedupuzha	Human dwelling	10º29’34”N	76º12’46”E
10	Kodungallur	Human dwelling	10º13’20”N	76º12’09”E
11	Poomala	Riparian	10º36’00”N	76º14’35”E
12	Ezhattumugham	Riparian	10º17’40”N	76º27’09”E
13	Vellayani	Human dwelling	8º25’53”N	76º59’09”E
14	Brennan college campus	Human dwelling	11º46’41”N	75º28’07”E
15	Kuruva	Riparian	11º50’48”N	76º04’20”E
16	Thennal resort, Kattikulam	Human dwelling	11º50’26”N	76º05’05”E
17	Bengalow kunnu	Forest	11º17’17”N	76º14’17”E
18	Nilambur Dippo	Human dwelling	11º16’44”N	76º15’11”E
19	Irrigation office	Human dwelling	11º16’18”N	76º13’43”E
20	Dhony temple	Forest	10º51’11”N	76º37’29”E
21	Idimuzhikkal	Human dwelling	11º09’48”N	75º52’39”E
22	Marthoma college, Tiruvalla	Human dwelling	9º24’02”N	76º35’02”E
23	Moyan modal school	Human dwelling	10º46’46”N	76º39’17”E
24	vettikkattiri	Human dwelling	10º43’53”N	76º16’56”E
25	Kanniyampuram	Human dwelling	10º46’14”N	76º21’26”E
26	Kinavallur	Human dwelling	10º48’32”N	76º33’56”E
27	Thumboormuzhi	Riparian	10º17’44”N	76º27’33”E

The study area in which the collection of antlion larvae was explained in Table 1 and the four different habitats of Genus Myrmeleon is described below

1. Abandoned area
The area in which no disturbance of animals, human being are considered as abandoned area.

2. Human Dwelling area
The area near to houses, schools, bus stops etc are considered as human dwelling area.

3. Forest area
The area near to forest boundaries are considered in this habitat. From the forest area up to 5 kms were considered as forest area in which abandoned areas, human dwelling area included.

4. Riparian
River banks were considered as riparian habitat.

Soil Texture Analysis
A total of 27 soil samples were collected from seven districts of Kerala namely Palakkad, Thrissur, Wayanad, Malappuram, Thiruvanathapuram, Kannur and Pathanamthitta. From this, twenty one soil samples were of the texture class sand and six soil samples were of the texture class fine sand (77.8% of soil samples were in texture class sand and 22.2% was fine sand).

Ten soil samples were collected and analysed the texture by examining the percentage of the soil components (sand, silt and clay) in Palakkad District. Seven soil samples are coming under the texture class sand and three samples coming under fine sand (Fig. 1). The soil samples collected from the study area Parali, Thiruvizhamkunnam, Parli river bank were fine sand in texture and that of Ottappalam, Manamthody, Moyan school, Kinavallur, Edathara, Dhoni, and Pezhumpara were sand in texture. Ottappalam, Kinavallur and Edathara were the study sites in which the absence of silt component noted.

Seven soil samples were tested from Thrissur district (Fig. 2) in which three samples were coming under texture class fine sand and four samples were texture class sand. The soil samples collected from Asarikkadu, Wadakkanchery and Nedupuzha were fine sand in texture and Thumburmuzhi, Vallathol Nagar, Kodungallur, Poomala and Ezhattumugham were sand in texture. Kuruva and Kattikulam of Wayanad District (Fig. 3), Benglakunnu, Nilambur Dippo, Nilambur Irrigation office and Idimuzhikkal of Malappuram District (Fig. 3) were tested and the result shows the sand texture in that place. The samples collected from Vellayani of Thiruvanathapuram (southern district of Kerala state) (Fig. 4), Brennan college campus of Kannur District (Eastern part of state) (Fig. 4), Tiruvalla of Pathanamthitta District (Fig. 4) was sand in texture.

In the case of Palakkad District the minimum and maximum sand percentage is noted from the soil samples collected from Pezhumpara & Moyan modal school area and Thiruvizhamkunnu & Parli river bank respectively. Pezhumpara is an abandoned area and Moyan modal school is a human dwelling area. Thiruvizhzmkunnu and Parli river banks are coming under forest and riparian respectively. In the case of Thrissur, the sand percentage was minimum in the soil collected from Thumboormuzhy and the sand percentage was maximum in the soil collected from Murikkumpara and Nedupuzha.

In case of Malappuram district all the four samples were observed similar percentage of sand particle.The data regarding habitat and texture was described in Table 2 and its relationship with the population density was explained in Table 3.

Table 2: Consolidated data of soil collection districts, habitat and its texture.

Sl No	District	Habitat	Texture class
1	Palakkad	Abandoned	Fine sand
2		Abandoned	sand
3		Abandoned	sand
4		Forest area	Fine sand
5		Forest area	sand
6		Human dwelling	sand
7		Human dwelling	sand
8		Human dwelling	sand
9		Human dwelling	sand
10		Riparian	Fine sand
11	Thrissur	Abandoned	Fine sand
12		Human dwelling	Fine sand
13		Human dwelling	Fine sand
14		Human dwelling	sand
15		Human dwelling	sand
16		Riparian	sand
17		Riparian	sand
18		Riparian	sand
19	Thiruvanathapuram	Human dwelling	sand
20	Kannur	Human dwelling	sand
21	Wayanad	Riparian	sand
22		Human dwelling	sand
23	Malappuram	Forest	sand
24		Human dwelling	sand
25		Human dwelling	sand
26		Human dwelling	sand
27	Pathanamthitta	Human dwelling	sand

Table 3: Relationship between population density of antlion larvae and texture of pit soil

Sl no	Habitat	Presence of antlion larvae (%)	Texture	
			Sand (%)	Fine sand (%)
1	Abandoned	42.1	50	50
2	Forest	9.3	66.7	33.3
3	Riparian	8.1	20	80
4	Human dwelling area	40.5	86.7	13.3
	Total	100	100	

From the soil sample collection area, also noted the number of individuals for identifying the relationship between the texture of soil and number of individuals (Population density). From the data it is understood that, the highest number of larvae were collected from abandoned areas followed by human dwelling area. The textures in these two habitats were sand, the population density shows a positive correlation with the texture class sand. Below 10% of the larvae were collected from Forest and riparian habitat. The texture of soil collected from four habitats and the presence of antlion larvae were explained in the table 3 and 4.

Table 4: Relationship between the habitat and population density of antlion larvae

Sl No	District	Habitat	Population density
1	Palakkad	Riparian	8.9
2		Human dwelling	33.3
3		Abandoned	15.7
4	Thiruvananthapuram	Human dwelling	7.8
5		Human dwelling	3.3
6		Human dwelling	3.3
7	Thrissur	Abandoned	11.1
8		Human dwelling	12.2
9		Human dwelling	2.2
10		Human dwelling	2.2

The sand, silt and clay content in each district, habitat was explained in Fig 1 to Fig4. Number of individuals are significantly positively correlated with silt (Pearson's correlation, 5% significance) in sandy soil (Fig. 5). Soil samples collected from seven districts (total of 27 sites) indicated that sand was the predominant content, 80% of every soil sample was sand, and there was slight variation in the silt and clay content.

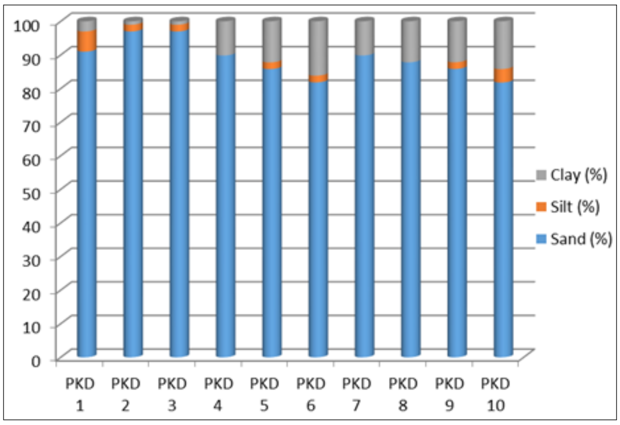

Fig 1: The sand, silt and clay percentage of soil samples in Palakkad District PKD1=AA, PKD2=FB, PKD3=RA, PKD4=RB, PKD5=HDA, PKD6=HDA, PKD7=HDA, PKD8=AA, PKD9=FB, PKD10=AA

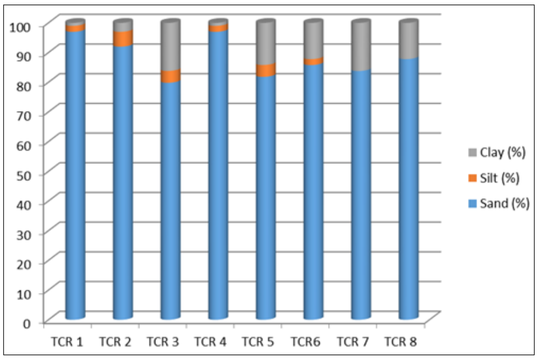

Fig 2: The sand, silt and clay percentage of soil samples in Thrissur District TCR1= HDA, TCR2=HDA, TCR3=RB, TCR4=HDA, TCR5=HDA, TCR6=HDA, TCR7=RB, TCR8=RB

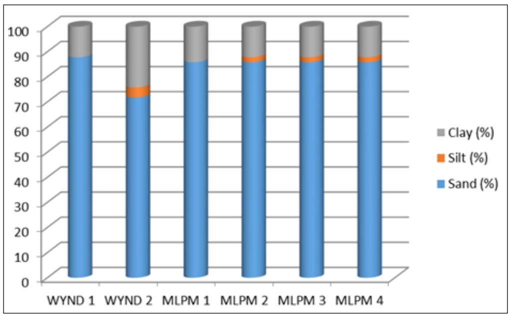

Fig 3: The sand, silt and clay percentage of soil samples in Wayanad and Malappuram Districts. WYND1=RB, WYND2=HDA, MLPM1=FB, MLPM2=HDA, MLPM3=HDA, MLPM4=HDA

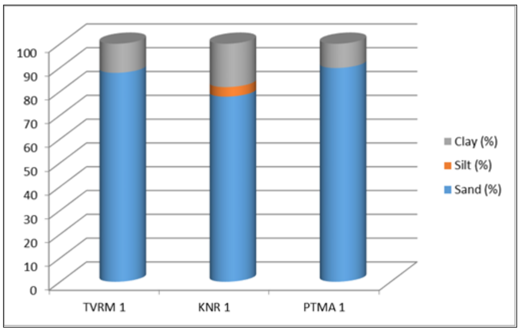

Fig 4: Sand, silt and clay percentage of soil samples in Thiruvananthapuram, Kannur and Pathanamthitta Districts TVRM1=HDA, KNR1=HDA, PTMA1=HDA

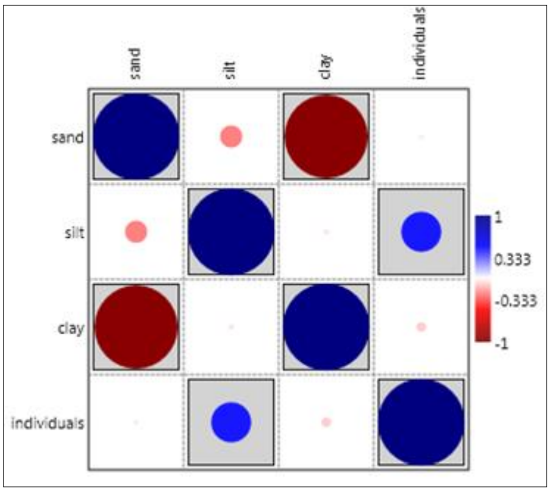

Fig 5: Correlation plot showing the relation between soil characters and Myrmeleon larvae.

Soil Chemical Analysis

Chemical composition of the media also has an important role in the pit building of antlion larvae. Higher the concentration of acidity, salinity, Calcium and magnesium are suppressive to larval development. But Potassium, Sulphate and Chlorides are good for larval development [6].

PCA for all 10 chemical parameters of soil where antlion larvae sampled and the number of individuals present were done. The soil chemical parameters are EC, OC, N, P, K, Ca, Mg, S and Cl. The Mg, OC, EC, and P^H were influenced the number of individuals in a positive manner. But, Ca and K show a negative correlation with the number of individuals. The Eigen values and map were given in Table 5 and Fig. 6 respectively.

Table 5: Eigen values

PC	Eigenvalue	% variance
1	2.26E+06	97.286
2	56452.5	2.4302
3	5133.78	0.221
4	1137.15	0.048953
5	232.276	0.009999
6	75.0003	0.003229
7	6.51953	0.000281
8	2.93664	0.000126
9	0.0668348	2.88E-06
10	0.00245453	1.06E-07

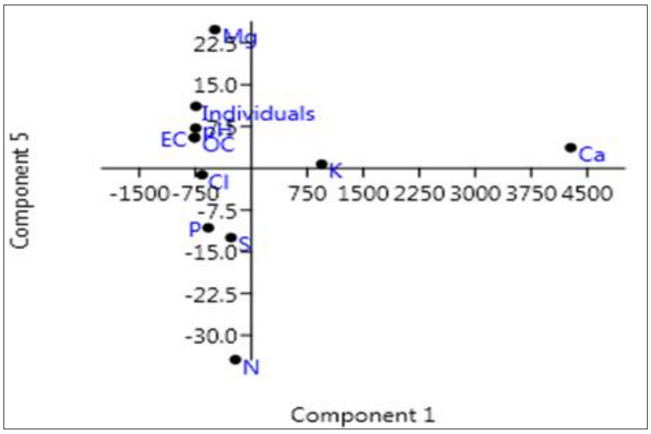

Fig 6: PCA map showing negative correlation between K & Ca and number of individuals.

The antlion larvae inhabited soil composed of sand, silt and clay particles. According to the International Union of Soil Sciences (IUSS), the soil particles were classified and this was used to interpret the soil texture in this study. Sand particles has low water and nutrient holding capacity, loose when dry and very low stickiness when wet. The silt components has low to medium water and nutrient holding capacity and shows some stickiness when wet. The clay component has high water and nutrient holding capacity, hard when dry and high degree of stickiness when wet.

From the 27 soil samples collected from study areas, 21 samples were classified under texture class sand and 6 samples were coming under fine sand. The sand texture was analyzed by evaluating the sand, silt and clay content of the soil samples. The correlation between the components of soil and number of individuals were performed and the result shows a positive correlation between the numbers of larvae with sand having highest silt content. It is assumed that, one reason behind the preference of sandy soil for pit building substrate was the nature of sand and silt with low water holding capacity and easily dry when wet compared to clay soil.

The study of Phogat *et al*., [8] described that the larvae build its pits in sand particle with low water holding capacity and low stickiness when wet and the present study also agrees with this result. For the survival of antlion larvae they choose the sand particle for making its pit. If the rainy season or wet condition occurs, the larvae took a dormancy period by burying deep into the sand until the sand become dry. It is a survival mechanism of pit building antlion larvae. Chemical composition of the media also has an important role in the pit building and development of antlion larvae. From this study, a negative correlation between calcium and potassium with the number of individuals were identified. The relationship between the chemical component of antlion inhabiting soil and its influence on larvae are not studied earlier and it can be studied in future. In general, higher the concentration of acidity, salinity, calcium and magnesium are suppressive to larval development. But potassium, sulphate and chlorides are good for larval development. Soils those are more favorable for the development of larvae are rich in potassium, magnesium and sulphates. The element that adversely affects the development of antlion is nitrogen [7].

Conclusion

The study gives a baseline data about the different biotic and abiotic parameters of soil inhabiting antlion larvae and its habitat. This is only confined to a single genus and the larval stage is completed in soil with three instars. The lengthy larval period successfully completed by the help of its habitat and a first information regarding the soil and habitat was depicted in this study.

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Research Article

AN ASSESSMENT OF ANTLION PREY, BY ANALYZING ITS PREY REMNANTS INSIDE PITS IN SELECTED DISTRICTS OF KERALA

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ABSTRACT

Surveys are direct knowledge from the natural conditions and it is the most reliable results rather than gathering from imitated artificial conditions. Hence a survey was done for understanding the diversity of antlion prey by analysing its prey remnants. It was very difficult and time consuming at the time of identification because the remnants after sucking the body juice only present inside the pit. The collection of antlion prey was made from January 2016-July 2016. Six varieties of antlion prey were found, which include four insect orders, one spider and millipede. A total of 74 prey remnants were collected from various locations of the different geographical regions, it is found that Hymenopterans are the most preferred prey of antlion, which include mostly ants. Antlion larvae also seen inside the pits, they were considered as the larvae which are not survived at that area. Coleopterans, in spite of its hard exoskeleton, are also preferred by antlion larvae.

INTRODUCTION

Insects make up the species diversity on the earth. Instead of creating species diversity, they play some major roles like pollinators, bio indicators, food for other animals etc. Irrespective of their colour, function, smell etc nobody can live without coming contact with a single insect. There are 30 insect orders are coming under Class Insecta and Neuroptera is one of the Order which include antlions. Antlions include a family called Myrmeleontidae and are soil dwelling creatures in the larval stage.

They are considered as a good predator of ants, hence the name antlion. Out of it, the other organisms seen in antlion pits are considered as an unravel thing. Antlion makes a conical shaped pit for predating other insects and anticipated a long wait for it and hiding in the pit expecting their prey. They suck the body fluids of prey by the help of hollow mandibles and throw the remnants of prey outside the pit, a mechanism called pit cleaning and wait for the next prey. They have an excellent mechanism for sensing the arrival of prey by the help of soil vibrations (Fertin *et.al.*, 2007). The characters like hard exoskeleton, size etc are the reasons for the variation in the prey. Mostly ants are soil dwelling insects, the chance to fall inside the pit is high compared to others.

Not only the physical character of prey, but also the season played a major role in the prey diversity in the pit (Maoge *et.al.*,). A study point out that the main potential prey of antlion in sudano guinean zone of Cameroon is Myrmicaria opaciventris (Ngamo *et.al.*, 2016) during dry season. So the potential prey changes according to the seasonal variation in that area. Studies regarding the modulation in the feeding prey capture, feeding kinematics of Myrmeleon crudelis etc (lambert *et.al.*, 2011) are the recent studies based on the prey capture. Associative learning enabled antlion to dig better pits and got prey efficiently, moult before the actual time (Guillette *et.al.*, 2009).

Every encounter with a prey is not successful, there are escaping of prey from pit also happening. Some defensive attacks by ants are seen and it will cause severe injury to antlion which leads to the death of antlion (Lucas, 1981). Mostly prey with large body size are very time consuming and it is not preferred by antlion. The body mass and successful predation is negatively correlated (Scharf *et.al.*, 2010). There are intraspecific competition and cannibalism are also present in between antlions.

MATERIALS AND METHODS

Study area: The study area selected for this work was three districts of Kerala, Such as Palakkad, Thrissur and Wayanad. Palakkad is the gateway to Kerala from the nearest state Tamil Nadu due to the presence of Palakkad gap, in the Western Ghats. Thrissur has a tropical humid climate with plentiful and seasonal rainfall. Wayanad district stands on the southern tip of Deccan Plateau and includes parts of the Western Ghats. It has a large amount of dry and moist deciduous forest. Palakkad and Thrissur are the nearest districts, but Wayanad seen after two

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districts of northern part of kerala. These three diverse districts gave an outlook about the prey species in antlion pits in the Kerala state.

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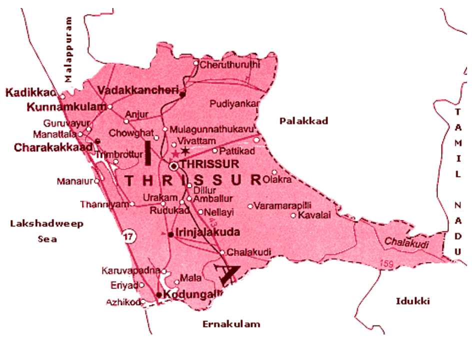


Study area- Source: Google map

METHODOLOGY

Analysis inside antlion pits shows the presence of antlion and some partially digested prey remnants. The collection of prey was made from three districts and nine areas of Kerala. The unusual insects inside antlion pits were carefully collected with a brush and transferred in to plastic boxes. The collection of antlion prey was made from January 2016-July 2016. The collected prey remnants were identified in the Order level by the help of the available literature etc. After taking photographs of prey remnants, the tabulation of data was done. The collected prey remnants were kept in the research laboratory of Research and Post Graduate Department of Zoology, St. Thomas College, Thrissur for further studies.

RESULT AND DISCUSSION

The prey remnants inside the pit found like a dried exoskeleton after consuming the body fluid by antlion. So the exoskeleton dried without any destruction. The lack of body fluid helps to dry the body parts. A total of 51 pits were analysed and collected 74 prey remnants. Among the data made from the study area it is clear that the most preferred prey is considered as hymenopterans (ants), because of its soft body and soil dwelling character.

Though the ants are walking without any laziness, the incidence inside the pit was more frequent. Antlion larvae also collected from the pits but it is not dead as a result of intraspecific competition or cannibalism. It is clear that some temperature variation affected and the larvae in that area found dead, the body dried and colour changed to blackish. Hence the analysis was done excluding the dead antlion.

Coleopterans also present in the category of antlion prey, though it is flying insects, it may accidently falling in to the pit. The hard exoskeleton remained exact and the body fluid sucks by antlion.

The collected data were undergone descriptive analysis, and got district wise prey preference status of antlion prey. The below table indicates the order of animals which is present in the pits of three districts of Kerala.

Table 1 Table showing the collection data of antlion prey remnants from antlion pits.

Order	Month	Place of collection	District	Number of individuals
	May	Parali	Palakkad	3
	April	Parali	Palakkad	4
	April	Parali	Palakkad	1
	April	Ottappalam	Palakkad	2
	April	Parali	Palakkad	2
	April	Ottappalam	Palakkad	3
	May	Ottappalm	Palakkad	1
	May	Parali	Palakkad	1
	May	Parali	Palakkad	2
	April	Pathirippala	Palakkad	2
	April	Pathirippala	Palakkad	1
	April	Pathirippala	Palakkad	1
	April	Asarikkad	Thrissur	2
	March	Asarikkad	Thrissur	2
	March	Asarikkad	Thrissur	1
	June	Parali	Palakkad	1
	July	Muthuvara	Thrissur	1
	July	Asarikkad	Thrissur	1
Hymenoptera	May	Vadakkanchery	Thrissur	1
	June	Parali	Palakkad	2
	May	Parali	Palakkad	2
	June	Parali	Palakkad	3
	May	Sulthan Bathery	Wayanad	4
	May	Sulthan Bathery	Wayanad	1
	March	Asarikkad	Thrissur	2
	March	Parali	Palakkad	2
	March	Parali	Palakkad	1
	March	Parali	Palakkad	1
	March	Parali	Palakkad	1
	March	Parali	Palakkad	1
	March	Parali	Palakkad	2
	March	Parali	Palakkad	3
	January	Panamuk	Thrissur	1
	February	Parali	Palakkad	1
	January	Ottappalam	Palakkad	1
	January	Parali	Palakkad	1
	February	Parali	Palakkad	1
	April	Asarikkad	Thrissur	1
	May	Sulthan Bathery	Wayanad	1
	June	Parali	Palakkad	1
	May	Parali	Palakkad	1
Coleoptera	March	Asarikkad	Thrissur	1
	January	Asarikkad	Thrissur	1
	January	Parali	Palakkad	1
	March	Edathara	Palakkad	1
Lepidoptera	June	Asarikkad	Thrissur	1
	March	Parali	Palakkad	1
Millipede	May	Sulthan Bathery	Wayanad	1
Araneae	June	Parali	Palakkad	1
Total				74

Table 2 Table showing district wise data of prey remnants

	Palakkad	Thrissur	Wayanad	Total
Hymenoptera	46	11	5	62
Coleoptera	4	3	1	8
Lepidoptera	1	1	0	2
Millipede	0	0	1	1
Araneae	1	0	0	1
	52	15	7	74

The data from the three districts taken separately and made pie diagram. In all the three districts, the antlion prefer hymenopterans and coleopterans. These two groups are the soil dwelling insects but in the case of spider and millipede they are rare, considered as accidently fallen groups.

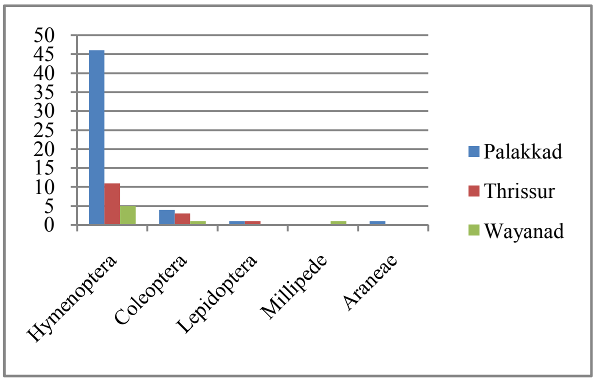


Fig 1 Graph showing district wise data of prey remnants

The different orders obtained from different districts are as follows:

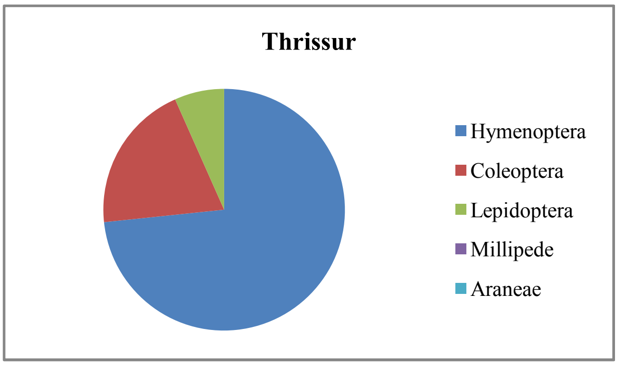


Fig 2 Pie diagram showing the diversity of different orders of prey in Thrissur district.

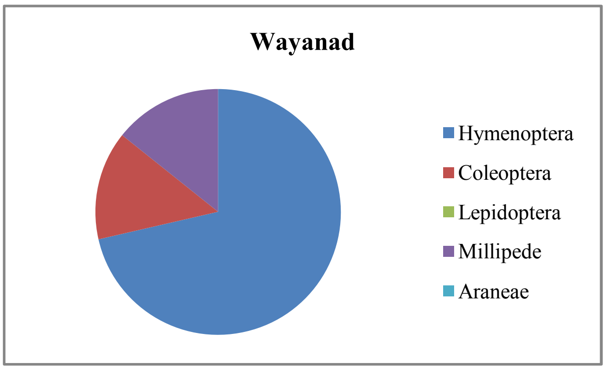


Fig 3 Pie diagram showing the diversity of different orders of prey in Wayanad district.

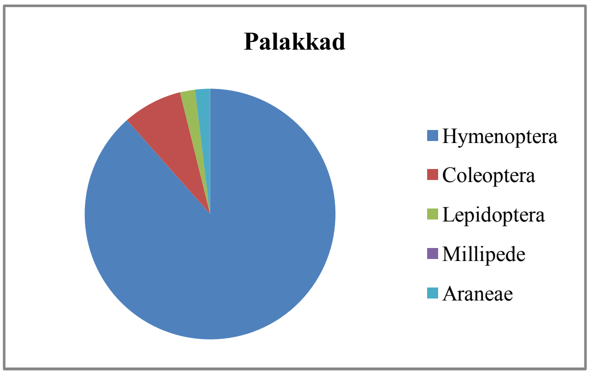


Fig 4 Pie diagram showing the diversity of different orders of prey in Palakkad district.

Though the data was taken monthly, based on that graphs are also prepared.

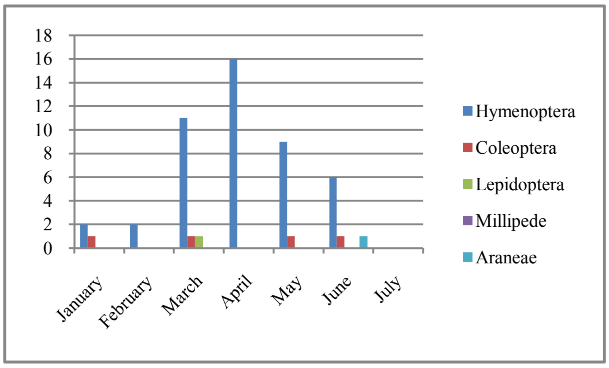


Fig 5 Monthly data of prey remnants collected from Palakkad District

In the three districts, the number of prey was high in March, April, May months. The activity of insects may be in increased level in summer season, or the successful predation is high in these three months.

Monthly data of different orders of prey remnants.

The presence of prey inside the antlion pit is high in the month of April, it may be because of the season in which the number of hymenopterans are high at Palakkad.

In the case of Thrissur district, the numbers of hymenopterans are high in the month of march. From Wayanad, the prey was got only in the month of May. Though it is a forest area, frequent rain was found. The insects inside the antlion pit may lost through rain water, it may be the main reason for the decrease in prey.

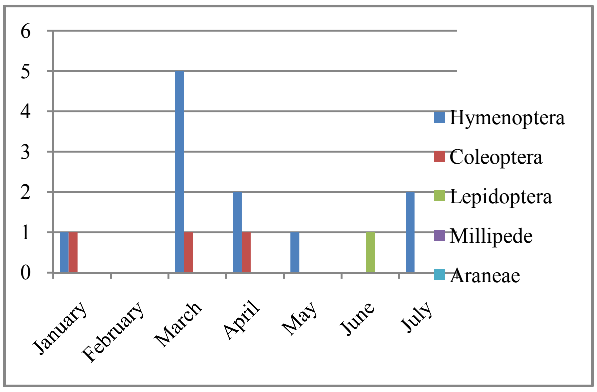


Fig 6 Monthly data of prey remnants collected from Thrissur District.

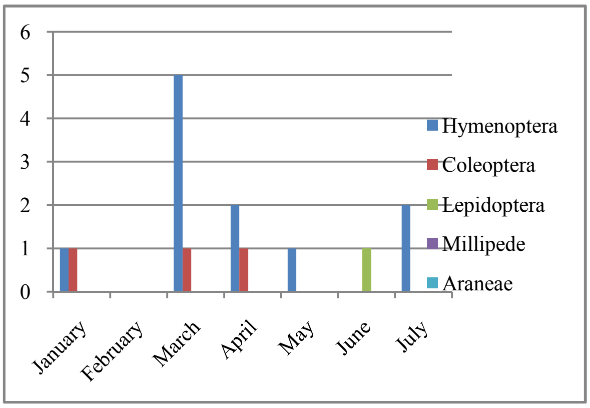


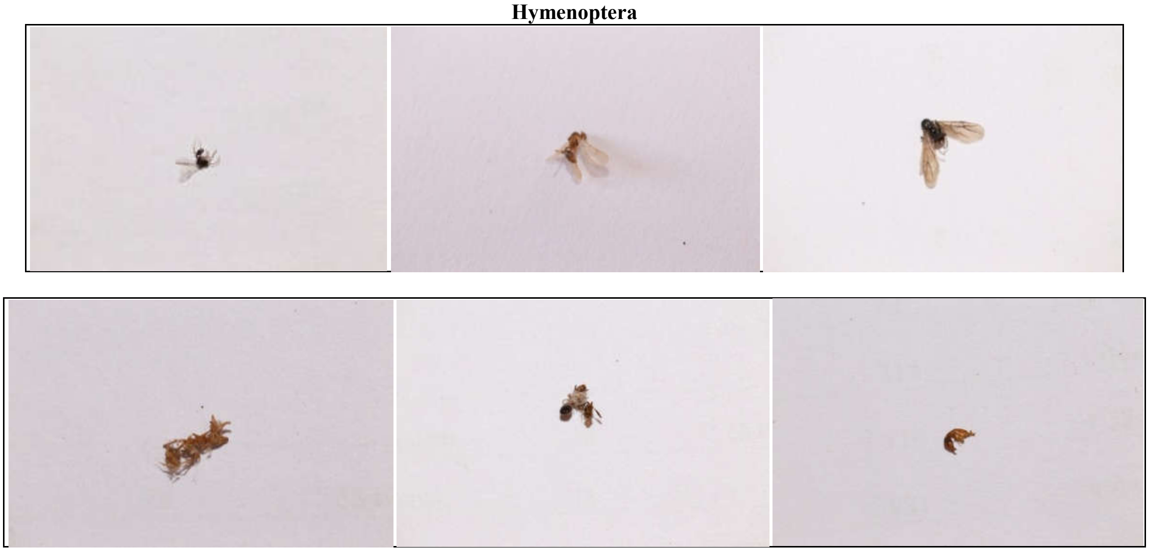
Fig 7 Monthly data of prey remnants collected from Wayanad District.

The number and variety of prey will change in the change in geography, rain, soil moisture, population change of organisms etc. Though ants are soil dwelling creatures, they are frequently fallen inside the pits hence the Hymenopterans seen more as prey. The prey species may change with the change in moisture content of soil because the presence of millipede also noted.

CONCLUSION

Antlion researches are rare when compared to other insects in India. The prey species may change from place to place and time to time. In the climatic and sesonal changes may influence the prey species but the ants are very common in our country, they remain constant. Cannibalism also prominent in the case of antlion, but the change in the exoskeleton gave the exact reason for their death.

Hymenoptera



Neuroptera

Coleoptera

Millipede

Aranea

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Statistical Analysis of Larval and Pit Size of Antlion (Family: Myrmeleontidae) with Short Notes on its Trailing Behaviour

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Abstract: ***Antlion larvae makes pits in the sandy soil with their mandibles. The natural predators on ants, they have a special strategy to predate. Pit building in loose sandy soil is the fascinating behaviour pattern shown by antlion larvae. The relationship between the antlion larval size and pit size were analysed using standard software. A total of sixty pits were analysed for this study and the measurements were made. Twelve of them are non resident pits. From correlation analysis, a high positive correlation between larval body width and pit diameter and a negative correlation between larval head length and pit depth were found. Also instar determination was done by using highly correlated parameters. From this result, we can predict the larval size of antlions inhabiting a pit by using its pit diameter. Approximately the diameter of pit 1.2-2.2, 2.2-3.2, 3.2-4.2 have the larvae with body size 2.9, 3.4, 3.8 respectively. A short note regarding the trailing behaviour of larvae is also included. The trailing was started within 3sec to 50 minutes from the introduction of larvae to the soil and started from the centre to the north of the tray in every experiment.***

Keywords: Antlion larval size, pit size, instar determination, trailing behaviour.

1. Introduction

Myrmeleontids, consisting of nerve - winged creatures commonly known as antlions, are the least studied insect group in India. They belong to Family Myrmeleontidae, Order Neuroptera with membraneous wings which are held roof like while resting. The life cycle includes a larval stage which is fascinating with so many behaviour patterns such as pit building, predatory and trailing. Antlion larva are sedentary predators, which dig in sandy soils and capture its preys, mostly ants.

The antlion larva has three instars in its life cycle. In each instar the size will increase progressively and it will stop prior to pupation. With the help of body size and pit characteristics we can determine the instar of pit dwelling larvae. Mainly head width is used as an indicator of body size (Arnett et al. 1999), from which we can decide the instar of antlion larvae. But here we are going to predict the stage of larvae in a pit with highly related parameter of pit. The pit characteristics are very significant in the case of antlion larvae hence it is a natural predator. Larva use their pit to capture insects mostly ants in the natural habitat. The diameter, depth etc of the pit is highly significant for the capture success of prey and also pit diameter was significantly correlated with larval size (Prado et al. 1993). But studies have revealed that there is a relationship between larval size and diameter of the pit (Simberloff et al. 1978, Griffiths 1980, Heinrich and Heinrich 1984).

There are studies regarding the change in Pit diameter and larval mass in different seasons. Pit diameter varied 7 fold and the larval mass varied 60 fold in early spring (Heinrich and Margaret 1984). Not only season causes the change in pit size but also the past pit- building experience and food limitation influence the pit size changes (Liang et al. 2010). Within each larval instar (Myrmeleon pictifrons) there is a linear, increasing relationship between larval size and pit size (Kitching 1984). Larval and adult body size increased with latitude, but decreased with elevation (Arnett et al. 1999)

2. Methodology

The study was conducted in the sunshade of an abandoned house in a village called Parli in Palakkad district, the central northern district of Kerala State bordering Tamilnadu, and the study was conducted from April 1 - May 30, 2015. A good number of pits were there but due to unexpected summer rain the antlion pits spread outside the sunshade were damaged. The remaining pits were studied for the correlation purpose. The depth and diameter were noted without disturbing the antlion larvae. Pit diameters and depth were measured to the nearest mm. The soil temperature of the study area was between 37^{o} C - 41^{o} C.

The following parameters were studied for the correlation purpose such as, Pit depth, Pit diameter, Larval head length, Larval head width, Larval body length and Larval body width. After measuring pit characteristics, larval size were measured on the spot. Non-resident pits were also taken note off. The larvae of non -resident pits either leave the pit because of food scarcity or by adverse physical conditions. The data's were analysed by standard statistical methods and software's.

The instar was determined by using the highly correlated parameters. From the correlation study the instar of the larvae inhabiting the pit can be predicted using the pit parameters. And also, the trailing behaviour of larvae was observed under laboratory condition. From this observation a short note was made. For this purpose, a single larva was placed on a single tray, and the trailing initiation and progress was analyzed prior to pit making. The dimension of the tray was 23cm X 23cm and soil depth was fixed to 3.2 cm in each experiment.

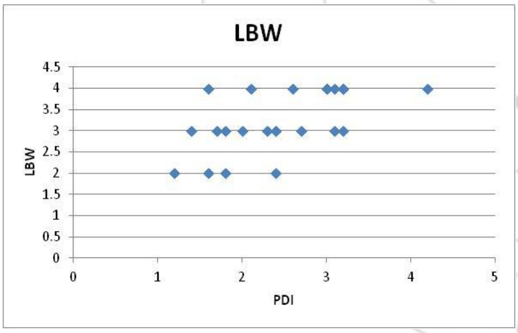
3. Results

Correlation						
	PDE	*PDI*	*LHL*	*LHW*	*LBL*	*LBW*
PDE	1					
PDI	-0.08251	1				
LHL	-0.22056	0.216193	1			
LHW	-0.16066	0.355969	0.681227	1		
LBL	0.021572	0.397212	0.371056	0.596104	1	
LBW	-0.1565	0.587012	0.58401	0.513509	0.518773	1

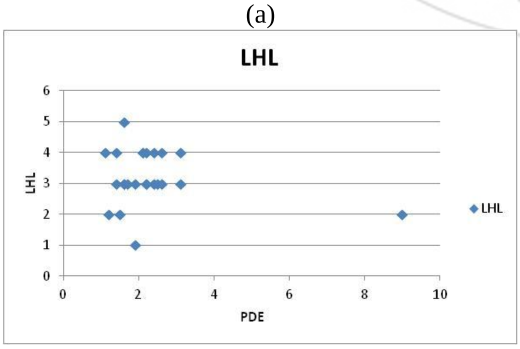
Fig. 1 Correlation results showing relationships between the different parameters of pit size and larval size.

PDE- Pit depth PDI- Pit diameter LHL-Larval head length LHW-Larval head width LBL- Larval body length LBW-Larval body width.

From the correlation results it was noted that, there is a negative correlation between larval head length and pit depth. That is, the pit depth increased with the decrease of larval head length. So smaller the head length of antlion larvae, bigger the depth of pits. Also, there is a high positive correlation between larval body width and pit diameter. That is the pit diameter increases with the increase in larval body width. There is no relationship between the larval body length and pit depth. The larval body width may help the larvae to make the pits with larger diameter. From this study, it is clear that other parameters of pit do not have any relationship with the inside dwelling larvae. Also there is a high positive correlation between pit depth and diameter. The pit depth increases with the increase in diameter.



(a)



(b)

Figure 2: (a) Positive relation between larval body width and pit diameter. (b) Negative relation between larval head length and pit depth.

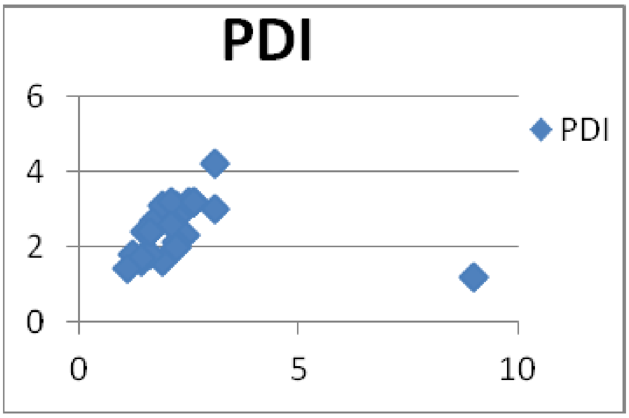


Figure 3: Graph showing the positive correlation between pit depth and diameter.

The high positive correlation results of larval body width and pit diameter were used for the instar determination study. Here the pit diameter is splitted into three classes according to their values, that are 1.2-2.2, 2.2-3.2, 3.2-4.2, from these classes the average larval body width in each diameter was calculated by taking its average. The result is furnished below.

Table 1: The expected larval body width as per diameter of pits

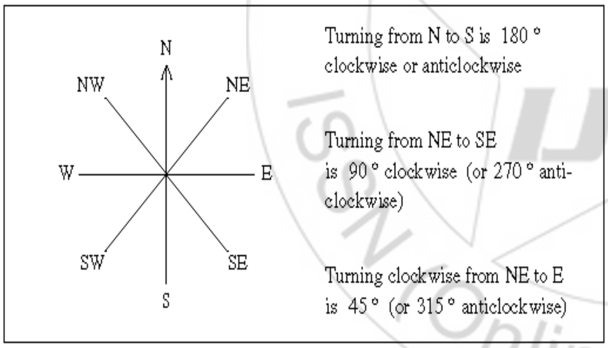
PDI	*LBW (number)*	*Mean LBW*
1.2-2.2	18	2.88
2.2-3.2	20	3.35
3.2-4.2	10	3.80

From this table, we can predict the larval size of pit-dwelling larvae by measuring its diameter. It is known that antlions have three larval instars during its life cycle. Here the average size of each larvae in each instar is determined. The average body width of first instar larvae is approximately 2.88 mm seen in the pit with diameter ranging from 1.2-2.2 cm. The average body width of second and third intar larvae is approximately 3.35 and 3.80mm respectively which is seen in pit diameter ranging from 2.2-3.2and 3.2-4.2 cm. Simply it can be determined that, the Pits with diameters ranging from 1.2-2.2, 2.2-3.2, 3.2-4.2 cm have the larvae with body width 2.9, 3.4, 3.8 mm respectively.

Figure 4: Showing the correlation between pit diameter and larval body width.

The trailing behaviour of antlion larvae was observed in laboratory condition. Single larvae placed in each tray were observed. From this study some predictions are made

(1) The trailing started between 3sec to 50 minutes from the introduction of larvae to the soil.
Most of the time it started trailing within 3 seconds.
(2) In all the experiments the larvae first moved from the centre portion of the tray to the periphery.
(3) The larvae make its pits only after the calculation of the boundary of the habitat or environment by moving on to the periphery of the tray.
(4) All the time the trailing started from the centre to the north of the tray, and moved closer back to the centre.
(5) After fixing the correct location for its pit- building, the backward rotation starts with the flipping of soil.



(a)

(b)

Figure 5: showing northward movement (a) and periphery movement (b).

4. Discussion

Antlions make their pits in shady places which are darker and cooler than areas exposed to sun (Klein 1982). Mostly, partially built buildings, abandoned houses, sandy areas are their favourite place to make pits. So many studies were conducted regarding pit building, foraging strategies etc., of which the pit diameter and pit depth are significant because it ensures the success of prey capture (Heinrich & Heinrich 1984, Scharf & Ovadia 2006). But the relationship between the larvae and its pits is somewhat new. The result shows a high positive correlation between the larval body width and pit diameter. It is known that antlions make their pit by the backward movement through soil and by flipping soil with the mandibles. So, the larvae having large body width cause the increase in diameter of pit while moving. It may help to stabilize the sand or soil from sliding down.

Some studies regarding pit size point out that the pit size will increase while short term starvation and it will decrease on long term starvation (Heinrich & Heinrich, 1984). But here, the study was emphasised on the larval size and pit size aspects. Many studies point out that there is a relationship between pit diameter and larval size without specifying whether it is body width, head length etc. But this study emphasises these aspects and finds an amazing relationship between pit and larval size parameters. Here there is a negative correlation between larval head length and pit depth, and also, there is a high positive correlation between larval body width and pit diameter. If we just measure the diameter of the pit, we can predict the body width of the larvae without seeing it.

Trailing behaviour is an important aspect regarding antlion and is only seen in antlion larvae. Experimentally it is proved that when a larvae is placed in the centre of the tray filled with sand, firstly the larvae moves to the edge of the tray in response to microclimatological factors (Heinrich and Margaret 1984). It is also true that the larvae was moved to the edge of the tray immediately after putting in to the tray, and interestingly it is seen that the larvae every time moves towards the north and comes back to the centre position and then it starts to build the pit. But the reason for northward movement is not known. Studies revealed that the amount of space used by an organism can be energetically determined (Swenson et al. 2007). There are so many pits which are non resident pits. The studies reveal the fact that almost 38% of the pits did not contain antlions (Boake et al.1984). As per this study, the percentage of non- resident pits is found to be 20%, may be due to unknown reasons.

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