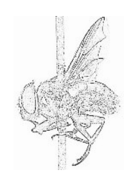

CHAPTER – 4

RESULTS

CHAPTER – 4

RESULTS

4.1. Blow fly fauna

The family Calliphoridae is encompassed of a group of flies having veterinary, ecological, medical and forensic significance with worldwide distribution. Currently, 1500 species of blow flies were reported from all over the world. In India, the family is represented by 9 subfamilies, 30 genera and 119 species. The subfamilies included are; Melanomyinae, Calliphorinae, Bengaliinae, Luciliinae, Rhiniinae, Helicoboscinae, Chrysomyinae, Ameniinae and Polleniinae. The blow flies in Kerala are represented by 4 subfamilies 8 genera and 17 species (Subramanian & Mohan, 1980, Nandi, 2004, Bharti, 2011, Radhakrishnan et al., 2012, Bharti and Singh 2017, Reject Paul and Binoy, 2021 and 2022). The description and distribution of the seventeen species found in Kerala are discussed below;

Subfamily: Bengaliinae

Genus: *Bengalia* Robineau- Desvoidy, 1830

***Bengalia jejuna* Fabricius, 1794**

Distribution: Kochi, Walayar, Thiruvananthapuram

Remarks/Source: (Bharti, 2011)

Distinguishing features: Absence of concavity on the posterior margin of eye, upper part of anepimeron with 9-11 black setulae, broad cercus narrowing down to pointed tip, 3rd and 4th tergite with marginal bands, bacilliform sclerite with an oblique distal margin, distiphallus with a constriction at the middle of dorsal wall, broad distal lip process with broad wing like membranes.

***Bengalia surcoufi* Senior-White, 1924**

Distribution: Kochi

Remarks/Source: (Nandi, 2004)

Distinguishing features: Brownish grey parafrontalia, first and second antennal segments reddish brown, pale palpi with black bristles, all segments of abdomen black banded, pale yellowish squamae, tarsi tips darkened.

Subfamily: Luciliinae

Genus: *Hemipyrellia,* Townsend, 1917

Hemipyrellia ligurriens* Wiedemann, *1830

Distribution: Foot of Nilgiri hills, Thrissur, Palakkad and Ernalulam, Kerala

Remarks/Source: (Nandi, 2004), collected from Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2021)

Distinguishing features: Genae and parafrontalia silver white, antennae tawny yellow to brownish, orange palpi, upper squama with creamish white short cilia and lower squama with light brown cilia, short hairs on the edges of tergites and first visible sternite, bare stem vein, 1st longitudinal vein without any setulae, 3rd longitudinal vein with short setulae on dorsal and ventral aspects.

Genus: *Lucilia* Robineau-Desvoidy, 1830

***Lucilia ampullacea* Villeneuve, 1922**

Distribution: Malabar Coast (Kerala)

Remarks/Source: (Nandi, 2004)

Distinguishing features: Third to fifth tergites without marginal band, basicostal scale brownish black, subcostal sclerite with upstanding hairs, post sutural acrosctichal 2, alar squama white with tuft of hair on the lower margin, and lower squama infuscated, tibiae black.

Lucilia papuensis Macquart, 1843

Distribution: Malabar Coast (Kerala)

Remarks/Source: (Nandi, 2004)

Distinguishing features: Frons broader than inter post ocelli distance, parafacialia broader than the third antennal segment, occiput with more than two irregular rows of black post ocular setae, posterior surface of post gena with black hairs, anterior pair of post sutural acrostichals present posterior to the second pair of post sutural dorsocentrals, alar and thoracic squama infuscated with a tuft of blackish brown hairs at the lower margin.

Lucilia sericata Meigen, 1826

Distribution: Calicut (Kerala), throughout India

Remarks/Source: (Priya and Sebastian, 2015, Nandi, 2004)

Distinguishing features: Parafrontalia with short decumbent bristles, cerebrale with 8-9 occipital bristles on either side, metallic green abdomen, non arched abdomen, abdomen metallic golden green with sparse pruniosity, absence of tuft of long hairs on sternites, hypopygium inconspicuous.

Subfamily: Chrysomyinae

Genus: *Chrysomya* Robineau-Desvoidy, 1830

Chrysomya megacephala Fabricius, 1794

Distribution: Throughout India, Thrissur, Palakkad and Ernakulam in Kerala

Remarks/Source: Calicut (Bharti and Singh, 2017), collected from Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2022)

Distinguishing features: parafrontalia slightly narrower than the breadth of frons, covered with golden tomentum. Antennae, arista and palpi orange, Parafacialia and genae completely orange, Anterior spiracles dark brown, Subcostal sclerite covered with brown felted pubescence and small erect hairs. A row of setulae on the upper posterior side on the stem vein. Upper calypter was with ventral hairs on the opaque white basal part.

***Chrysomya chani* Kurahashi, 1979**

Distribution: Western Ghats, Thottilpalam, Calicut, Thrissur, Palakkad and Ernakulam, Kerala

Remarks/Source: Western Ghats (Bharti, 2014), Calicut (Bharti and Singh, 2017), Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2021)

Distinguishing features: Fuscous to black coloured genae and parafacialia, setulae and hairs on parafacialia and parafrontalia were black in colour, brown to fuscous 1st , 2nd and 3rd antennal segments, black hairs on the venter of tergite V, prothoracic spiracle fuscous black in colour, black coloured epaulet and basicosta, dense basal tuft of black hairs on the subcostal sclerite, black setae on the upper margin of 3rd longitudinal vein, base of alar squamae white and ventrally bare except for fringe.

***Chrysomya nigripes* Aubertin, 1932**

Distribution: Calicut, Kerala, India

Remarks/Source: (Bharti and Singh, 2017)

Distinguishing features: Parafrontalia and parafacialia with grey tomentum, genae grey, antennae dark brown, anterior spiracle white, only one katepisternal setae developed, all hairs on the tergite V black, prothoracic stigma white, hind margins of second and third segments of abdomen dark banded, basicostal scale dark brown, sub costal sclerite with pale hairs, squama white.

Chrysomya rufifacies Macquart, 1842

Distribution: Throughout India, Calicut, Thrissur, Palakkad and Ernakulam in Kerala

Remarks/Source: Nandi, 2004, Calicut (Bharti and Singh, 2017) Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2021)

Distinguishing features: Third antennal segment brownish red on the inner surface. Parafrontalia narrowed with a black colour in the upper half, lower half covered with silver tomentum with upstanding white hairs, parafacialia and genae light yellowish and covered with white hairs, anterior spiracle white, few white hairs present on the tergite V, and upper squama white. The lower squama was slightly fuscous with white hairs.

Chrysomya albiceps Wiedemann, 1819

Distribution: Periyar Lake, in the Periyar Tiger Reserve, Thekkady, Kerala,

Remarks/Source: (Radhakrishnan et al., 2012)

Distinguishing features: Third antennal segment blackish brown, proepimeral seta absent, two katepiternal setae, dorsal part of thorax glossy with a little dusting, anterior spiracle white, few white hairs on the posterior edge of tergite V with incision, black transverse narrow marginal abdominal bands on the 3^{rd} and 4^{th} segments.

Subfamily: Rhiniinae

Genus: *Idiella* Braeuer and Berensteamn, 1889

Idiella euidielloides Senior-White, 1922

Distribution: Cardamom Estate (Kerala)

Remarks/Source: (Arce et al., 2020)

Distinguishing features: Basicosta black, sternopleuron and mesopleuron with distinct piliferous spots, first and second tergite with few black lateral bristles, posteroventral surface of hind tibia with longer hairs, tibial hairs not exceeding the width of tibia.

Idiella mandarina **Wiedemann, 1830**

Distribution: Thiruvananthapuram, Kerala

Remarks/Source: (Nandi, 2004)

Distinguishing features: Frontal stripe brownish black, white parafrontalia with black spots, genae shining black, antennae brown, black palpi, lower half of the occiput with dense hairs, pleurae with dense golden hairs, tibiae and first tarsal joint brown and rest of tarsi black.

Genus: *Stomorhina* Rondani, 1861

Stomorhina discolor **Fabricius, 1794**

Distribution: Cardamom Estate (Kerala)

Remarks/Source: : (Nandi, 2004)

Distinguishing features: Frontal stripe dark brown, parafacialia and parafrontalia white with shining black spots, epistome and genae shining black, antennae and palpi brown, green thorax densely grey dusted with small black spots, anterior lower meso pleuron and anterior sternopleuron glossy black, abdominal segments with black hind margins with a black median stripe, hind femur yellowish at base, tibiae and tarsi brownish yellow.

Genus: *Cosmina* Robineau-Desvoidy, 1830

Cosmina bicolor Walker, 1856

Distribution: Nilgiris (Kerala)

Remarks/Source: (Nandi, 2004)

Distinguishing features: Parafrontalia greyish with black spots, parafacialia silvery white, antennae yellowish brown, palpi black, propleuron hairy, mesopleuron metallic green, sub median mesonotal stripes broad, abdominal segments with a median stripe, strong bristles close to the apex of fifth sternite, hypopygium without strong spines, epaulet reddish brown.

Cosmina simplex Walker, 1858

Distribution: Kochi, Kerala

Remarks/Source: (Nandi, 2004)

Distinguishing features: Parafacialia silvery white with black spots, parafrontalia greyish with shining black spots, genae shining black, antennae yellowish brown, thorax copper green with black spots, long bristles on the entire surface of fifth visible sternite, hypopygium with curved laterally directed spines.

Genus: *Strongyloneura* Bigot, 1886

Strongyloneura prolata Walker, 1860

Distribution: Chalakudy, Kerala

Remarks/Source: (Nandi, 2004)

Distinguishing features: Mesopleuron without bristle on its upper part, third sternite without tuft of hair, fourth sternite with tuft of hair, fifth sternite and hypopygium are well developed, last sternite projected posteriorly and widely uncovered by corresponding tergites, bend of vein M_{1+2} gently curved.

4.1.1. Identification

Chrysomya megacephala

Morphological identification

Adult fly

Blow fly samples collected during the current study were identified up to the level of species using existing literature and taxonomic keys. Morphological features of head, thorax, abdomen and wings of *C. megacephala* are discussed below.

Head

Eyes were holoptic in males and dichoptic in females. Facets of upper two-thirds in the male eyes were enlarged and was clearly demarcated from the smaller facets below. In females, the facets were uniformly small. In males, the parafrontalia was reduced to a fine line covered with golden tomentum. The right and left paprafrontalia slightly narrower than the breadth of the frons. Antennae, arista and palpi orange in colour. Outer vertical bristles were absent in males. Parafacialia and genae completely orange in colour. Hairs and setulae on the parafacialia yellowish. In females, the frontal stripe is broader at the middle of frons than in male fly (Fig. 4.1 B-C).

Thorax

Thorax was bluish green with anterior spiracles dark brown. Femora was not swollen in both sexes (Fig. 4.1. D).

Abdomen

Bluish green. First segment bluish black and second and third segments were banded posteriorly. Absence of median incision in tergite V of females. Ventral aspect of tergite V

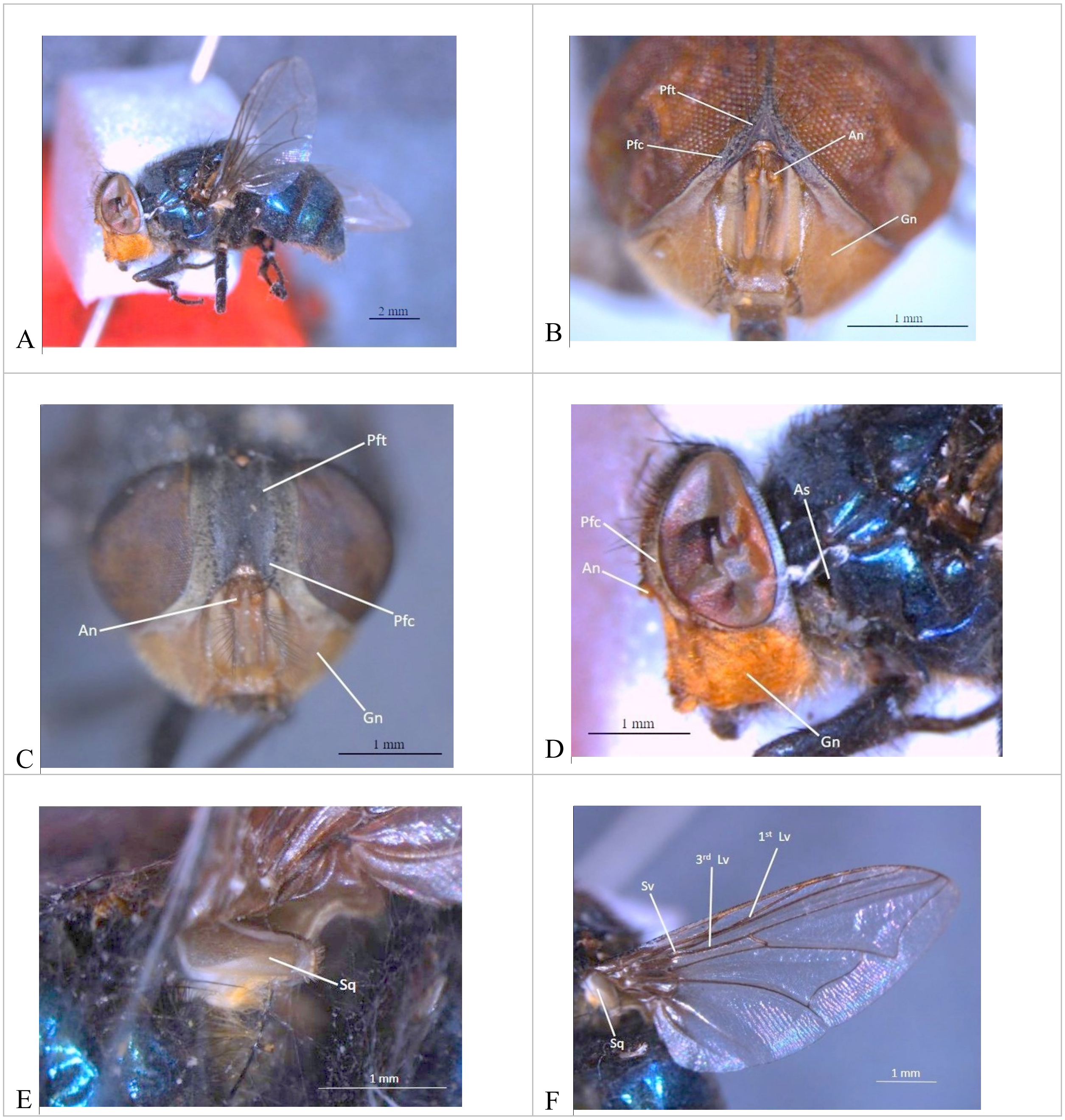

Fig. 4.1. Characteristics of *C. megacephala* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E.Wing base showing calypter F. Wing venation with setulae

An- Antenna, As - Anterior spiracle, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft-parafrontalia , Sq- Squama, Sv - Stem vein

was intermixed with yellow hairs. Golden hairs were seen on the sternites and tergites (Fig. 4.1. D-E).

Wings

Darkened at the base and hyaline in nature. Basicostal scale dark brown to black. Sub costal sclerite covered with brown felted pubescence and also with small erect hairs. A row of setulae were seen on the upper posterior margin on the stem vein. Squama showing upper calypter with ventral hairs on the opaque white basal part. Except the pale base, upper and lower calypters were brown (Fig. 4.1. F).

Molecular characterisation

Molecular sequencing of CO I gene

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *C. megacephala* using the primers; LCO (forward) and HCO (reverse). The DNA sequence interpret (Fig. 4.2), conceptual translational product (Fig.4.3), Agarose Gel Electrophoresis of PCR products (Fig.4.4), and the electropherogram (Fig. 4.5 a & b) are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.6). The isolated sequence was submitted in GenBank, NCBI with accession No: MW 522614 (Appendix II).

```
CGGAGCTTGA TCCGGAATAG TAGGAACTTC ATTAAGTATT TTAATTCGAG CTGAATTAGG
ACACCCTGGA GCATTAATTG GAGACGACCA AATTTATAAT GTAATTGTAA CAGCTCACGC
TTTTATTATA ATTTTCTTTA TAGTAATGCC AATTATAATT GGAGGATTTG GAAATTGACT
AGTTCCTTTA ATGTTAGGAG CTCCAGATAT AGCTTTCCCA CGAATAAATA ATATAAGTTT
CTGACTTTTA CCTCCTGCAT TAACTTTATT ATTAGTAAGT AGTATAGTAG AAAATGGGGC
TGGAACAGGA TGAACTGTTT ACCCACCTTT ATCTTCTAAT ATTGCTCATG GAGGAGCATC
AGTTGATTTA GCTATTTTCT CCTTACACTT AGCAGGAATT TCTTCAATTT TAGGAGCTGT
AAATTTTATT ACAACTGTAA TTAATATACG ATCTACAGGA ATTACATTTG ATCGAATACC
TTTATTTGTA TGATCTGTAG TTATTACTGC TCTATTATTA TTATTATCTT TACCAGTATT
AGCTGGAGCT ATTACTATAT TATTAACTGA CCGAAATCTA AATACTTCAT TCTTTGATCC
AGCAGGAGGA GGAGATCCTA TTTTATATCA ACATTTAA
```

Fig. 4.2. Partial coding sequence of mitochondrial COI gene of *C.megacephala.*

(>MW522614.1 *C. megacephala* isolate CMRP02 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (638 bp)

```
GAWSGMVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMI

FFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAGT

GWTVYPPLSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMP

LFVWSVVITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHL
```

Fig. 4.3. Translational product of mitochondrial COI gene of *C.megacephala*

>QQX23394.1 cytochrome c oxidase subunit I, partial Amino acid sequences

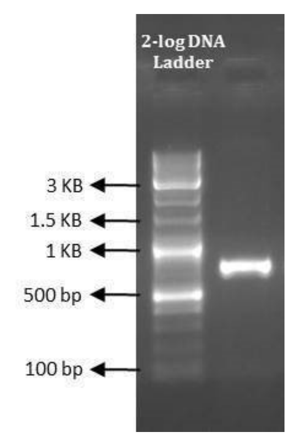

Fig. 4.4. Agarose Gel Electrophoresis of the PCR product

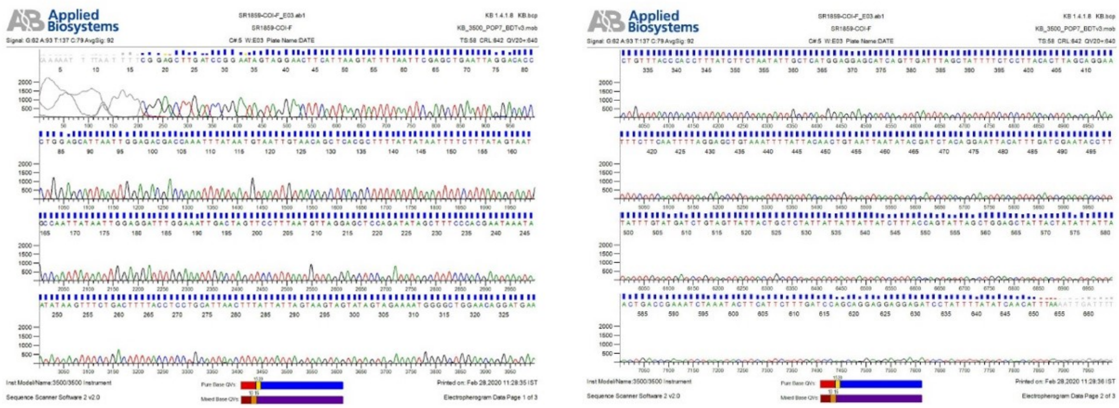

Fig. 4.5 a. Electropherogram of mitochondrial COI gene of *C.megacephala* using forward primer

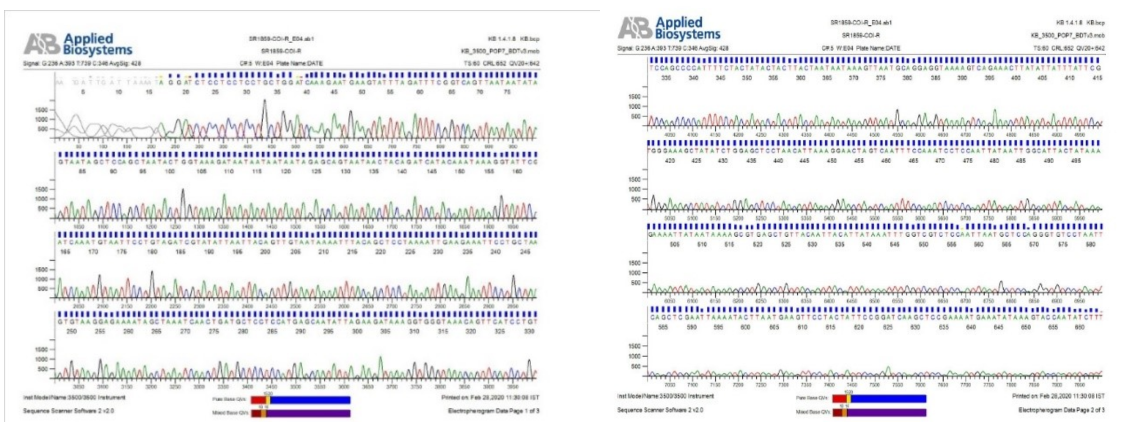

Fig. 4.5. b. Electropherogram of mitochondrial COI gene of *C.megacephala* using reverse primer

Fig. 4.6. Phylogenetic tree based on COI sequence, SR1859-COI-F_E03 of *C.megacephala* by Maximum Likelihood method

This analysis involved 28 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were 638 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *C.megacephala* with strong boot strap support.

Chrysomya rufifacies

Morphological identification

Adult fly

Head

Facets of eyes in both sexes were small. Third antennal segment brownish red on the inner surface. Parafrontalia narrow with a black colour in the upper half. The lower half covered with silver tomentum with white erect hairs. Frons greyish in colour. Parafacialia

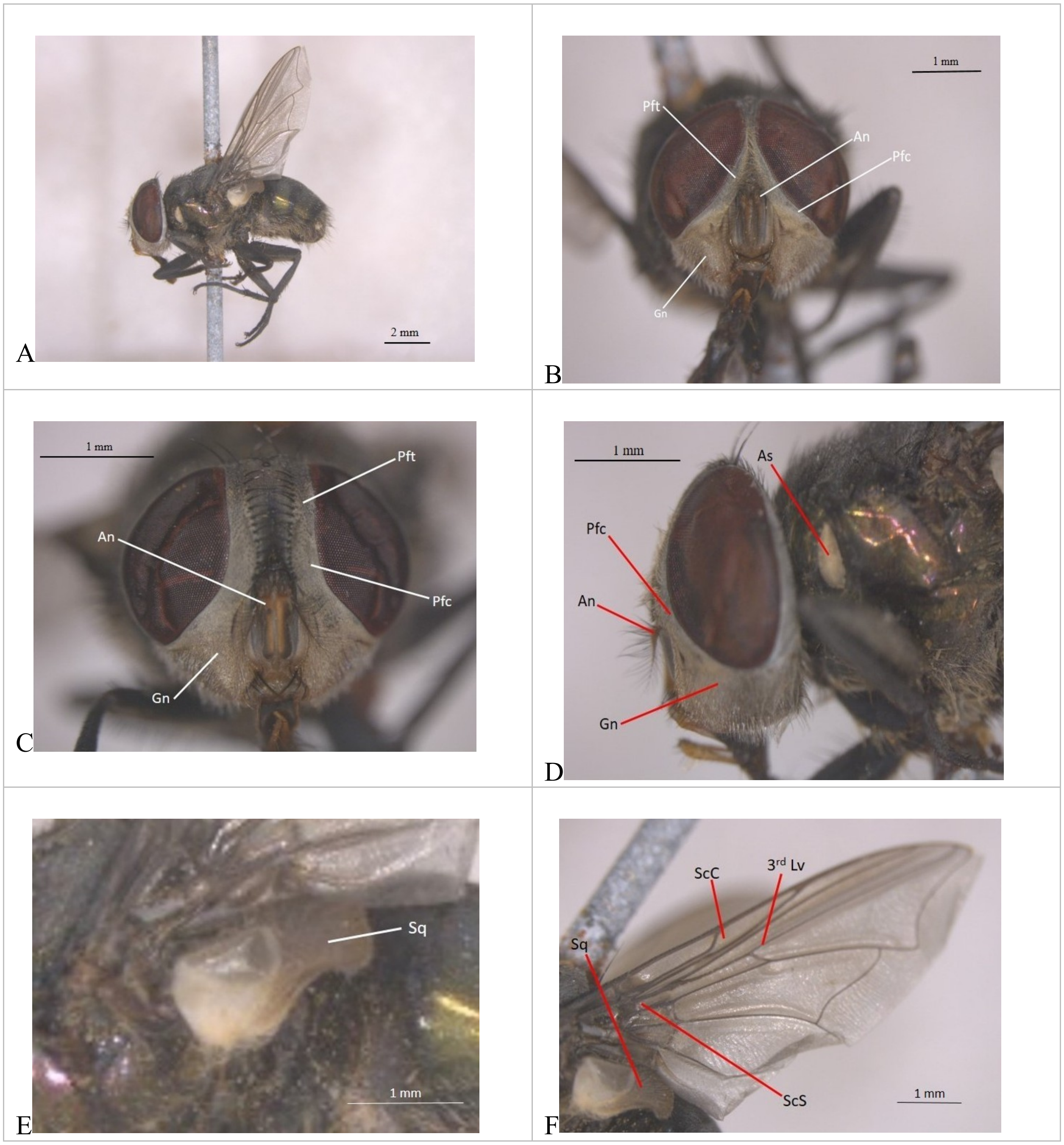

Fig. 4.7. Characteristics of *C. rufifacies* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E.Wing base showing calypter F. Wing venation with setulae

An- Antenna, As - Anterior spiracle, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , ScC - Subcostal cell, ScS - subcostal sclerite, Sq- Squama, Sv - Stem vein

and genae light yellowish in colour and covered with white hairs. Antennae brown in colour. The palpi and epistome orange in colour (Fig. 4.7 B-C).

Thorax

Dorsum of the thorax shiny and greenish blue colour with no dusting. Anterior spiracle white. First quarter of 3rd and one sixth of the 4th abdominal segments very narrow. Proepimeral setae present, two setae present on the katepisternum. Median incision present on the posterior edge of tergite V of females. Few white hairs present on the tergite V (Fig. 4.7 D-E).

Abdomen

Second and third segments dark banded on the posterior margins. Fine golden hairs were present on the venter.

Wings

Hyaline in nature and infuscated at the base of the subcostal cell. Basicostal scale was brownish black. Subcostal sclerite with no hairs. Upper squama was white in colour. The lower squama was slightly fuscous in colour with white hairs. (Fig. 4.7 F).

The length of the fly was 9-10mm.

Molecular characterisation

Molecular sequencing of CO I gene

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *C. rufifacies* using the primers; LCO (forward) & HCO (reverse). The DNA sequence interpret (Fig. 4.8), conceptual translational product (Fig.4.9), Agarose Gel Electrophoresis of PCR product (Fig.4.10), and the electropherogram (Fig. 4.11 a & b), are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.12). The isolated sequence was submitted in GenBank, NCBI with accession No: OM019083.1 (Appendix II).

```
GGAACTTCTT TAAGAATCCT AATTCGAGCT GAATTAGGAC ATCCTGGAGC ACTAATTGGG
GATGACCAAA TTTATAATGT AATTGTAACA GCTCATGCTT TTATTATAAT TTTCTTTATA
GTAATACCAA TTATAATTGG AGGATTTGGA AATTGACTAG TCCCTCTAAT ACTAGGAGCC
CCAGATATAG CTTTCCCACG AATAAATAAT ATAAGTTTTT GACTTTTACC TCCTGCATTA
ACTTTACTAT TAGTAAGTAG TATAGTAGAA AATGGAGCTG GAACAGGATG AACTGTTTAT
CCACCTTTAT CATCTAATAT TGCACATGGT GGAGCATCAG TTGATTTAGC TATTTTTTCT
TTACACTTAG CTGGAATTTC ATCAATTTTA GGAGCCGTAA ATTTTATTAC AACTGTTATT
AATATACGAT CTACAGGAAT TACATTTGAT CGAATACCTT TATTTGTATG ATCTGTAGTT
ATTACTGCTC TTCTTTTATT ATTATCATTA CCAGTATTAG CAGGTGCAAT TACTATATTA
TTAACTGATC GAAATTTAAA TACTT
```

Fig. 4.8. Partial coding sequence of mitochondrial COI gene of *C.rufifacies*
(> OM019083.1 *C. rufifacies* isolate CRRP04 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (565 bp))

```
GTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPI

MIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAGTGWTVYPP

LSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVWSVV

ITALLLLLSLPVLAGAITMLLTDRNLNT
```

Fig. 4.9. Translational product of mitochondrial COI gene of C. *rufifacies*
(> UHQ29699.1 cytochrome c oxidase subunit I, partial Amino acid sequences)

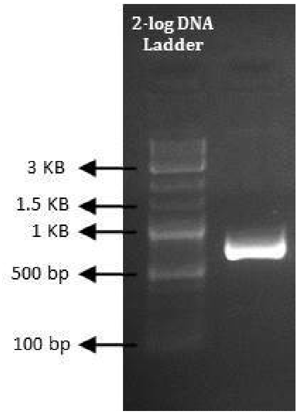

Fig. 4.10. Agarose Gel Electrophoresis of the PCR product

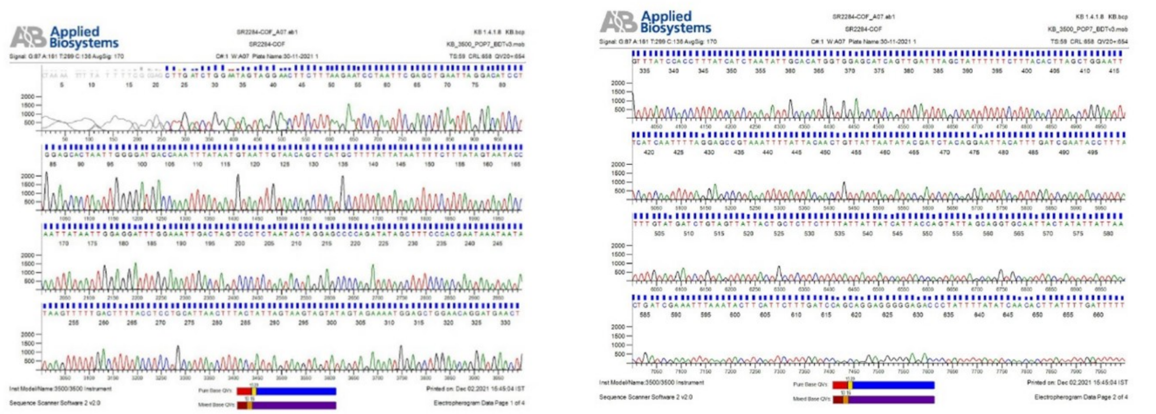

Fig. 4.11 a. Electropherogram of the mitochondrial COI gene of *C.rufifacies* using forward primer

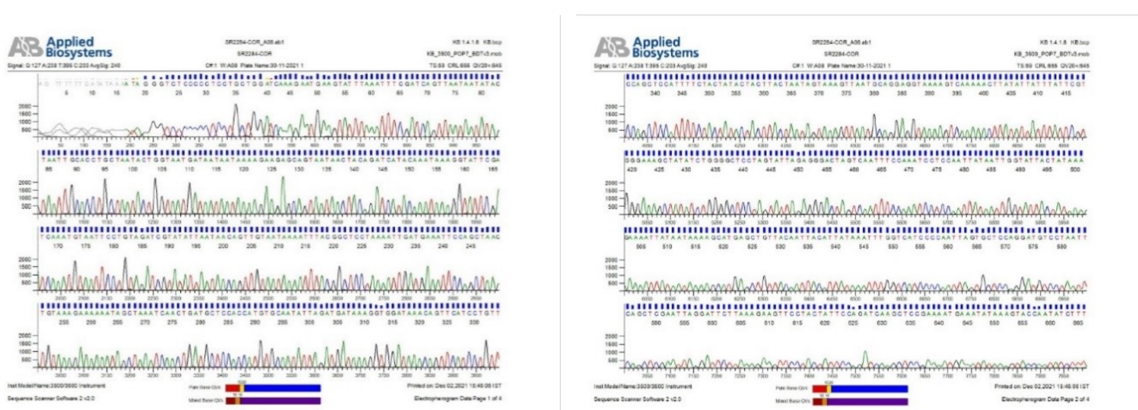

Fig. 4.11 b. Electropherogram of the mitochondrial COI gene of *C.rufifacies* using reverse primer

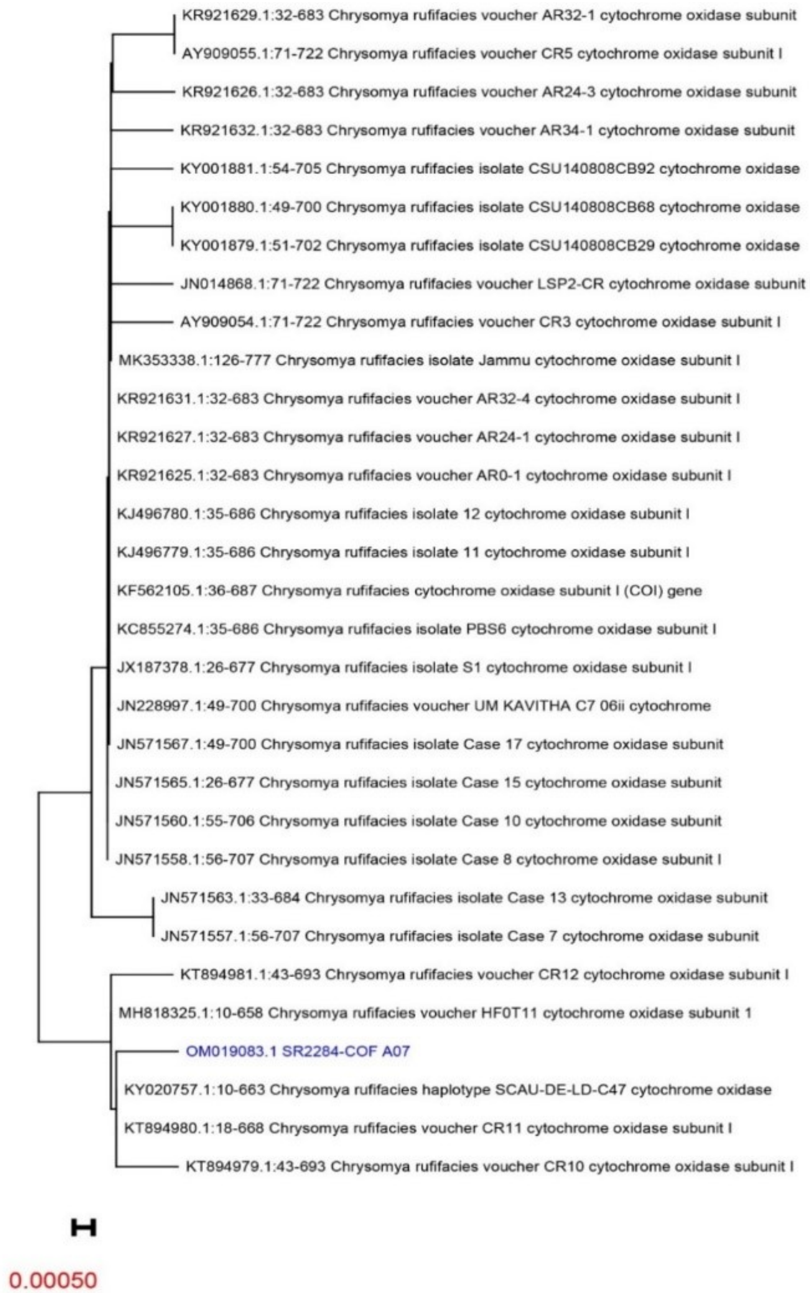

Fig. 4.12. Phylogenetic tree based on COI sequence *SR2284-COF_A07* of *C. rufifacies* by Maximum Likelihood method

This analysis involved 31 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were 655 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *C. rufifacies* with strong bootstrap support.

Chrysomya chani

Morphological identification

Adult fly

Head

Eyes holoptic in males and dichoptic in females. The upper facets of the eye were enlarged and clearly demarcated from the small facets. Facial ridge was present. Genae and parafacialia were fuscous to black in colour. Setulae and hairs on parafacialia and parafrontalia were blackish in colour. 1st and 2nd antennal segments were brown to fuscous in colour. 3rd segment was fuscous in colour (Fig. 4.13. B-C).

Thorax

Thorax was bluish green coloured with anterior spiracle fuscous black in colour. Black hairs were present on the pleura. Median cleft was present on the female tergite V with black hairs on the venter (Fig. 4.13. D-E).

Wings

Wings hyaline. Epaulet and basicosta are black coloured. Dense basal tuft of black hairs present on the subcostal sclerite. Black setae present on the upper margin of 3rd longitudinal vein. Base of alar squamae is white in colour and ventrally it is bare except for fringe (Fig. 4.13. F).

Molecular characterisation

Molecular sequencing of CO I gene

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *C. chani* using the primers; LCO (forward) & HCO (reverse).

The DNA sequence interpret (Fig. 4.14), conceptual translational product (Fig. 4.15), Agarose Gel Electrophoresis of PCR product (Fig. 4.16), and the electropherogram (Fig. 4.17 a-b), are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.18).The isolated sequence was submitted in GenBank, NCBI with accession no: MW600494.1 (Appendix II).

```
TTCGGAGCTT GATCCGGAAT AGTAGGAACT TCATTAAGAA TTTTAATTCG AGCTGAATTA
GGACACCCTG GAGCATTAAT TGGAGATGAC CAAATTTATA ATGTAATTGT AACAGCTCAC
GCTTTTATTA TAATTTTTTT TATAGTAATA CCAATTATAA TTGGAGGATT TGGAAATTGA
TTAGTTCCTT TAATACTAGG AGCCCCAGAT ATAGCTTTCC CACGAATAAA TAATATAAGT
TTCTGACTTT TACCTCCTGC ATTAACTTTA TTATTAGTAA GTAGTATAGT AGAAAATGGA
GCTGGAACAG GATGAACTGT TTATCCACCT TTATCTTCTA ATATTGCTCA TGGAGGAGCA
TCAGTTGATT TAGCTATTTT TTCTTTACAT TTAGCAGGAA TTTCTTCAAT TTTAGGAGCT
GTAAATTTTA TTACTACAGT AATTAATATA CGATCTACAG GAATTACATT TGATCGAATA
CCTCTATTTG TTTGATCAGT AGTTATTACT GCTTTATTAT TATTATTATC TTTACCAGTA
TTAGCAGGAG CTATTACTAT ATTATTAACT GATCGAAATT TAAATACTTC ATTCTTTGAT
CCAGCA
```

Fig: 4.14. Partial coding sequence of mitochondrial COI gene of *C. chani*

> MW600494.1 *C. chani* isolate CCRP03 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (606 bp)

```
FGAWSGMVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIM

IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAG

TGWTVYPPLSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRM

PLFVWSVVITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPA
```

Fig. 4.15. Translational product of mitochondrial COI gene of *C. chani*

>QRQ47096.1 cytochrome c oxidase subunit I, partial Amino acid sequences

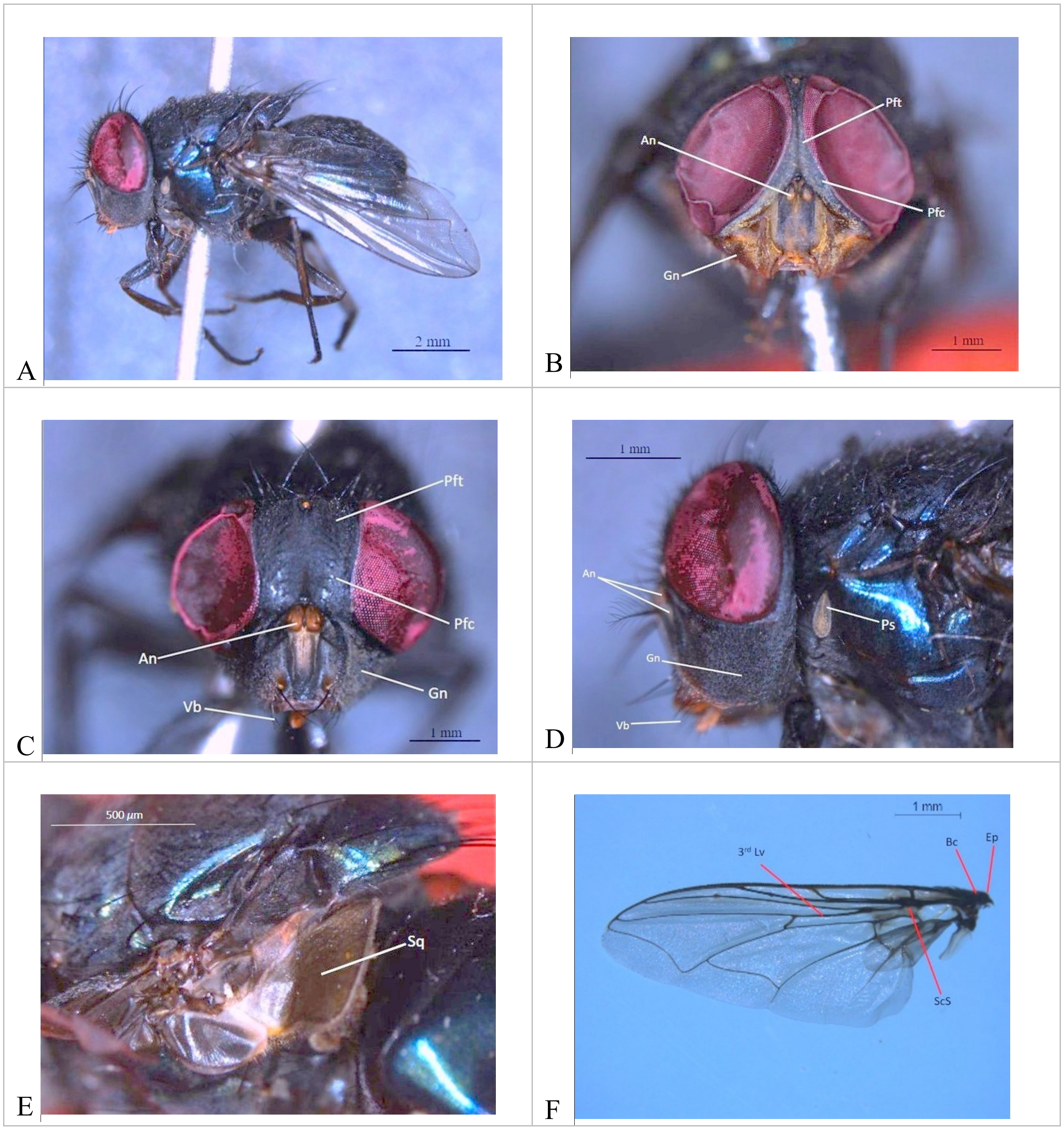

Fig. 4.13. Characteristics of *C. chani* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E. Wing base showing calypter F. Wing venation with setulae

An- Antenna, As - Anterior spiracle, Bc – Basicosta, Ep – Epaulet, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , ScC - Subcostal cell, ScS - subcostal sclerite, Sq- Squama, Sv - Stem vein, Vb - Vibrissae

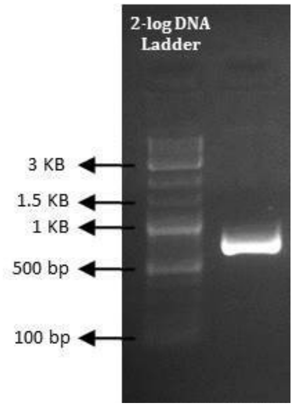

Fig. 4.16. Agarose Gel Electrophoresis of the PCR product

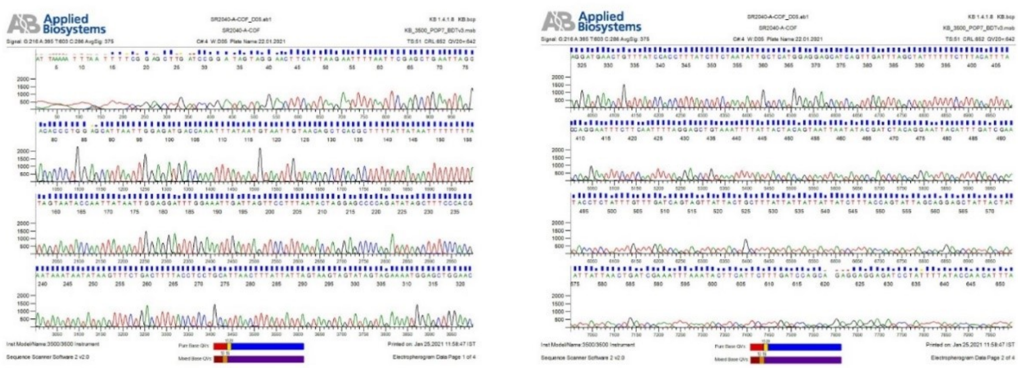

Fig. 4.17 a. Electropherogram of the mitochondrial COI gene of *C. chani* using forward primer.

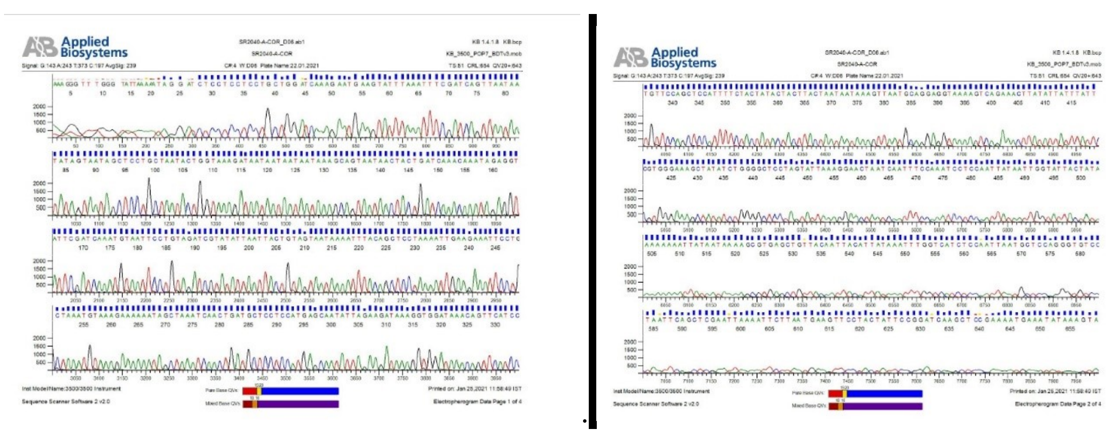

Fig. 4.17 b. Electropherogram of the mitochondrial COI gene of *C. chani* using reverse primer

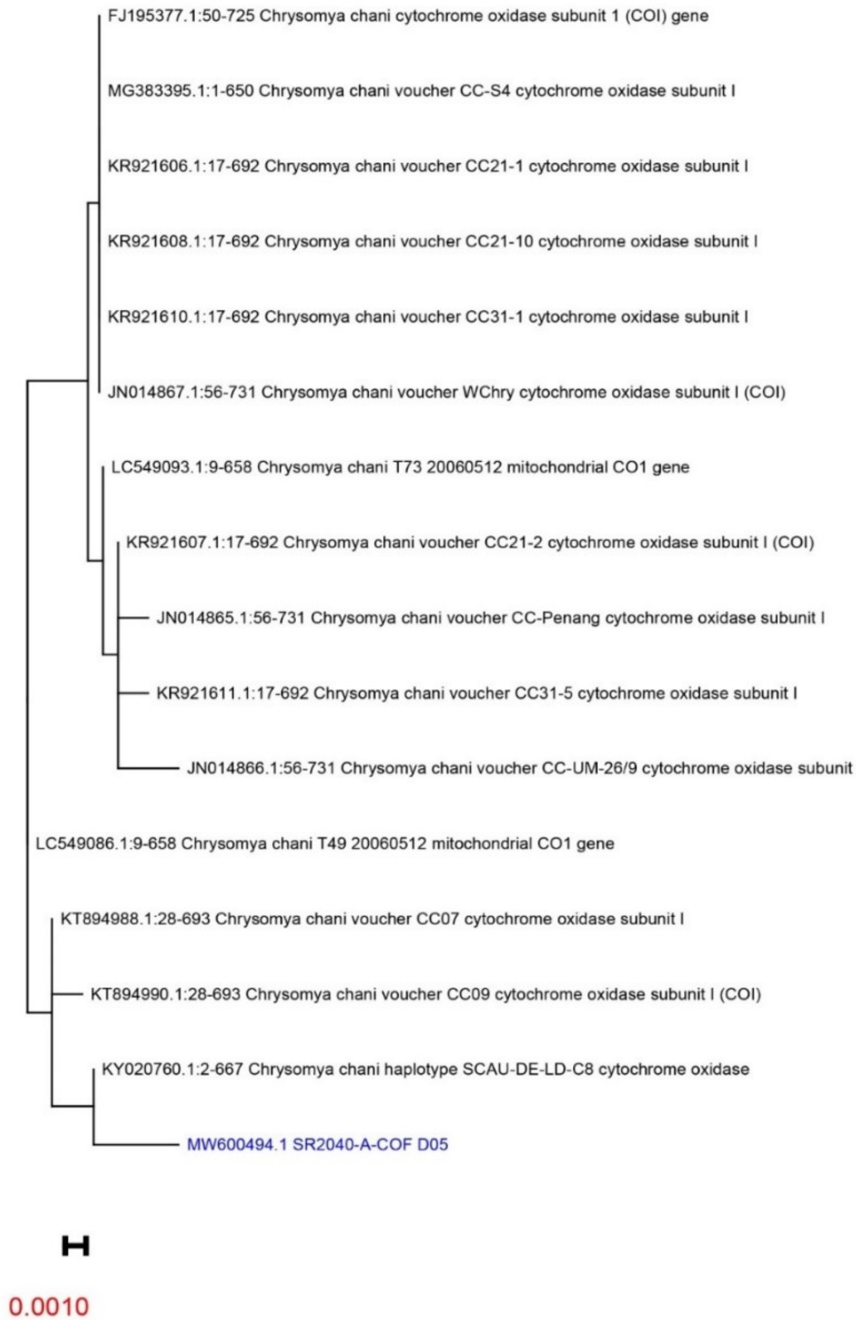

Fig. 4.18. Phylogenetic tree based on COI sequence, *SR2040-A-COF_D05* of *C. chani* by Maximum Likelihood method

This analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were 685 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *C. chani* with strong boot strap support.

Hemipyrellia ligurriens

Morphological identification

Adult fly

Head

The length of the third antennal segment is shorter than the distance between the eyes in males. Parafrontalia is narrower. Genae and parafrontalia silver white. Antennae tawny yellow to brownish. Palpi orange in colour. Parafrontalia and frons are with similar width (Fig. 4.19. B-C).

Thorax

Greenish purple shining present on the thorax. Anterior part of the thorax and lower part of hypopleuron heavily dusted. The squama whitish. The upper squama creamish white with short cilia and lower squama with light brown cilia (Fig. 4.19. D-E).

Abdomen

Greenish purple shining is present on the abdomen. Posterior margins of the abdomen dark. In males, sparse short hairs are seen on the edges of tergites and first visible sternite.

Wings

Wings hyaline. Setulae are not present on the stem vein and 1st longitudinal vein, but short setulae are present on the dorsal and ventral aspects of 3rd longitudinal vein (Fig. 4.19. F).

Length of the adult fly is 9-10 mm

Molecular characterisation

Molecular sequencing of CO I gene

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *H. ligurriens* using the primers; LCO (forward) and HCO (reverse). The DNA sequence interpret (Fig. Fig. 4.20), conceptual translational product (Fig.4.21), Agarose Gel Electrophoresis of PCR product (Fig.4.22), and the electropherogram (Fig. 4.23 a & b), are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.24). The isolated sequence was submitted in GenBank, NCBI with accession no: MN831480.1 (Appendix II).

```
CAGGAATAAT TGGAACTTCA TTAAGAATTC TAATTCGAGC TGAATTGGGA CACCCTGGAG
CTTTAATTGG AGATGACCAA ATCTATAATG TAATTGTAAC AGCTCATGCT TTTATTATAA
TTTTTTTTAT AGTAATACCA ATTATAATTG GAGGATTTGG AAATTGATTA GTTCCTTTAA
TATTAGGAGC CCCAGATATA GCATTCCCTC GAATAAATAA TATAAGTTTT TGACTTTTAC
CTCCTGCATT AACTTTATTA TTAGTAAGCA GTATAGTAGA AAACGGAGCT GGAACAGGAT
GAACAGTTTA CCCTCCTTTA TCATCTAATA TTGCCCATGG AGGAGCTTCT GTAGATCTAG
CTATTTTCTC TTTACATTTA GCAGGAATTT CATCAATTTT AGGAGCTGTA AATTTCATTA
CAACAGTAAT TAATATACGA TCAACAGGTA TTACTTTTGA TCGAATACCT TTATTTGTTT
GATCTGTAGT AATTACAGCT TTATTACTTT TATTATCATT ACCAGTATTA GCAGGAGCTA
TTACTATACT TTTAACAGAC CGAAATCTAA ACACTTCATT CTTTGATCCA GCTGGAGGAG
GAGATCCAAT TTTATATCAA CATTTATTTT GATTTTTTGG TCACCAGA
```

Fig. 4.20. Partial coding sequence of mitochondrial COI gene of *H. ligurriens*

(>MN831480 *H. ligurriens isolate* HLRP01 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (648 bp)

```
GMIGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMIFFMV

MPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAGTGWTV

YPPLSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVW

SVVITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHQ
```

Fig. 4.21. Translational product of mitochondrial COI gene of *H. ligurriens*

(> QNG41879.1 cytochrome c oxidase subunit I, partial Amino acid sequences)

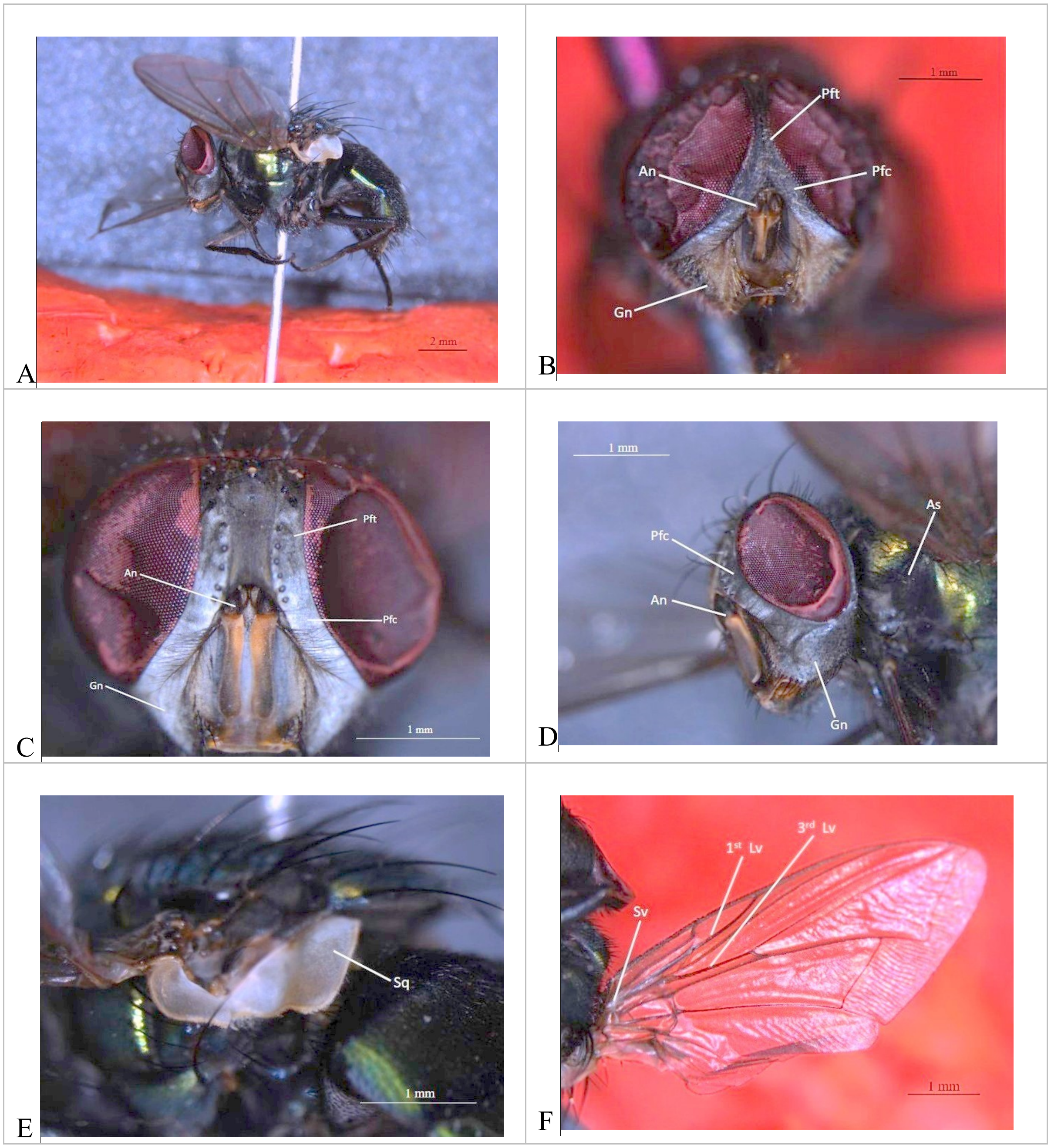

Fig. 4.19. Characteristics of *H. ligurriens* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E. Wing base showing calypter F. Wing venation with setulae

An- Antenna, As - Anterior spiracle, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , Sq- Squama, Sv - Stem vein

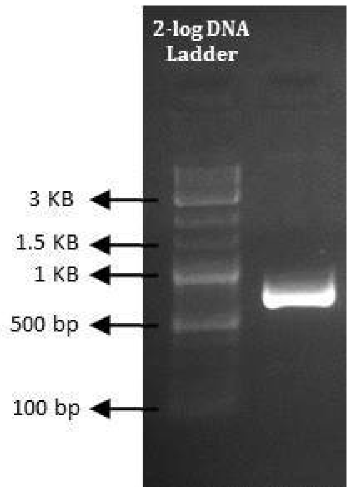

Fig. 4.22 Agarose Gel Electrophoresis of the PCR product

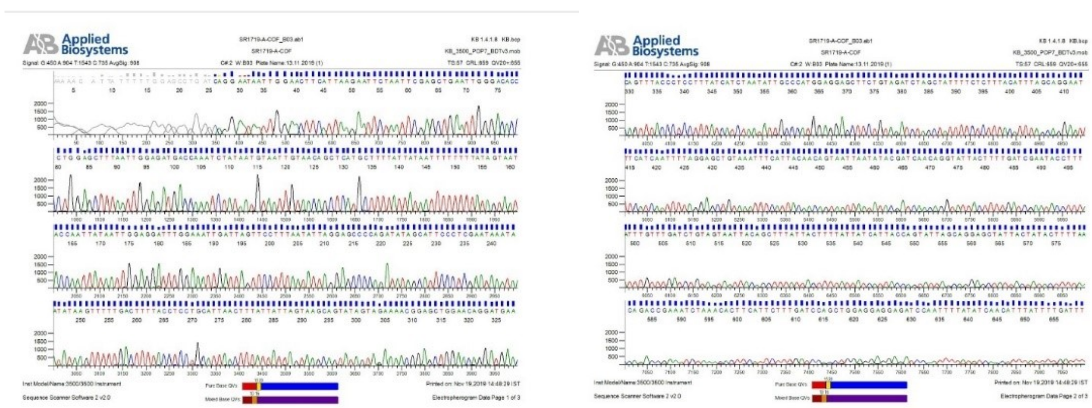

Fig. 4.23 a. Electropherogram of the mitochondrial COI gene of *H. ligurriens* using forward primer

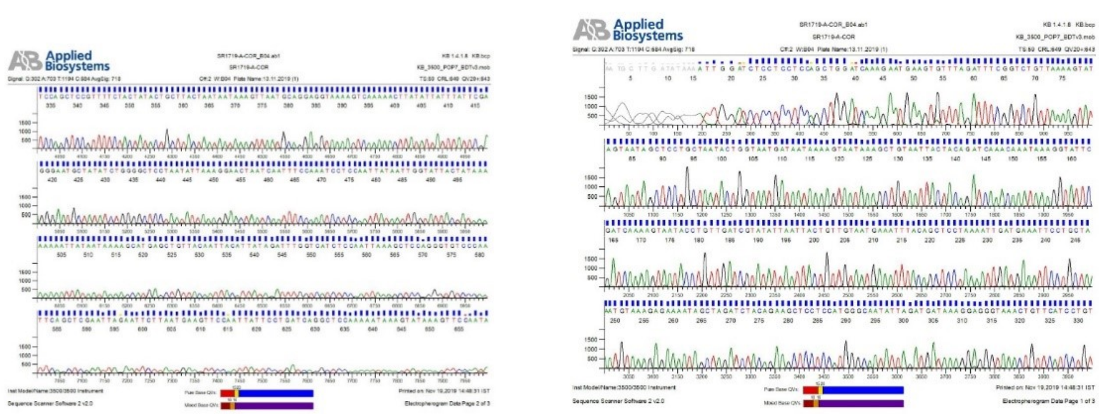

Fig. 4.23. b. Electropherogram of the mitochondrial COI gene of *H. ligurriens* using reverse primer

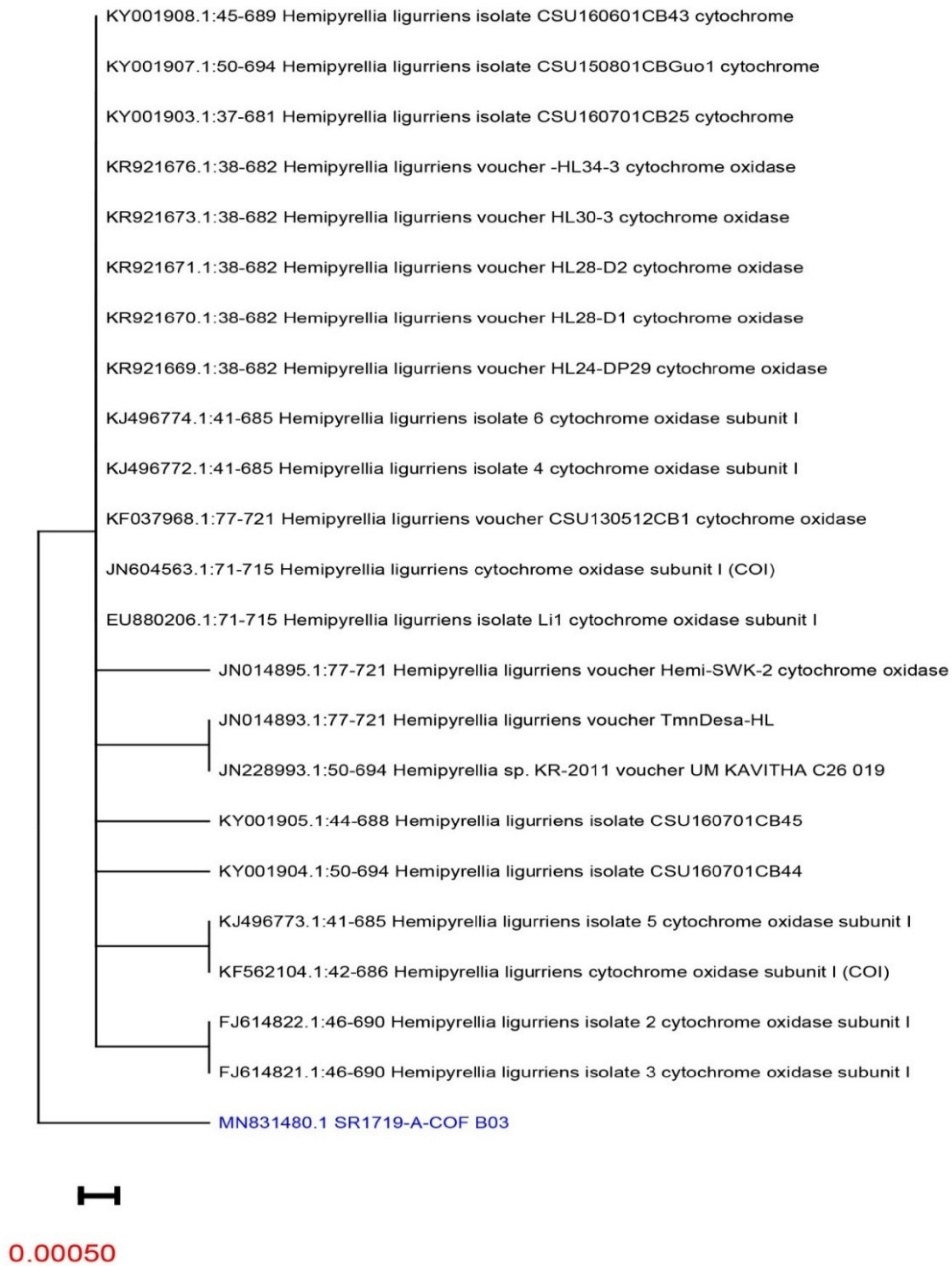

Fig.4.24. Phylogenetic tree based on COI sequence, *SR1719-A-COF_B03* of *H. ligurriens* by Maximum Likelihood method

This analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were 648 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *H.ligurriens* with strong boot strap support.

4.2. Seasonal differences in blow fly population in Central Kerala

Abundance

Seasonal differences in the number of adult blow flies, the number of male and female flies and the difference in abundance between seasons and also between years was considered for data analysis.

4.2.1. *Chrysomya megacephala:*

Effect of season on the abundance of *C. megacephala* was found to be significant (F = 52.773; P = 0.01). The abundance of *C. megacephala* was significantly higher in monsoon (70.37 ± 9.52) in comparison with summer (65.33 ± 9.23) and winter (45.56 ± 7.88) (Table 4.1).The interaction between years and seasons were found to be non-significant (F = 1.567; P= 0.419) indicating that seasonal variations were same in all years.

Table 4.1. Abundance (%) of *C. megacephala* between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	75.44 ± 8.56	65.44 ± 9.68	45.56 ± 7.88	62.15 ± 9.68
2020	67.67 ± 6.44	66.78 ± 8.51	42.00 ± 10.15	58.81 ± 8.51
2021	68.00 ± 11.67	63.78 ± 10.28	50.11 ± 9.03	60.63 ± 10.28
Overall Season	70.37 ± 9.52^{a}	65.33 ± 9.23^{b}	45.89 ± 9.35^{c}	60.53 ± 9.23
Between year F-value = 0.879^{ns}; (P-value = 0.419) Between season F-value = 52.773**; (P-value < 0.001)) Interaction between season and year F-value = 1.567^{ns}; (P-value = 0.192)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season on the abundance of *C. megacephala* male flies was found to be significant (F = 6.357; P = 0.003). The abundance of male flies was significantly lower in monsoon (23.90 ± 6.82) in comparison with summer (28.13 ± 5.74) and winter (30.30 ± 7.37) The interaction between years and seasons were found to be non-significant (F =0.557; P= 0.694) indicating that seasonal variations were same in all years (Table 4.2).

Table 4.2. Abundance (%) of *C. megacephala* male flies between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	24.56 ± 9.74	29.55 ± 5.32	29.61 ± 6.78	27.91 ± 5.32
2020	21.66 ± 5.03	27.39 ± 5.50	27.90 ± 6.87	25.65 ± 5.50
2021	25.49 ± 4.69	27.46 ± 6.71	33.39 ± 8.09	28.78 ± 6.71
Overall Season	23.90 ± 6.82^{b}	28.13 ± 5.74^{a}	30.30 ± 7.37^{a}	27.45 ± 5.74
Between year F-value = 1.574^{ns}; (P-value = 0.214) Between season F-value = 6.357**; (P-value = 0.003)) Interaction between season and year F-value = 0.557^{ns}; (P-value = 0.694)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season on the abundance of *C. megacephala* female flies was found to be significant (F = 6.357; P = 0.003). The abundance of female flies was significantly higher in monsoon (76.50 ± 6.18) in comparison with summer (71.21 ± 6.55) and winter (67.94 ± 10.29). The interaction between years and seasons were found to be non-significant (F = 0.557; P = 0.694) indicating that seasonal variations were same in all years. (Table 4.3)

Table 4.3. Abundance (%) of *C. megacephala* female flies between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	76.99 ± 8.38	68.47 ± 7.17	68.60 ± 10.55	71.35 ± 7.17
2020	78.02 ± 4.94	72.61 ± 5.50	71.53 ± 8.18	74.06 ± 5.50
2021	74.51 ± 4.69	72.54 ± 6.71	63.68 ± 11.43	70.24 ± 6.71
Overall Season	76.50 ± 6.18^{a}	71.21 ± 6.55^{b}	67.94 ± 10.29^{b}	71.88 ± 6.55
Between year F-value = 1.574^{ns}; (P-value = 0.214) Between season F-value = 6.357**; (P-value = 0.003)) Interaction between season and year F-value = 0.557^{ns}; (P-value = 0.694)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

4.2.2. *Chrysomya rufifacies:*

Effect of season (F = 4.006; P = 0.022) and year (F = 3.935; P = 0.024) on the abundance of *C. rufifacies* was found to be significant. The abundance of *C. rufifacies* was significantly lower in winter (38.44 ± 8.99) in comparison with monsoon (44.22 ± 7.59) and summer (43.56 ± 9.38). The abundance was significantly higher in 2021 (45.56 ± 7.91) compared to 2019 (39.48 ± 8.73) and 2020 (41.19 ± 6.46). The interaction between years and seasons were found to be non-significant (F = 1.567; P = 0.419) indicating that season wise variations were same in all years (Table. 4.4).

Table 4.4. Abundance (%) of *C. rufifacies* between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	45.44 ± 9.10	37.78 ± 8.73	35.22 ± 6.83	39.48 ± 8.73^{B}
2020	42.33 ± 8.03	41.78 ± 6.46	39.44 ± 9.10	41.19 ± 6.46^{AB}
2021	44.89 ± 5.82	51.11 ± 7.91	40.67 ± 10.72	45.56 ± 7.91^{A}
Overall Season	44.22 ± 7.59^{a}	43.56 ± 9.38^{a}	38.44 ± 8.99^{b}	42.07 ± 9.38
Between year F-value = 3.935*; (P-value = 0.024) Between season F-value = 4.006*; (P-value = 0.022) Interaction between season and year F-value =1.890^{ns}; (P-value = 0.122)				

* Significant at 0.05 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect of season (F = 1.654; P = 0.198), effect of year (F = 2.921; P = 0.060), and the interaction between year and season (F = 0.902; P = 0.468) were found to be non-significant on the abundance of *C. rufifacies* male files. The above results showed that there were no significant variations in the abundance of male flies between seasons and between years (Table 4.5).

Table 4.5. Abundance (%) of *C. rufifacies* male flies between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	30.18 ± 6.01	27.21 ± 7.17	30.35 ± 10.02	29.24 ± 7.17
2020	32.81 ± 10.32	34.74 ± 8.97	34.4 ± 8.9	33.98 ± 8.97
2021	28.65 ± 3.74	26.42 ± 6.76	34.83 ± 5.35	29.96 ± 6.76
Overall Season	30.55 ± 7.16	29.45 ± 8.32	33.19 ± 8.26	31.06 ± 8.32
Between year F-value = 2.921^{ns}; (P-value = 0.060) Between season F-value = 1.654^{ns}; (P-value = 0.198) Interaction between season and year F-value = 0.902^{ns}; (P-value = 0.468)				

ns non-significant

Effect of season (F = 1.654; P = 0.198), effect of year (F = 2.921; P = 0.060), and the interaction between year and season (F = 0.902; P = 0.468) were found to be non-significant on the abundance of *C. rufifacies* female files. The above results showed that there were no significant variations in the abundance of female flies between seasons and between years (Table 4.6).

Table 4.6. Abundance (%) of *C. rufifacies* female flies between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	70.11 ± 6.31	72.5 ± 6.94	72.24 ± 10.98	71.61 ± 6.94
2020	67.19 ± 10.32	65.85 ± 9.53	63.69 ± 7.29	65.58 ± 9.53
2021	71.35 ± 3.74	69.58 ± 8.68	64.97 ± 5.30	68.63 ± 8.68
Overall Season	69.55 ± 7.24	69.31 ± 8.58	66.96 ± 8.76	68.61 ± 8.58
Between year F-value = 2.921^{ns}; (P-value = 0.060) Between season F-value = 1.654^{ns}; (P-value = 0.198) Interaction between season and year F-value = 0.902^{ns}; (P-value = 0.468)				

ns non-significant

4.2.3. *Chrysomya chani:*

Effect of season on the abundance of *C. chani* was found to be significant (F = 33.586; P = < 0.001). The abundance of *C. chani* was significantly higher in monsoon (30.19 ± 5.00) in comparison with summer (22.22 ± 5.18) and winter (19.78 ± 4.85) (Table 4.7). The interaction between years and seasons were found to be non-significant (F = 1.814; P = 0.135) indicating that season wise variations were same in all years.

Table 4.7. Abundance (%) of *C. chani* between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	33.78 ± 2.99	22.78 ± 4.92	19.33 ± 4.66	25.30 ± 4.92
2020	28.22 ± 5.91	22.56 ± 6.27	18.22 ± 5.14	23.00 ± 6.27
2021	28.56 ± 3.94	21.33 ± 4.74	21.78 ± 4.55	23.89 ± 4.74
Overall Season	30.19 ± 5.00^{a}	22.22 ± 5.18^{b}	19.78 ± 4.85^{b}	24.06 ± 5.18
Between year F-value = 1.520^{ns}; (P-value = 0.226) Between season F-value = 33.586**; (P-value < 0.001)) Interaction between season and year F-value =1.814^{ns}; (P-value = 0.135)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season (F = 1.446; P = 0.242) and effect of year (F = 1.162; P-value = 0.319), were found to be non-significant on the abundance of *C.chani* male files. The interaction between year and season was found to be significant (F = 2.832; P = 0.031). In the year 2021, abundance of male flies was significantly lower in monsoon (26.99 ± 6.70) compared to summer (36.11 ± 4.86) and winter (36.01 ± 6.29). During winter, the abundance of male flies was significantly higher in the year 2021(36.01 ± 6.29%) in comparison to 2019 (27.00 ± 4.56) and 2020 (28.46 ± 7.61) (Table 4.8).

Table 4.8. Abundance (%) of *C. chani* male flies between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	32.06 ± 5.04	31.28 ± 7.27	27.00 ± 4.56^{B}	30.11 ± 7.27
2020	32.35 ± 10.84	32.64 ± 8.76	28.46 ± 7.61^{B}	31.15 ± 8.76
2021	26.99 ± 6.70^{b}	36.11 ± 4.86^{a}	36.01 ± 6.29^{aA}	33.04 ± 4.86
Overall Season	30.47 ± 8.01	33.34 ± 7.17	30.49 ± 7.25	31.43 ± 7.17
Between year F-value = 1.162^{ns}; (P-value = 0.319) Between season F-value = 1.446^{ns}; (P-value = 0.242) Interaction between season and year F-value =2.832^{*}; (P-value = 0.031)				

* Significant at 0.05 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect of season (F = 1.446; P = 0.242) and effect of year (F = 1.162; P = 0.319), were found to be non-significant on the abundance of *C. chani* female files. The interaction between year and season was found to be significant (F = 2.832; P = 0.031). In the year 2021, abundance of female flies was significantly higher in monsoon (73.01 ± 6.70) compared to summer (63.89 ± 4.86) and winter (63.99 ± 6.29). During winter, the abundance of female flies was significantly lower in the year 2021(63.99 ± 6.29%) in comparison to 2019 (73.00 ± 4.56) and 2020 (71.54 ± 7.61) (Table 4.9).

Table 4.9. Abundance (%) of *C. chani* female flies (%) between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	67.94 ± 5.04	68.72 ± 7.27	73.00 ± 4.56^{A}	69.89 ± 7.27
2020	67.65 ± 10.84	67.36 ± 8.76	71.54 ± 7.61^{A}	68.85 ± 8.76
2021	73.01 ± 6.70^{a}	63.89 ± 4.86^{b}	63.99 ± 6.29^{bB}	66.96 ± 4.86
Overall Season	69.53 ± 8.01	66.66 ± 7.17	69.51 ± 7.25	68.57 ± 7.17
Between year F-value = 1.162^{ns}; (P-value = 0.319) Between season F-value = 1.446^{ns}; (P-value = 0.242) Interaction between season and year F-value =2.832^{*}; (P-value = 0.031)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

4.2.4. *Hemipyrellia ligurriens:*

Effect of season on the abundance of *H. ligurriens* was found to be significant (F = 47.470; P = < 0.001). The abundance of *H. ligurriens* was significantly higher in monsoon (30.85 ± 7.42) in comparison with summer (18.56 ± 5.01) and winter (17.63 ± 4.58). The interaction between years and seasons were found to be non-significant (F =2.180; P=0.080) indicating that season wise variations were same in all years (Table 4.10).

Table 4.10. Abundance (%) of *H. ligurriens* between seasons and between years

Year	Monsoon	Summer	Winter	Overall year
2019	30.89 ± 5.82	15.33 ± 3.08	15.89 ± 4.40	20.7 ± 3.08
2020	34.11 ± 8.72	19.22 ± 4.99	17.89 ± 4.86	23.74 ± 4.99
2021	27.56 ± 6.67	21.11 ± 5.26	19.11 ± 4.4	22.59 ± 5.26
Overall Season	30.85 ± 7.42^{a}	18.56 ± 5.01^{b}	17.63 ± 4.58^{b}	22.35 ± 5.01
Between year F-value = 2.049^{ns}; (P-value = 0.136) Between season F-value = 47.470**; (P-value < 0.001) Interaction between season and year F-value = 2.180^{ns}; (P-value = 0.080)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season (F = 2.251; P = 0.113), effect of year (F = 0.587; P = 0.559), and the interaction between year and season (F = 1.421; P = 0.236) were found to be non-significant on the abundance of *H. ligurriens* male files. The above results showed that there were no significant variations in the abundance of *H. ligurriens* male flies between seasons and between years (Table 4.11).

Table 4.11. Abundance (%) of *H. ligurriens* male flies between season and between years

Year	Monsoon	Summer	Winter	Overall year
2019	36.95 ± 8.45	31.21 ± 8.23	26.18 ± 7.9	31.44 ± 8.23
2020	33.35 ± 7.08	35.17 ± 5.13	33.04 ± 10.6	33.85 ± 5.13
2021	35.44 ± 7.16	29.98 ± 11.11	32.64 ± 6.04	32.69 ± 11.11
Overall Season	35.25 ± 7.44	32.12 ± 8.49	30.62 ± 8.67	32.66 ± 8.49
Between year F-value = 0.587^{ns}; (P-value = 0.559) Between season F-value = 2.251^{ns}; (P-value = 0.113) Interaction between season and year F-value = 1.421^{ns}; (P-value = 0.236)				

ns non-significant

Effect of season (F = 2.251; P = 0.113), effect of year (F = 0.587; P = 0.559), and the interaction between year and season (F = 1.421; P = 0.236) were found to be non-significant on the abundance of *H.ligurriens* female files. The above results showed that there were no significant variations in the abundance of *H.ligurriens* female flies between seasons and between years (Table 4.12).

Table 4.12. Abundance (%) of *H. ligurriens* female flies between season and between years

Year	Monsoon	Summer	Winter	Overall year
2019	66.23 ± 7.66	68.79 ± 8.23	73.82 ± 7.9	69.61 ± 8.23
2020	66.65 ± 7.08	66.28 ± 7.79	66.96 ± 10.6	66.63 ± 7.79
2021	64.56 ± 7.16	70.02 ± 11.11	67.36 ± 6.04	67.31 ± 11.11
Overall Season	65.81 ± 7.08	68.36 ± 8.94	69.38 ± 8.67	67.85 ± 8.94
Between year F-value = 0.587^{ns}; (P-value = 0.559) Between season F-value = 2.251^{ns}; (P-value = 0.113) Interaction between season and year F-value = 1.421^{ns}; (P-value = 0.236)				

ns non-significant

4.3. Life history of Blow flies

4.3.1. *Chrysomya megacephala*

Morphology of eggs and larval instars

Egg

Creamy white.The caudal end slightly wider than the anterior end and oblong (Fig. 4.25. A).

Larvae

In total, there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

First instar

Larvae whitish cream. Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.25. B).

Cephalopharyngeal skeleton is incompletely developed with no uniform sclerotisation. Dense pigmentation is present on the dorsal cornua and it is long, pointed and slightly curved (Fig. 4.26. A).

Anterior spiracles not developed completely. Two slits were present on the posterior spiracles and were brown in colour (Fig. 4.26. B).

Second instar

The second instar larvae were muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae are prominent with a broad conical base especially in the outer

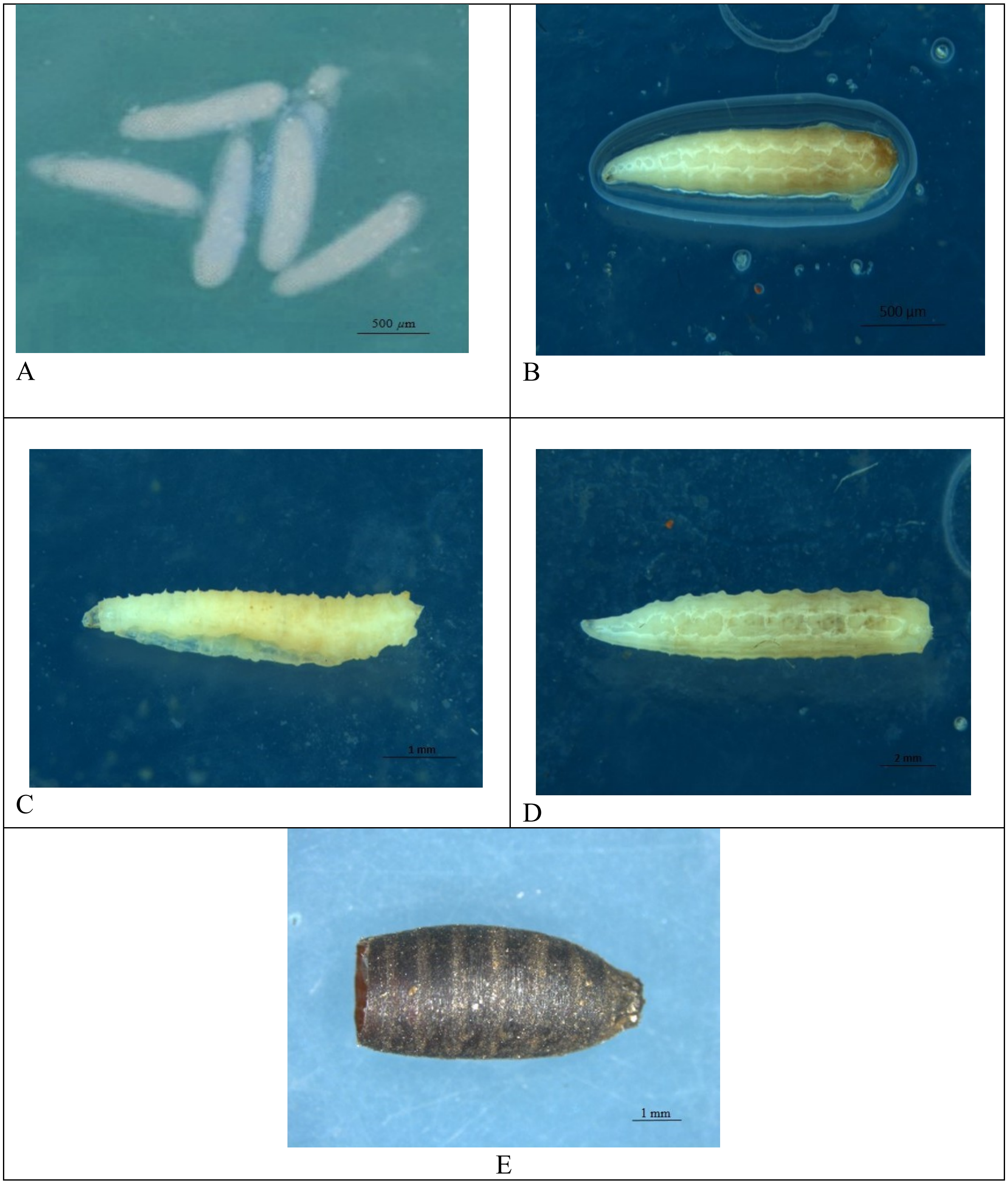

Fig. 4.25. Eggs, different larval instars and pupal case of *C. megacephala*

(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupal case

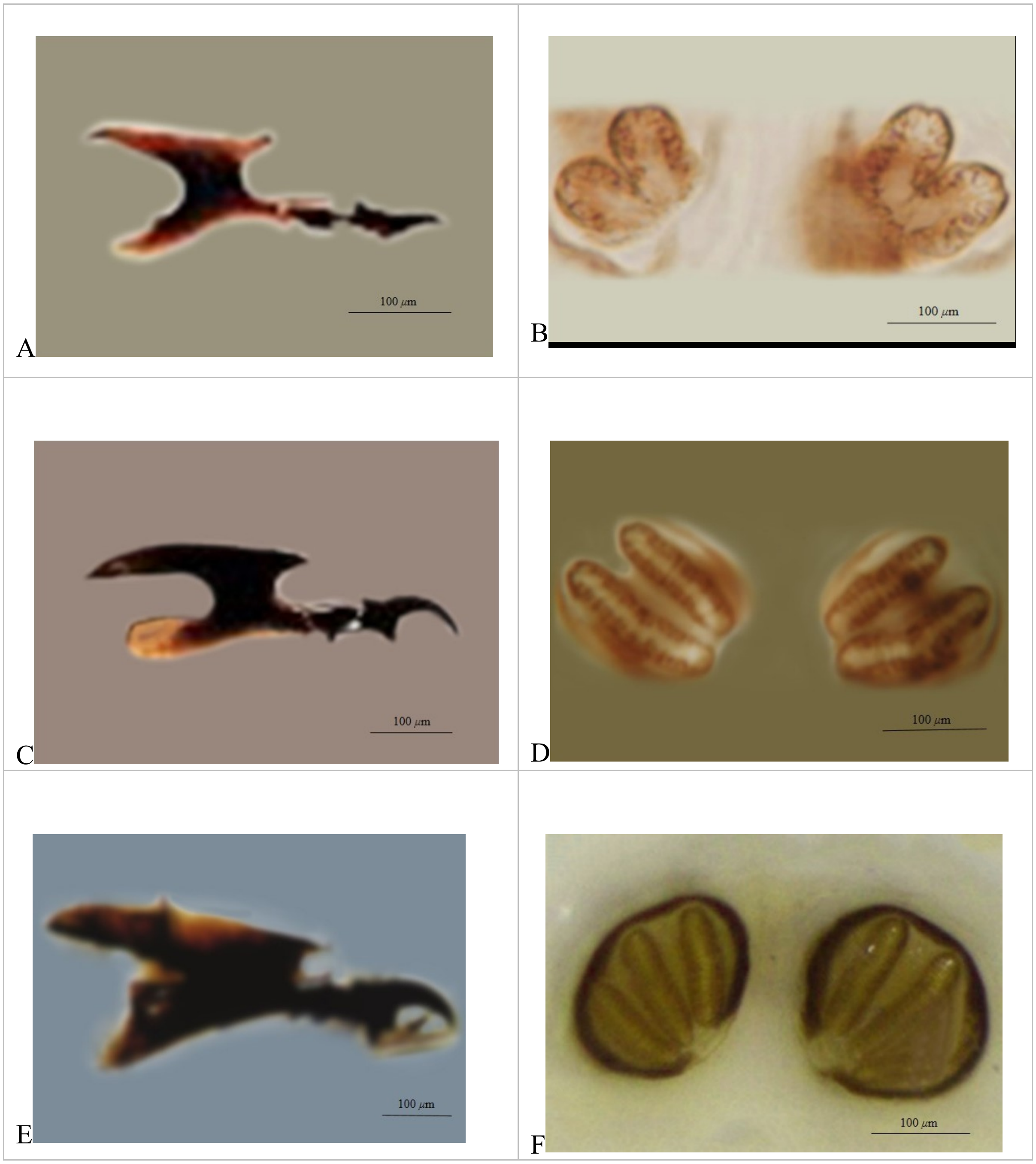

Fig. 4.26. Cephalopharyngeal skeleton and posterior spiracle of *C. megacephala* larvae (A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar

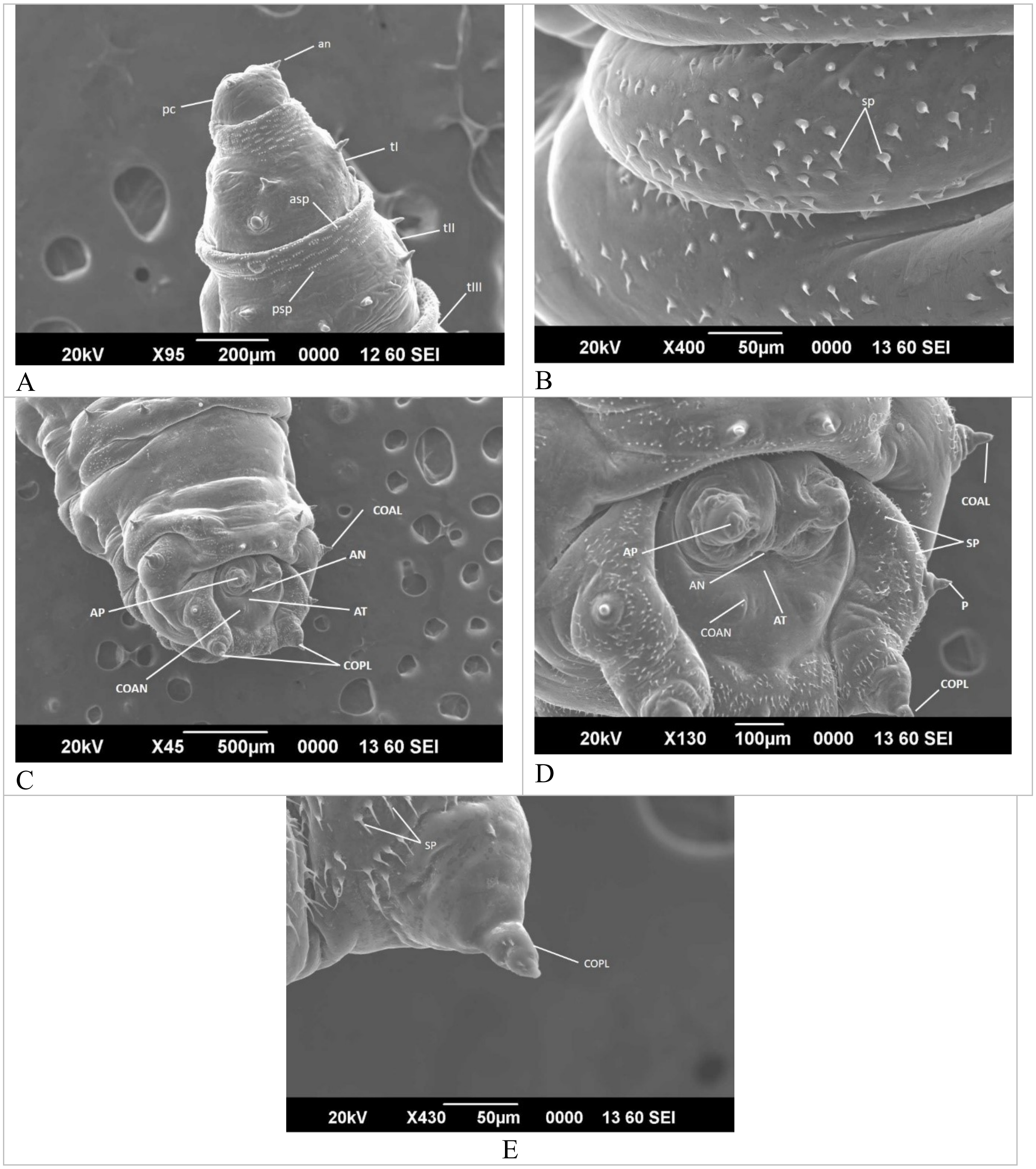

Fig. 4.27. SEM micrographs of first instar larvae of *C. megacephala*

A) pseudocephalon showing antennal complex (an), and anterior spinous process (asp) of second thoracic segment, thoracic segments (t I - t III) B) spines on thorax (sp), C) & D) anal segment displaying anterolateral cones (coal), postero lateral cones (copl), anus (an), anal cone (coan), anal process (ap), anal tubercle (at), spinous process (sp), E) anal segment displaying posterolateral cones (copl) and spinous process (sp)

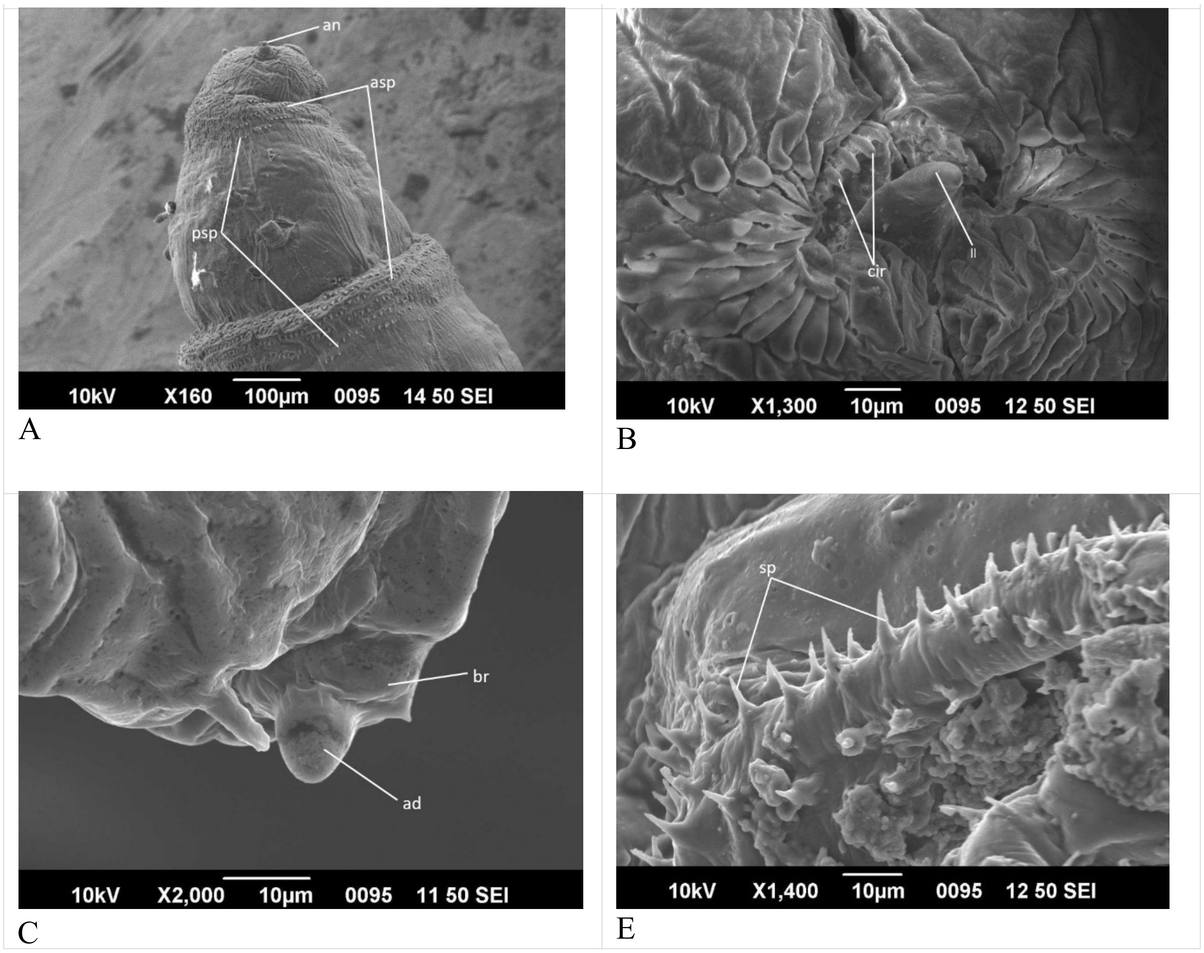

Fig .4.28. SEM micrographs of second instar larvae of *C. megacephala*

A) pseudocephalon showing antennal complex (an), anterior and posterior spinous process (asp & psp) B) pseudocephalon showing cirri (cr) and labial lobe (ll) C) pseudocephalon showing antennal complex having antennae (an) and basal ring (br) D) spines with flat base and sharp tips between first an d second thoracic segments (sp)

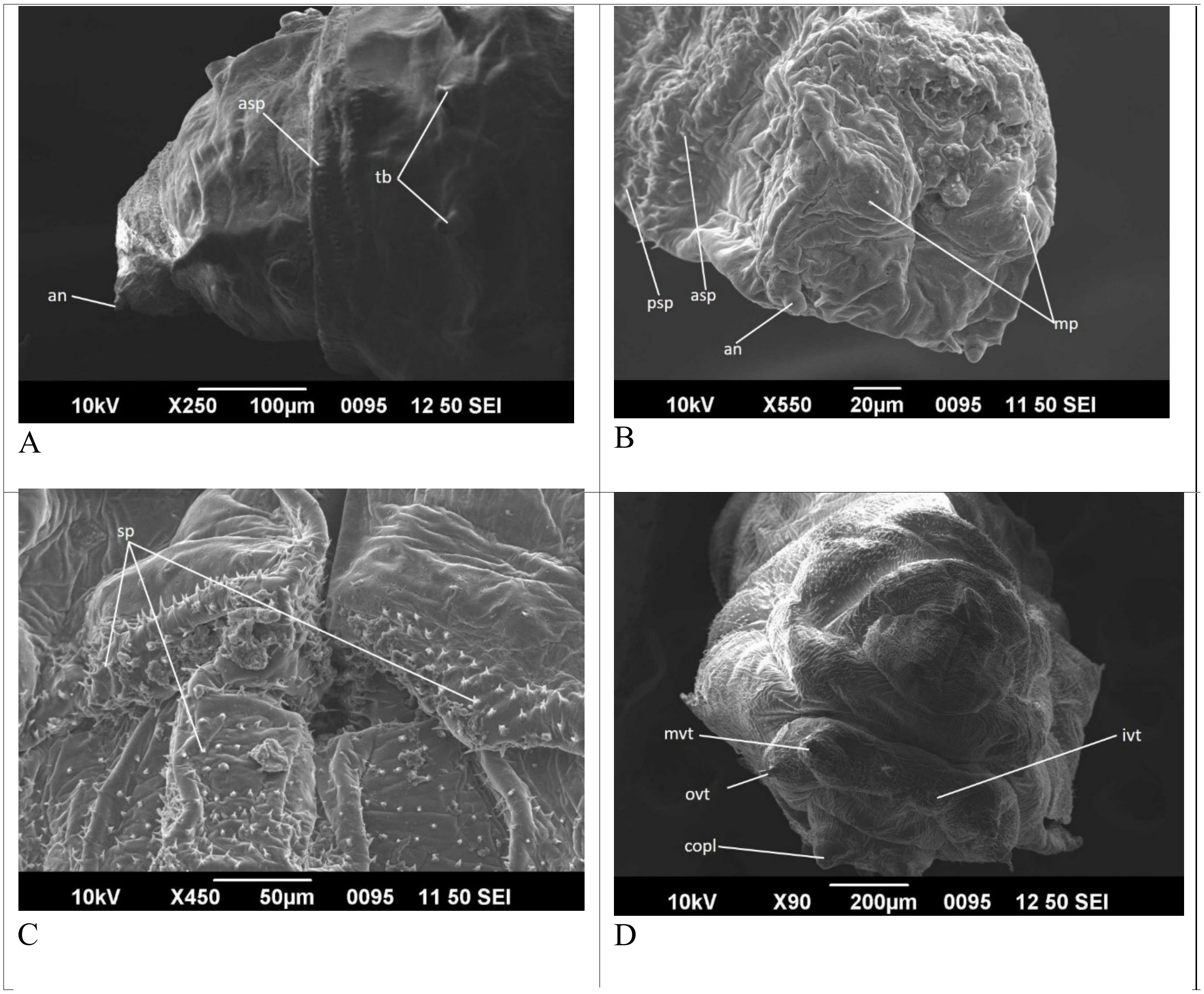

Fig.4.29. SEM micrographs of third instar larvae of *C. megacephala*

A) pseudocephalon showing antennal complex (an), anterior spinous process (asp) and tubercles (tb) B) pseudocephalon showing antennal complex (an), maxillary palpus (mp), anterior and posterior spinous process (asp & psp) of the first and second thoracic segment C) spines with flat broad base and sharp tips on first thoracic segment (sp) D) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt), postero lateral cones(copl)

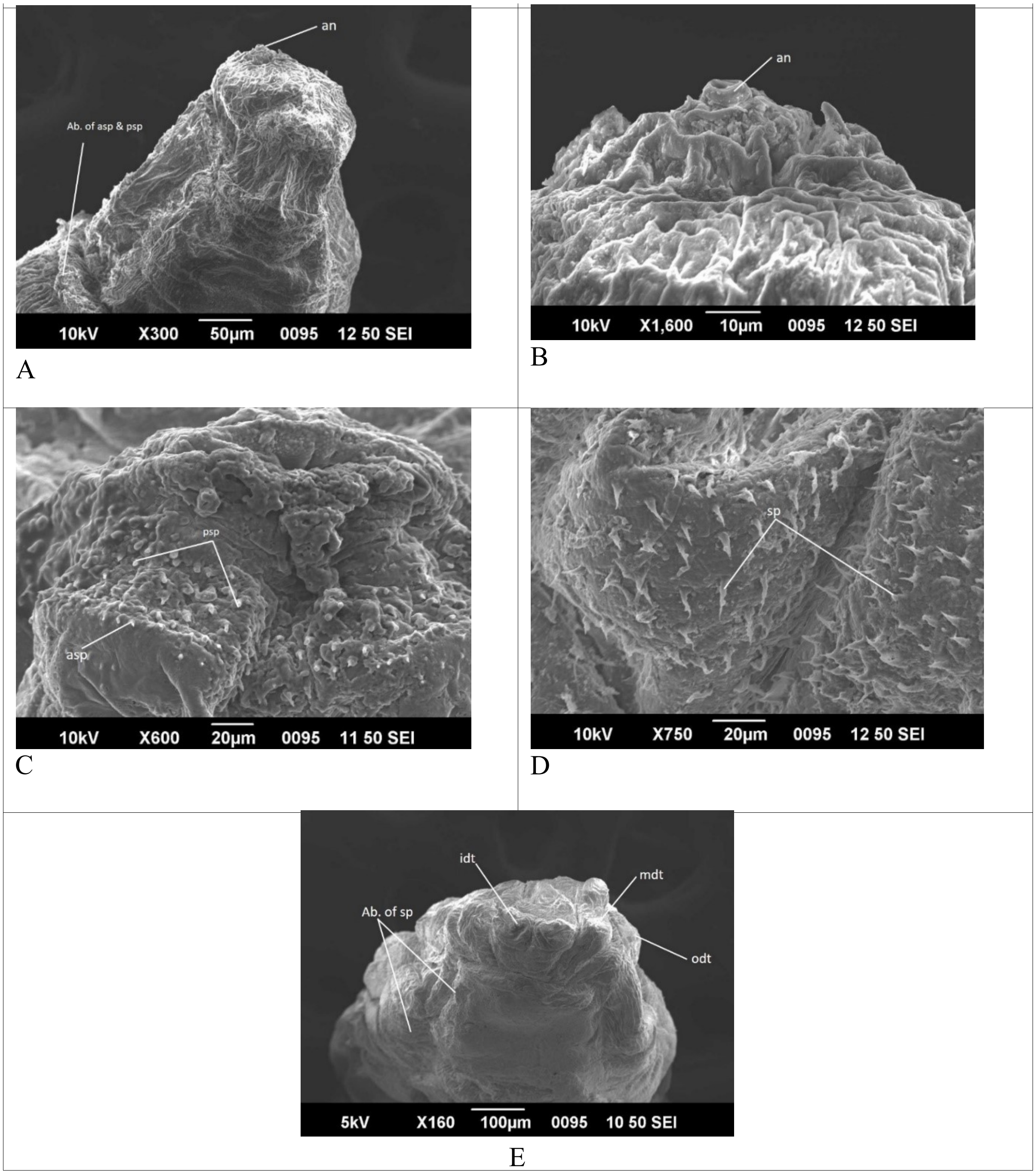

Fig.4.30. SEM micrographs of post feeding stage of *C. megacephala*

A) pseudocephalon showing antennal complex (an), rudimentary anterior and posterior spinous process (asp & psp) B) pseudocephalon showing rudimentary antennal complex (an), C) rudimentary anterior and posterior spines (asp & psp) with flat broad base and sharp tips on first thoracic segment D) third and fouth thoracic segments showing slender filiform spines (sp) E) anal segment displaying inner, middle and outer dorsal tubercles (idt, mdt & odt), rudimentary spines (sp)

dorsal and outer ventral papillae. Papillae were surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar. (Fig. 4.25.C)

Cephalopharyngeal skeleton pigmentation is uniform. Postero dorsal process projected upwards. Dorsal cornua is pointed and long and is structurally similar to the first instar larva but with larger size. The ventral cornua is shorter than the dorsal cornua (Fig. 4.26. C).

Anterior spiracles are yellow while the posterior spiracles are deep brown in colour with two spiracular slits (Fig. 4.26. D).

Third instar

Cream to light yellow in colour. All segments from 2-11 are with spinous bands. The spines acuminate and were arranged singly in rows and have dark points on the tips. The spinous bands were found to be restricted to the lateral and ventral surfaces. The middle dorsal tubercles were moderately sized in comparison to the inner and outer tubercles. The lateral, ventral and dorsal tubercles were large and found to be fully developed (Fig. 4.25. D).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua reduced in length and with uniform width and longer than the ventral cornua. Parastomal and accessory sclerites and anterodorsal process were present (Fig. 4.26. E).

Posterior spiracles were clearly seen with three spiracular slits. A dark pigmented incomplete peritreme was seen surrounding the three slits with a bent in the middle slit (Fig. 4.26. F).

Puparium is brown in colour (Fig. 4.25.E)

Morphology of larval instars using SEM

First Instars

Shape was muscoid having 12 segments. Dorsal organs and terminal organs were present in pairs. Mouth hooks branched with three to four rows of curved sharp tipped spines. Posterior spiracular discs were present in the caudal segment with two slits. The spiracular regions surrounded with fine spiracular hairs (Fig. 4.27).

Second instar

The ultra structural details were mostly similar to that of the first instar. The antennal dome and maxillary palpii were similar to the first instar. The labium and mouth hooks were well developed. Three lobes were present in the labium. The sensillae in the terminal organs were seen as two separate groups. The caudal segment have post spiracular discs with two spiracular slits. The slits were surrounded by fine spiracular hairs (Fig. 4.28).

Third instar

Body size relatively large. The ultra structural details were almost similar to the second instar. The posterior spiracular discs were positioned in a depression with three slits (Fig. 4.29).

Post feeding stage

Stage showed a rudimentary antennal complex, maxillary process, anterior and posterior spinous process (asp & psp) of the second thoracic segment, short spines with flat broad base and sharp tips on the first thoracic segment and rudimentary spines on the anal segment (Fig. 4.30).

Life cycle

Seasonal life cycle data is provided in Appendix III.

Mating

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to the 8th day of emergence. The duration of mating was seen as 10 ± 3 minutes.

Fecundity

The mature female fly laid an average of 345.48 ± 26.09 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small foldings, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 9.37 ± 1.15 days after mating. The next batches of eggs were laid after an interval of 4.39 ± 0.38 days. An average of 2485.44 ± 257.9 eggs were laid by the fly during its life span. The fly stopped laying eggs by 52nd day. The egg took an average of 18 hrs for hatching (Table 4. 13 – 4. 16).

Development of Larvae and Pupae

First Instar

The average length of the first instar was 1.5 ± 0.1 mm and the average dry weight was 1.47 ± 0.12 mg. The first ecdysis was completed after 17 hrs of growth. The cuticle was found to be loosening approximately one to two hours before the ecdysis. (Table 4.17, 4.22 and 4.28).

Second Instar

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 5.14 ± 1.32 mm and the average dry weight was 10.19 ± 0.50 mg. The second moulting was completed after 22 hours (Table 4.18, 4.23 and 4.28).

Third Instar

This stage took 40 hours to enter into the post feeding stage. Till then, third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 10.91 ± 1.02 mm and the average dry weight was 30.71 ± 1.09 mg (Table 4.19, 4.24 and 4.28).

Post feeding stage

This non-feeding stage is characterized by shortening of body length. Larva spent 30 hours in this stage before pupation. The average length of the post feeding stage was 10.60 ± 0.44 and the average dry weight was 29.63 ± 0.78 mg (Table 4.20, 4.25 and 4.28).

Pupa

The average period of pupation was 99 hours. The colour of the pupae was greyish black. The average length and width of the pupa was 6.8 mm and 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4.28).

Adult fly

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 69 days where as the males have lesser longevity and live for only 16 days. The average length of the fly was 9-10mm.

Total life cycle period

The total life cycle period from egg till the emergence of adult fly was found to be 227 ± 59 hours (Table 4.28)

Survival

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rates were 86.32 ± 6.50%, 84.22 ± 7.27%, 75.98 ± 8.03%, 69.26 ± 4.82% and 69.4 ± 5.38% for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *C. megacephala* was 77.04 ± 9.65% (Table 4. 29 – 4. 31).

4.3.2. *Chrysomya rufifacies*

Morphology of eggs and larval instars

Egg

Creamish white in colour.The caudal end was slightly wider than the anterior end and oblong (Fig. 4.31 A).

Larvae

In total there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

First instar

Larvae whitish cream in colour. Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.31. B).

Cephalopharyngeal skeleton incompletely developed and with no uniform sclerotisation. Dense pigmentation is present on the dorsal cornua and it is long, pointed and slightly curved (Fig. 4.32. A).

Anterior spiracles are not developed completely. Two slits were present on the posterior spiracles and were brown in colour (Fig. 4.32. B).

Second instar

The second instar larvae were muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae are prominent with a broad conical base especially in the outer dorsal and outer ventral papillae. These papillae were surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar. (Fig. 4.31. C).

Cephalopharyngeal skeleton pigmentation is uniform. Postero dorsal process projected upwards. Dorsal cornua is pointed and long and is structurally similar to the first instar larva but with larger size. The ventral cornua is shorter than the dorsal cornua (Fig. 4.32. C).

Anterior spiracles are yellow while the posterior spiracles are deep brown in colour with two spiracular slits (Fig. 4.32. D).

Third instar

Creamish yellow in colour and muscoid in shape. All segments from 2-11 are with spinous bands. The body was hairy with long and stout tubercles on all segments. The tubercles had a broad base and tapered with pointed spines at the tip. The spines were

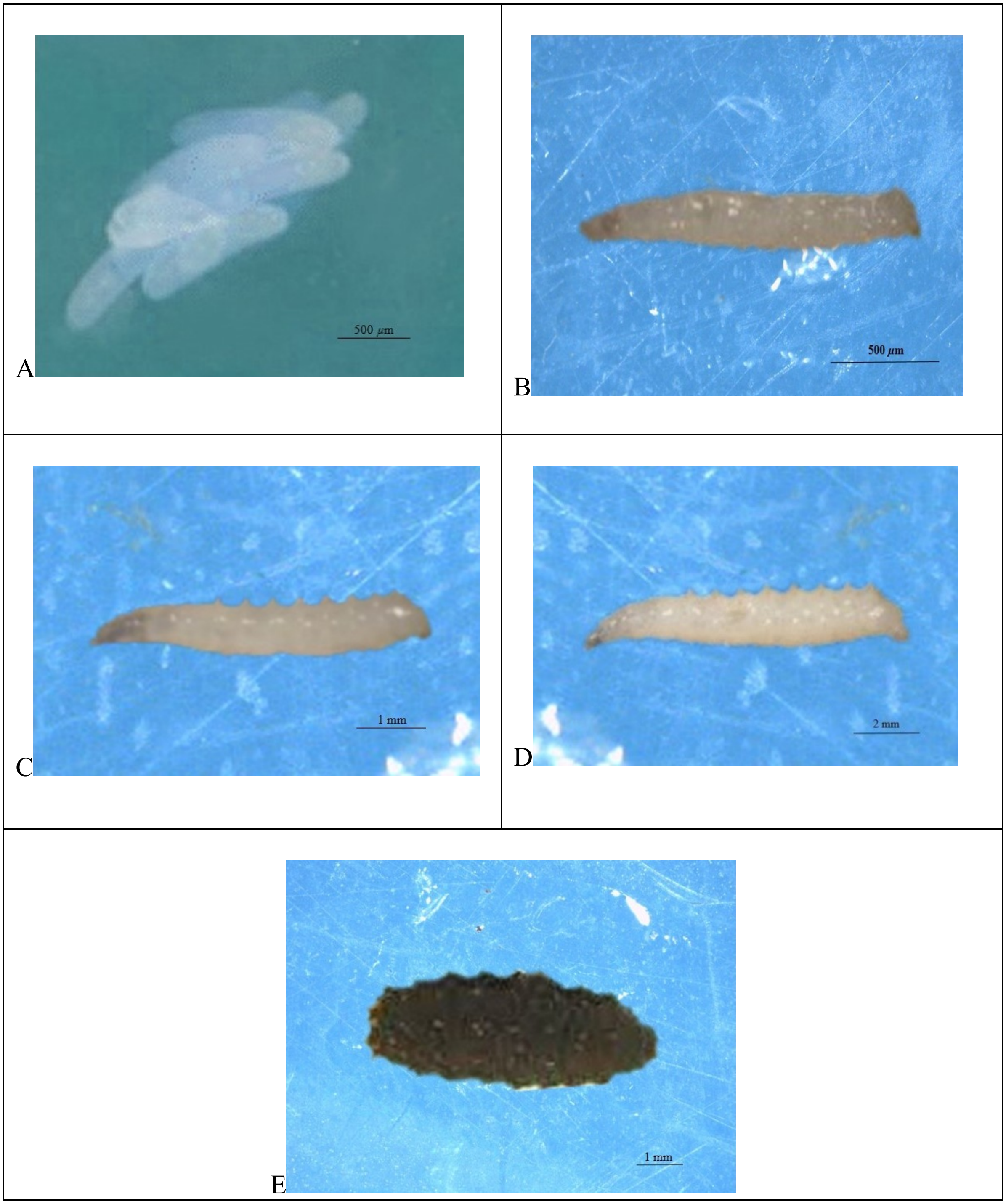

Fig. 4.31. Eggs, different larval instars and pupa of *C. rufifacies*

(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupa

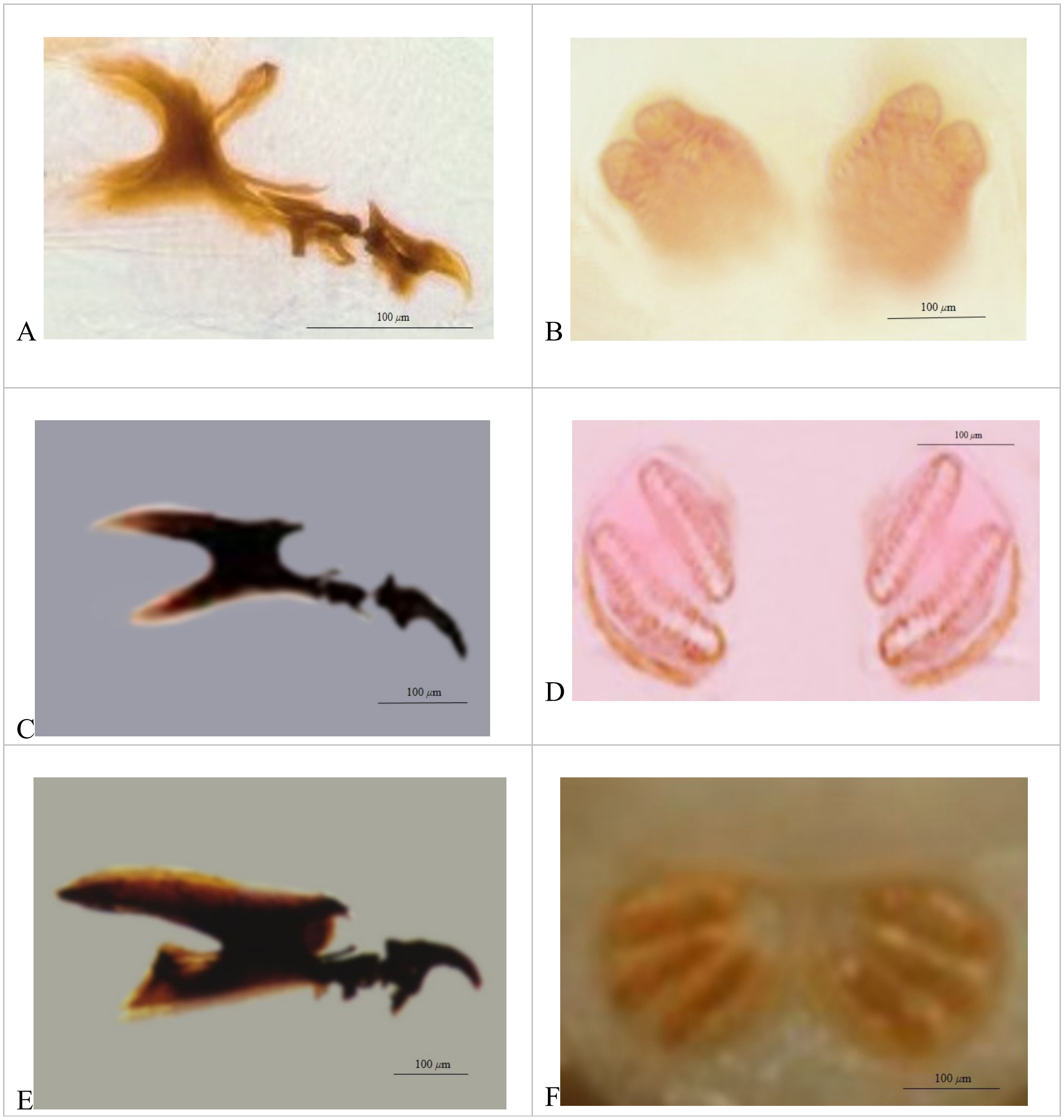

Fig. 4.32. Cephalopharyngeal skeleton and posterior spiracle of *C. rufifacies* larvae

(A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar

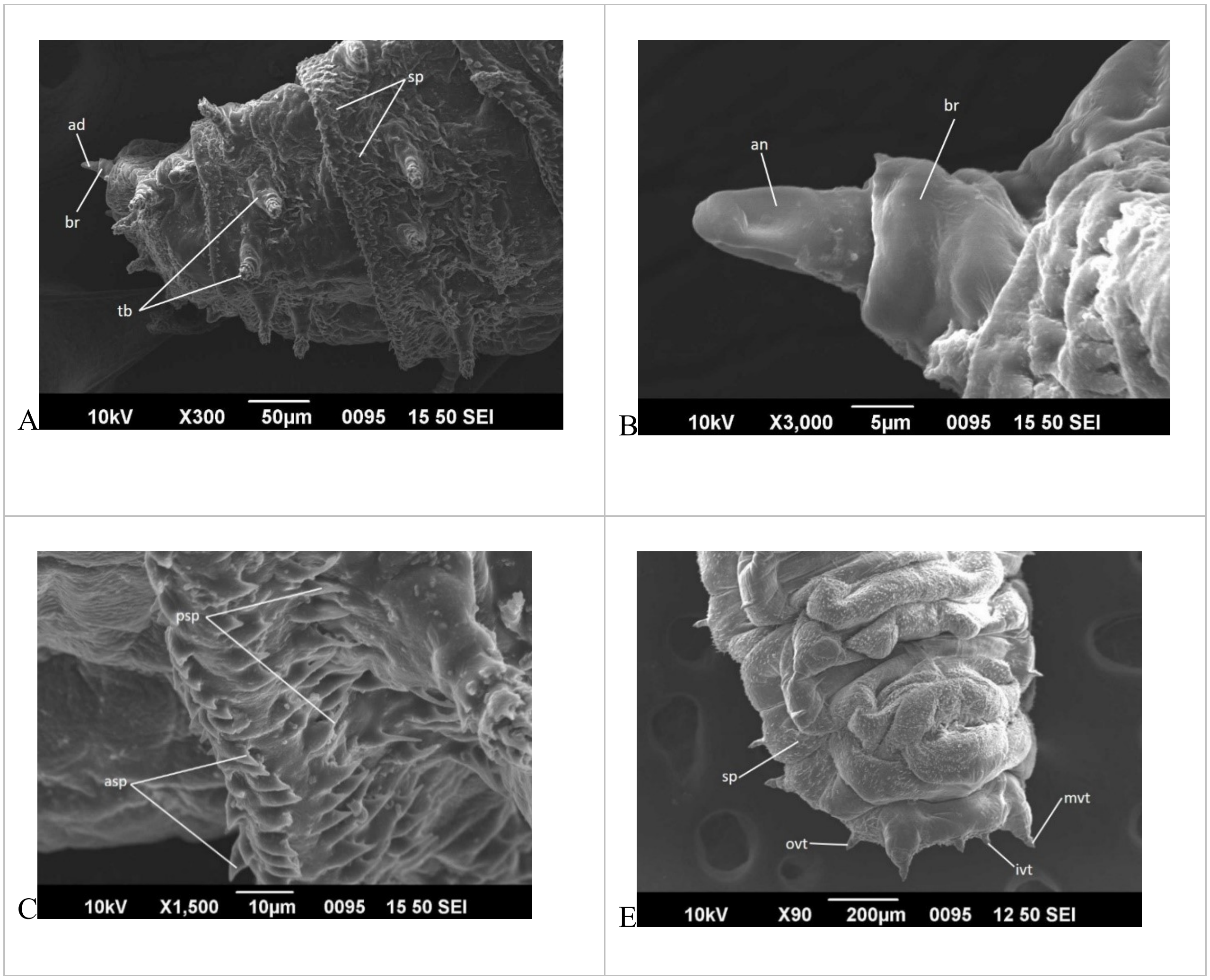

Fig. 4.33. SEM micrographs of first instar of *C. rufifacies*

A) pseudocephalon showing antennal complex (an), spinous process of second thoracic segment (sp), long tubercles on body (tb) B) antennal complex showing antenna (an), basal ring (br) C) spines with flat broad triangular with less sharp tips on first thoracic segment (sp) D) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt), spinous process (sp)

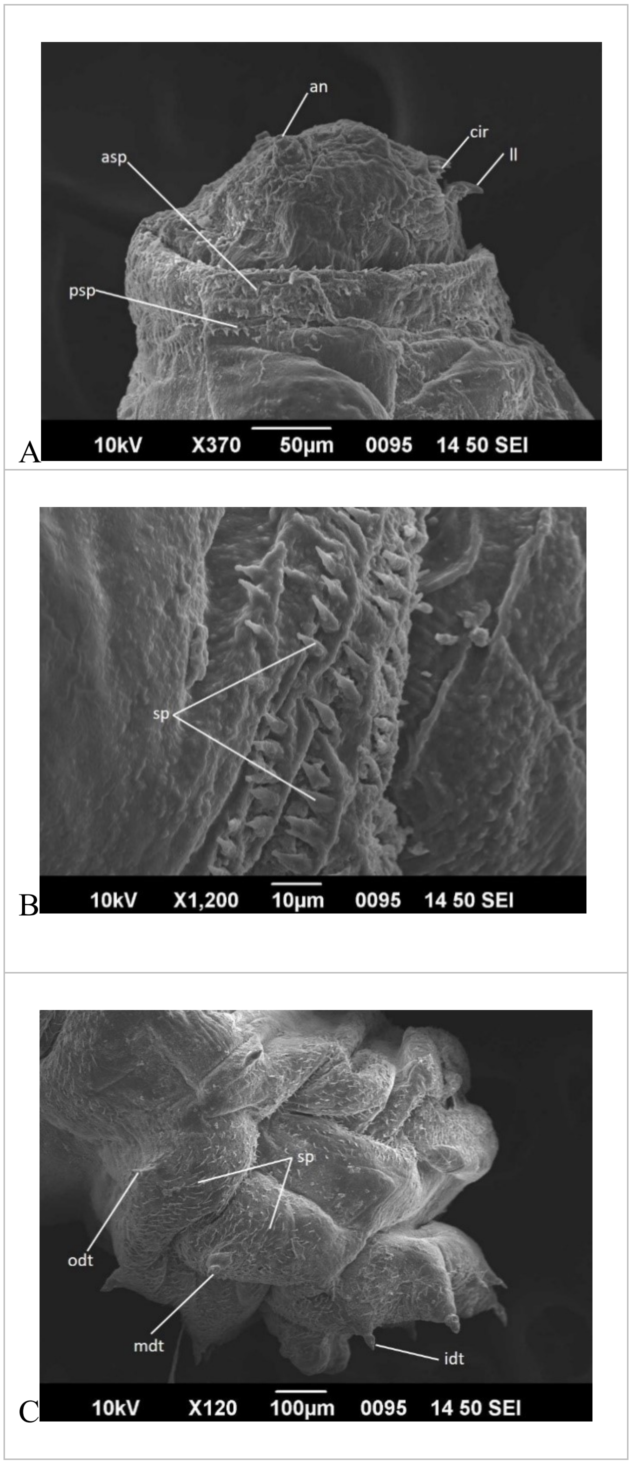

Fig. 4.34. SEM micrographs of second instar of *C. rufifacies*

A) pseudocephalon showing antennal complex (an), cirri (cr) and labial lobe (ll), anterior and posterior spinous process (asp & psp) of the first and second thoracic segment B) spines with flat broad triangular with less sharp tips on second thoracic segment (sp) C) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt), spinous process (sp)

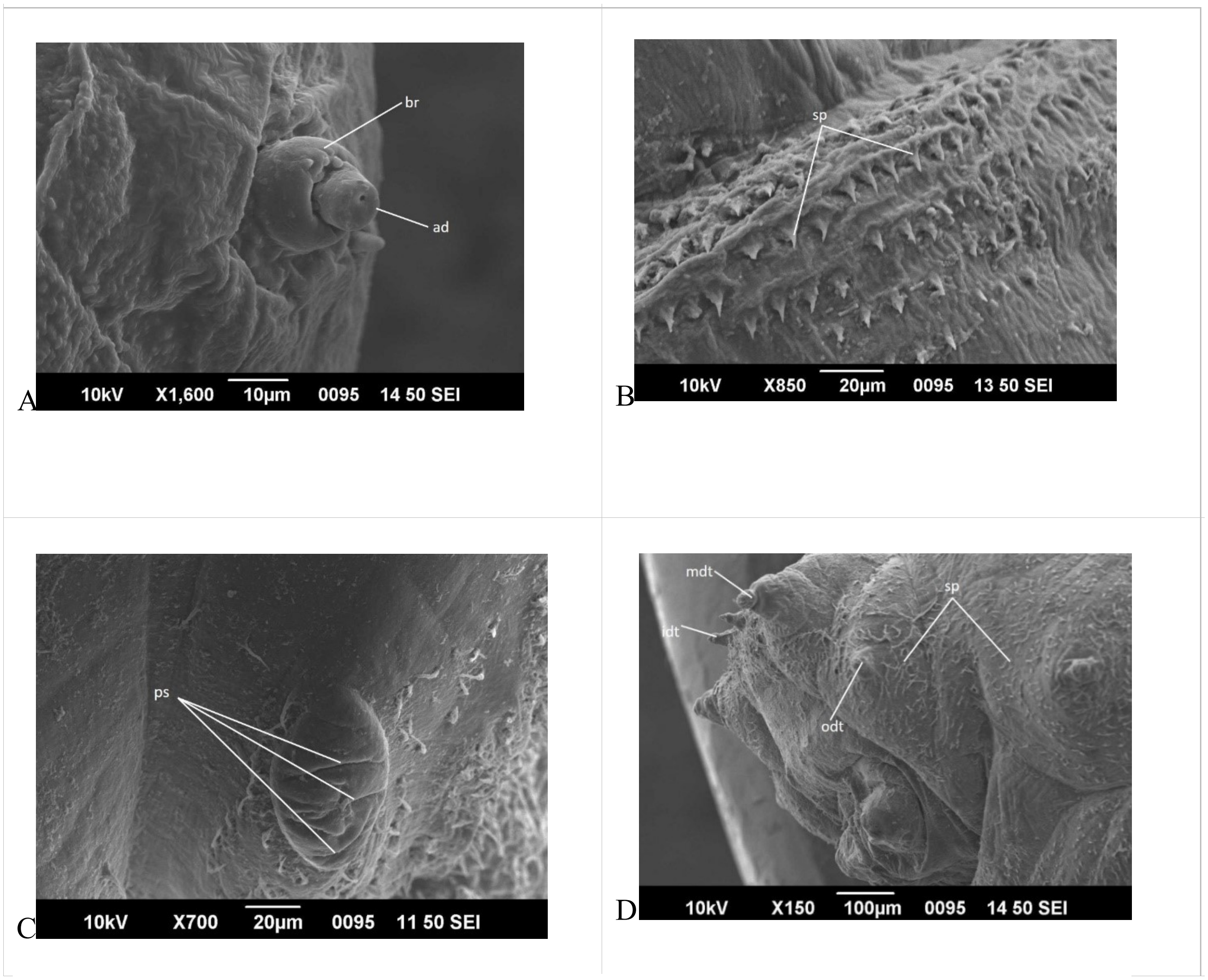

Fig. 4.35. SEM micrographs of third instar of *C. rufifacies*

A) pseudocephalon showing antennal complex showing antenna (an), basal ring (br) B) flat broad triangular spines with less sharp tips on second thoracic segment (sp) C) anal segment showing posterior spiracles (ps) D) anal segment displaying inner, medial and outer dorsal tubercles (idt, mdt & odt), spinous process (sp)

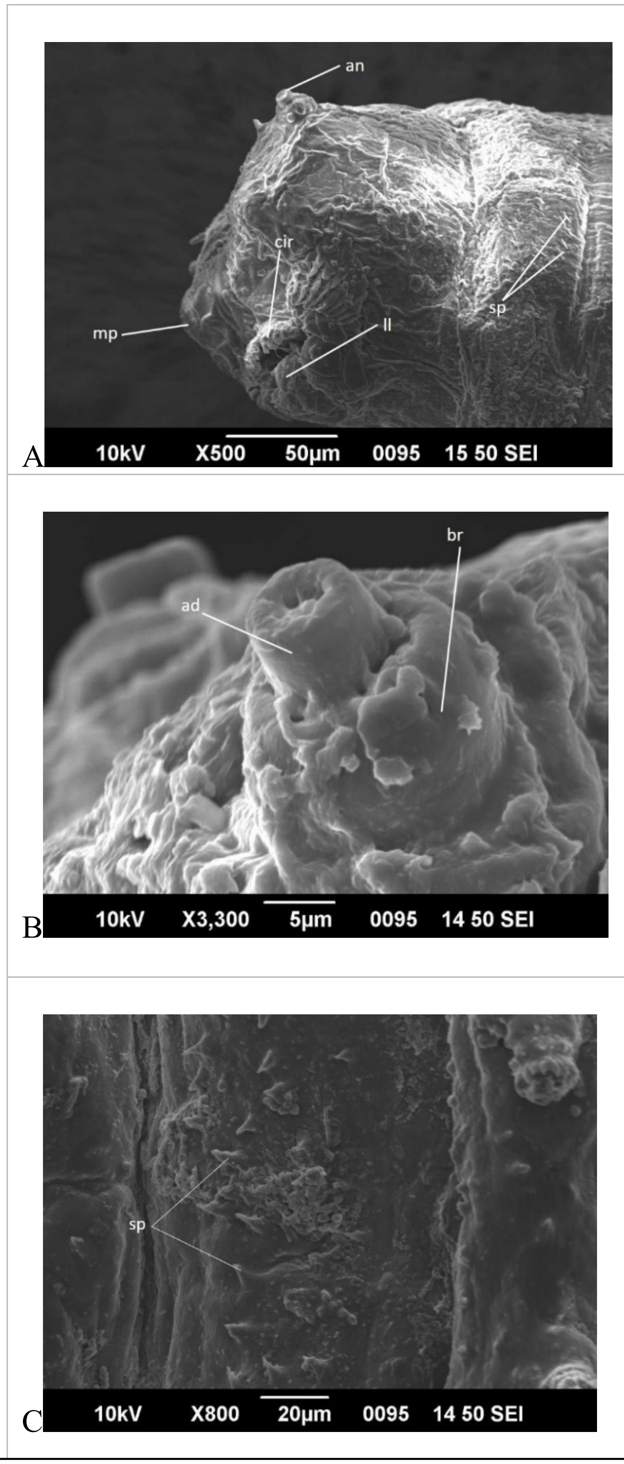

Fig. 4.36. SEM micrographs of post feeding instar of *C. rufifacies*

A) pseudocephalon showing, antennal dome (an), maxillary process (mp), rudimentary cirri (cir), rudimentary labial lobe (ll), spinous process (sp) of the second thoracic segment B) pseudocephalon showing rudimentary antennal complex antenna (an), basal ring (br) C)rudimentary spines second thoracic segment (sp)

arranged singly in rows and were having dark points on the tips. Spines were present on the anterior and posterior margins on the ventral and lateral surfaces of all the three thoracic segments. The middle, inner and outer dorsal tubercles, middle, inner and outer ventral tubercles were found to be fully developed. Outer dorsal tubercles were prominent (Fig. 4.31. D).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua reduced in length and with uniform width. The ventral and dorsal cornua were smaller in size. Parastomal and accessory sclerites were absent. Anterodorsal process was present (Fig. 4.32. E).

Posterior spiracles were clearly seen with three spiracular slits. Densely dark pigmented incomplete peritreme was seen surrounding the three slits with a medial bent in the middle slit (Fig. 4.32. F).

Puparium is brown in colour (Fig. 4.31. E).

Morphology of larval instars using SEM

First instar

Shape was muscoid. Dorsal organs were represented by a pair of antennal domes. Terminal organs were represented by a pair of maxillay palpii. Papillae were present as two separate groups. Oral cirri were curved spines and present in rows. Six to eight rows of pointed spinous process were seen on the demarcating areas between prothorax and metathorax. Six pairs of long tubercles with slender spines were present on the anal segment (Fig. 4.33).

Second instar

Shape was muscoid and similar to first instar. Antennal dome and maxillary palpii were well developed. Labium was trilobed. Pointed spines were present on the junction between the neighbouring body segments. Thick long tubercles were present on body segments with fine spines at the tip. Six pairs of well-developed tubercles were present on the anal segment (Fig. 4.34).

Third instar

Shape was vermiform and muscoid similar to second instar. Labium was not trilobed.Tubercles were elongated and present all over the body except on the pseudocephalon and anal segments. Tubercles were thicker at the bases and terminated to a narrow end with many sharp tipped fine spines. Antennal dome and terminal organs were well developed. The dorsal organs were seen present on the dorsolateral aspect of the terminal organ. The sensillae in the terminal organ were similar to the second instar. Three to four rows of anterior and posterior spines were present on the inter segmental junctions. Six pairs of well-developed marginal tubercles were present on the anal segment. Broad posterior spiracular hairs were present (Fig. 4.35).

Post feeding stage

Spines on the thorax, antennal dome, terminal organs, oral cirri and tubercles were rudimentary in nature. (Fig. 4.36).

Life Cycle

Seasonal life cycle data is provided in Appendix III.

Mating

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to 7th day of emergence. The duration of mating was seen as 9 ± 3 minutes.

Fecundity

The mature female fly laid average of 247.74 ± 28.43 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small folding, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 8.15 ± 0.99 days after mating. The next batches of eggs were laid after an interval of 4.44 ± 0.38 days. An average of 1842.26 ± 97.99 eggs were laid by the fly during its life span. The fly stopped laying eggs by 39th day. The egg took an average of 16 hrs for hatching (Table 4. 33 - 4. 36).

Development of Larvae and Pupae

First Instar

The average length of the first instar was 1.66 ± 0.20 mm and the average dry weight was 1.53 ± 0.07 mg. The first ecdysis was completed after 19 hours of growth. The cuticle was found to be loosening approximately one to two hours before the ecdysis (Table 4. 37, 4. 42 and 4. 48).

Second Instar

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 5.05 ± 1mm and the average dry weight was 10.22 ± 0.84 mg. The second moulting was completed after 23 hours (Table 4. 38, 4. 43 and 4. 48).

Third Instar

This stage took 37 hours to enter into the post feeding stage. Till then, the third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 10.61 ± 0.51 mm and the average dry weight was 31.49 ± 1.29 mg (Table 4. 39, 4. 44 and 4. 48).

Post feeding stage

This non feeding stage is characterized by shortening of body length. Larva spent 27 hours in this stage before pupation. The average length of the post feeding stage was 10.61 ± 0.43 mm and the average dry weight was 29.96 ± 1.01 mg (Table 4. 40, 4. 45 and 4. 48).

Pupa

The average period of pupation was 91 hours. The colour of the pupae was greyish black. The average length and width of the pupa were 6.7 mm & 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4. 48,).

Adult fly

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 71 days where as the males have lesser longevity and live for only 18 days. The average length of the fly was 10-11 mm.

Total life cycle period

The total life cycle period from egg till the emergence of adult fly was found to be 212.78 ± 8.98 hours (Table 4. 48).

Survival

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rates were 82.47 ± 5.45 %, 81.90 ± 6.16 %, 76.03 ± 4.66 % , 72.27 ± 5.92% and 72.33 ± 6.14 % for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *C. rufifacies* was 77.00 ± 7.17% (Table 4.49 – 4.51).

4.3.3. *Chrysomya chani*

Morphology of eggs and larval instars

Egg

Creamish white in colour. The caudal end was slightly wider than the anterior end and generally it was oblong (Fig. 4.37. A).

Larvae

In total, there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

First instar

Larvae whitish cream in colour.Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.37. B).

Cephalopharyngeal skeleton incompletely developed with no uniform sclerotisation. Dense pigmentation present on the dorsal cornua and it is long, pointed and slightly curved. (Fig. 4.38. A).

Anterior spiracles not developed completely. Two slits present on the posterior spiracles and brown in colour (Fig. 4.38. B).

Second instar

The second instar larvae muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae prominent with a broad conical base especially in the outer dorsal and outer ventral papillae. Papillae surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar. (Fig. 4.37. C).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua reduced in length and with uniform width. Dorsal cornua is longer than the ventral cornua. Parastomal and accessory sclerites are present. Anterodorsal process is present (Fig. 4.38. C).

Anterior spiracles are yellow and posterior spiracles were brown in colour with two slits (Fig. 4.38. D).

Third instar

Cream to light yellow in colour. All segments from 2-11 were with spinous bands. Characteristic acuminate spines present on the first and second thoracic segments were clearly visible. The spinous bands were found to be restricted to the lateral and ventral

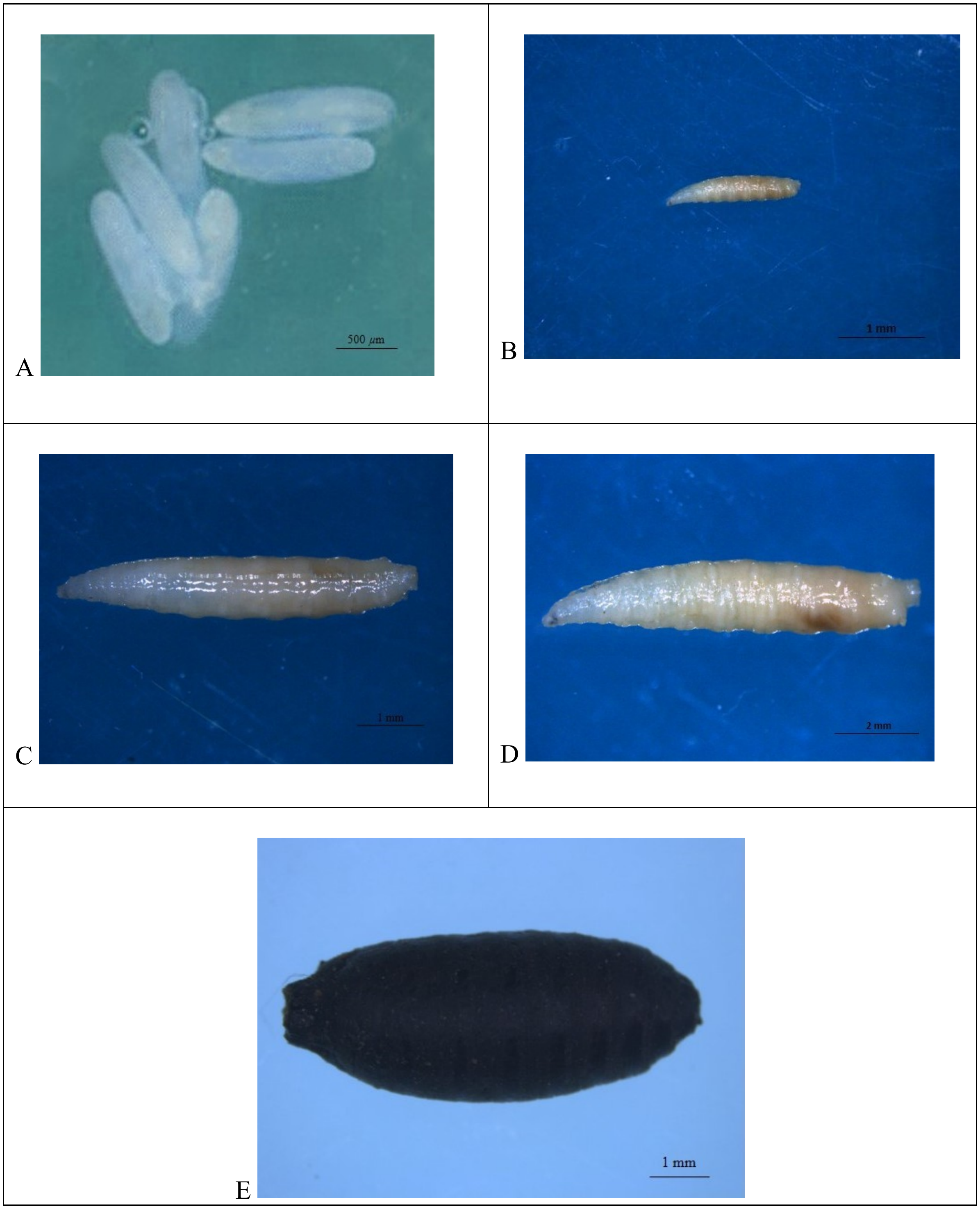

Fig. 4.37. Eggs, different larval instars and pupa of *C. chani*

(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupa

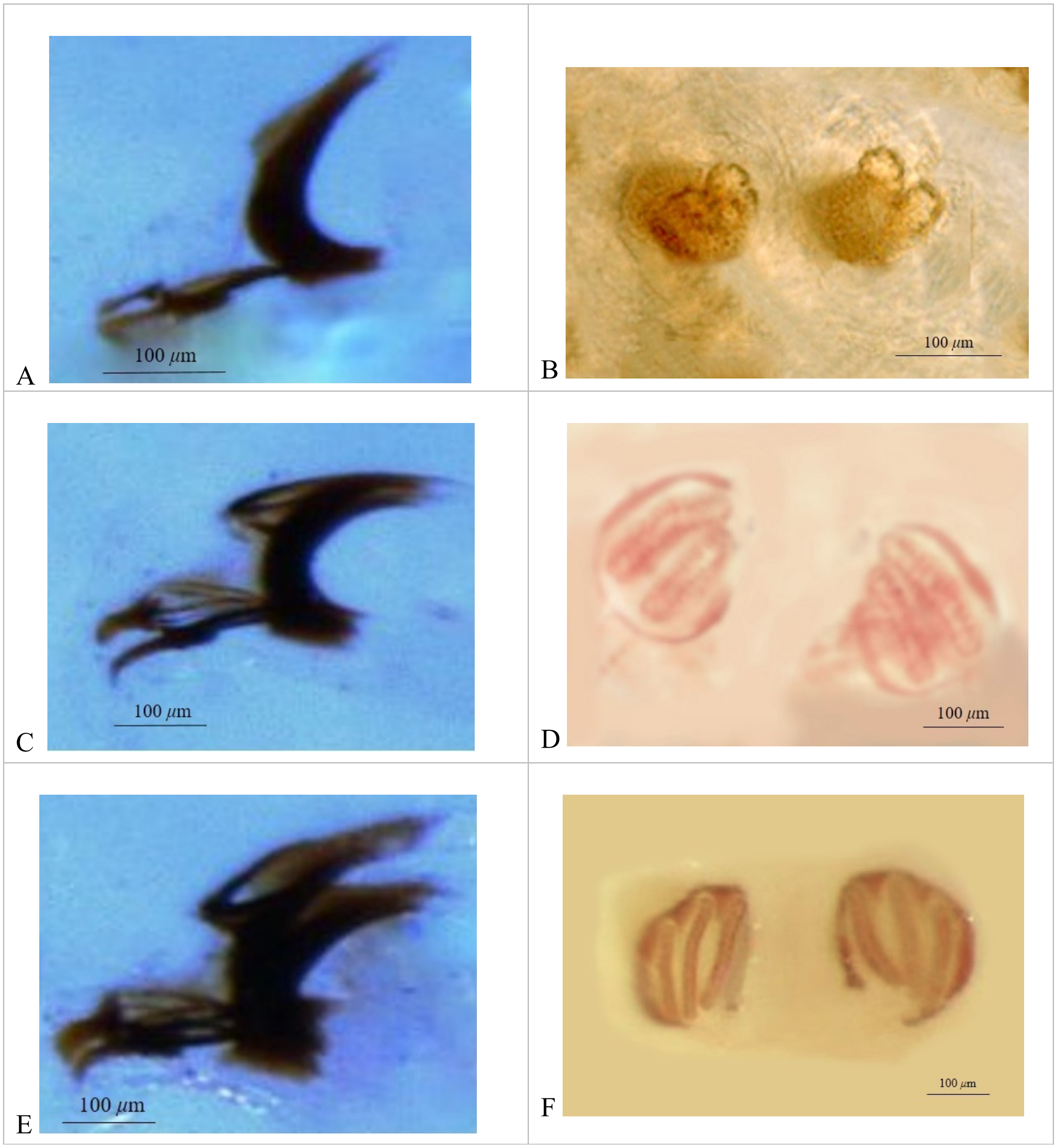

Fig. 4.38 Cephalopharyngeal skeleton and posterior spiracle of *C.chani* larvae

(A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar

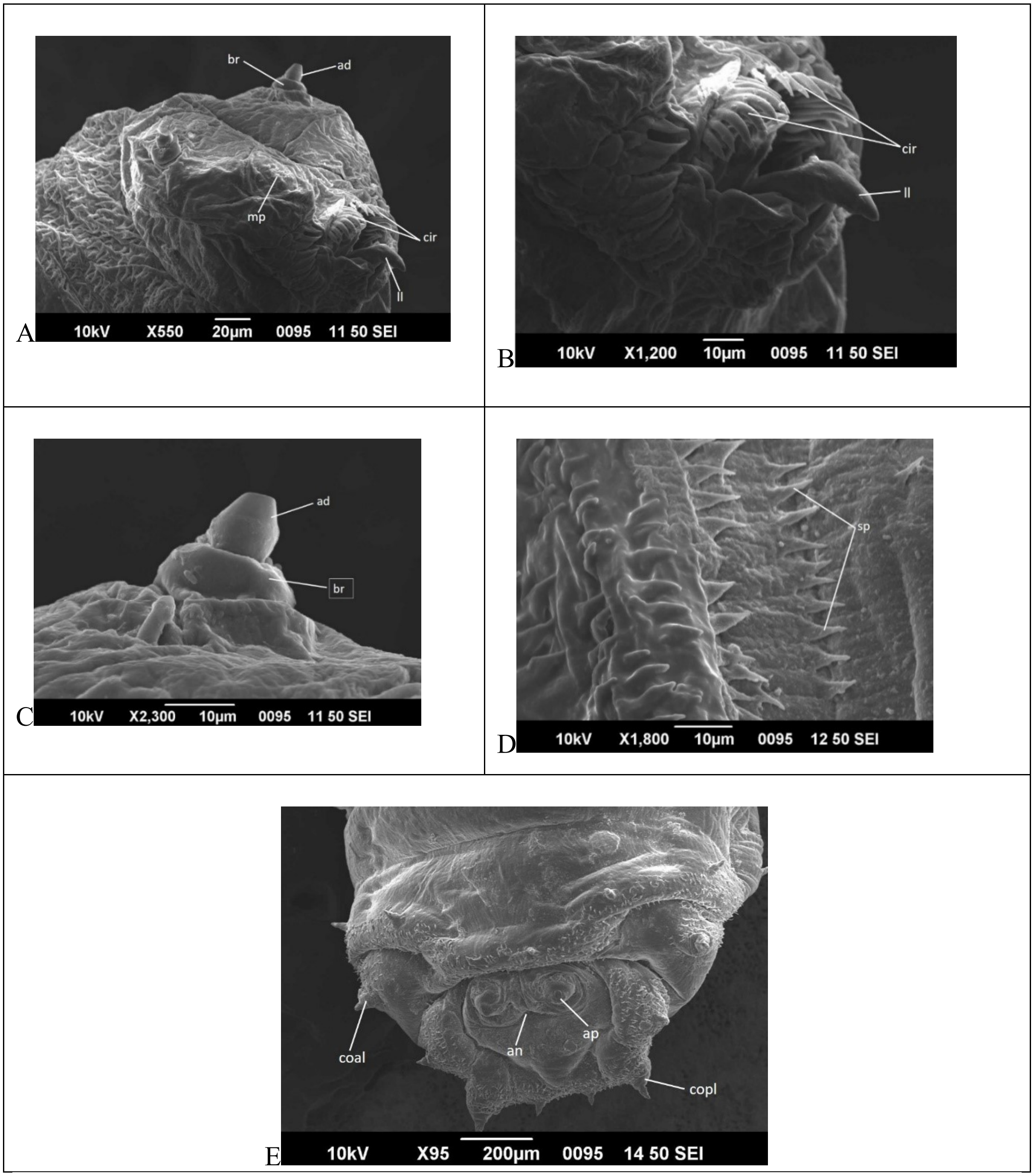

Fig. 4.39. SEM micrographs of first instar larvae of *C. chani*

A) pseudocephalon showing antennal complex (an), maxillary palpus (mp), cirri (cr), labial lobe (ll) B) cirri (cr), labial lobe (ll) C) antennae (an) and basal ring (br) D) spines between first and second thoracic segments (sp) E) anal segment displaying anterolateral cones (coal), postero lateral cones (copl), anus (an), anal process (ap)

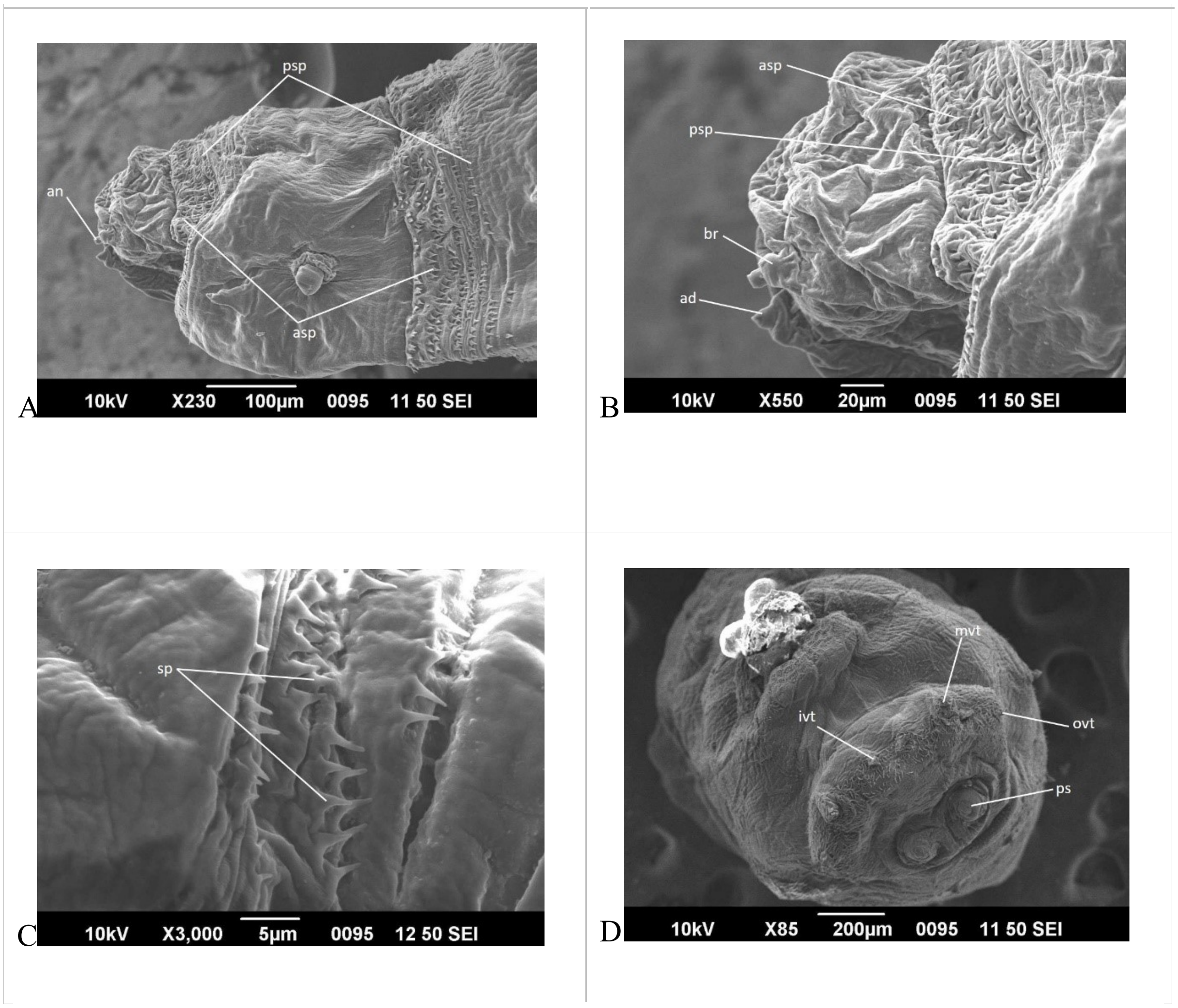

Fig. 4.40. SEM micrographs of second instar larvae of *C. chani*

A) pseudocephalon showing antennal complex (an), anterior and posterior spinous process (asp) B) antennae (an) and basal ring (br), anterior and posterior spinous process (asp & psp) C) spines on first and second thoracic segment (sp) D) anal segment displaying inner, middle and outer ventral tubercles (ivt, mvt & ovt), posterior spiracles (ps)

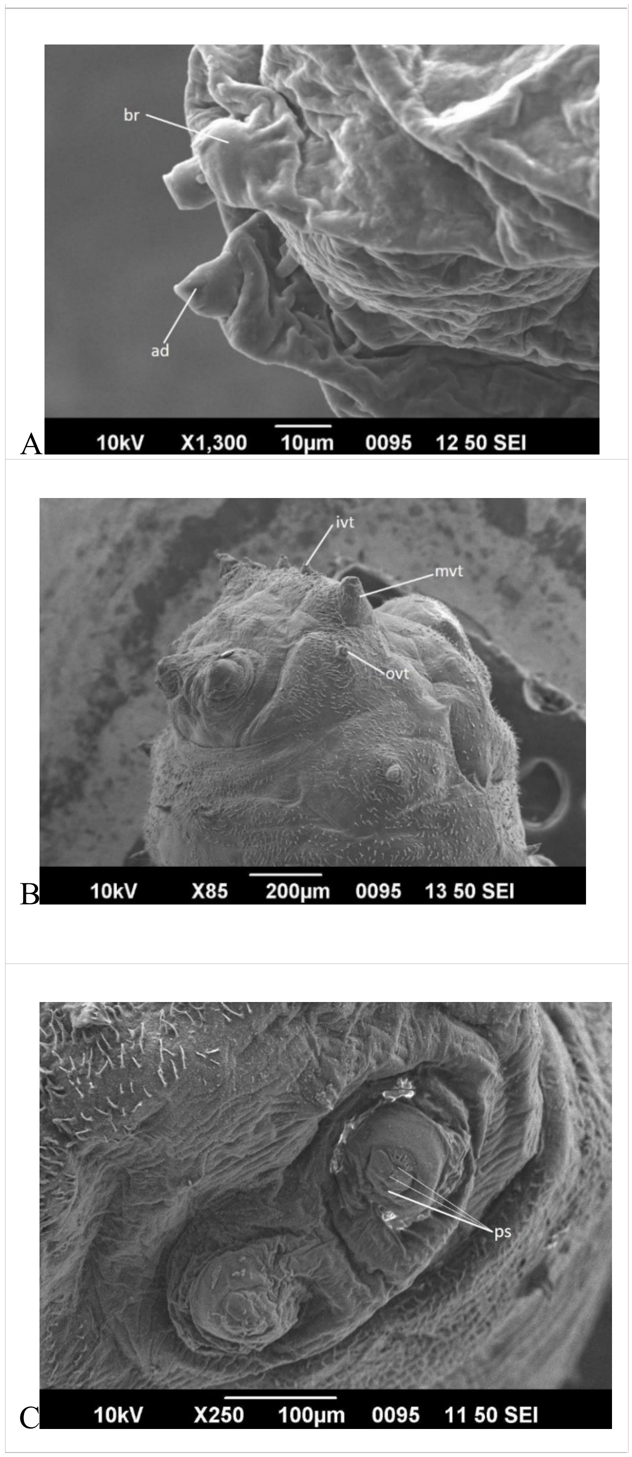

Fig. 4.41. SEM micrographs of third instar larvae of *C.chani*

A) pseudocephalon showing antennal complex with antennae (an) and basal ring (br) B) anal segment displaying inner, middle and outer ventral tubercles (ivt, mvt & ovt) C) posterior spiracles (ps)

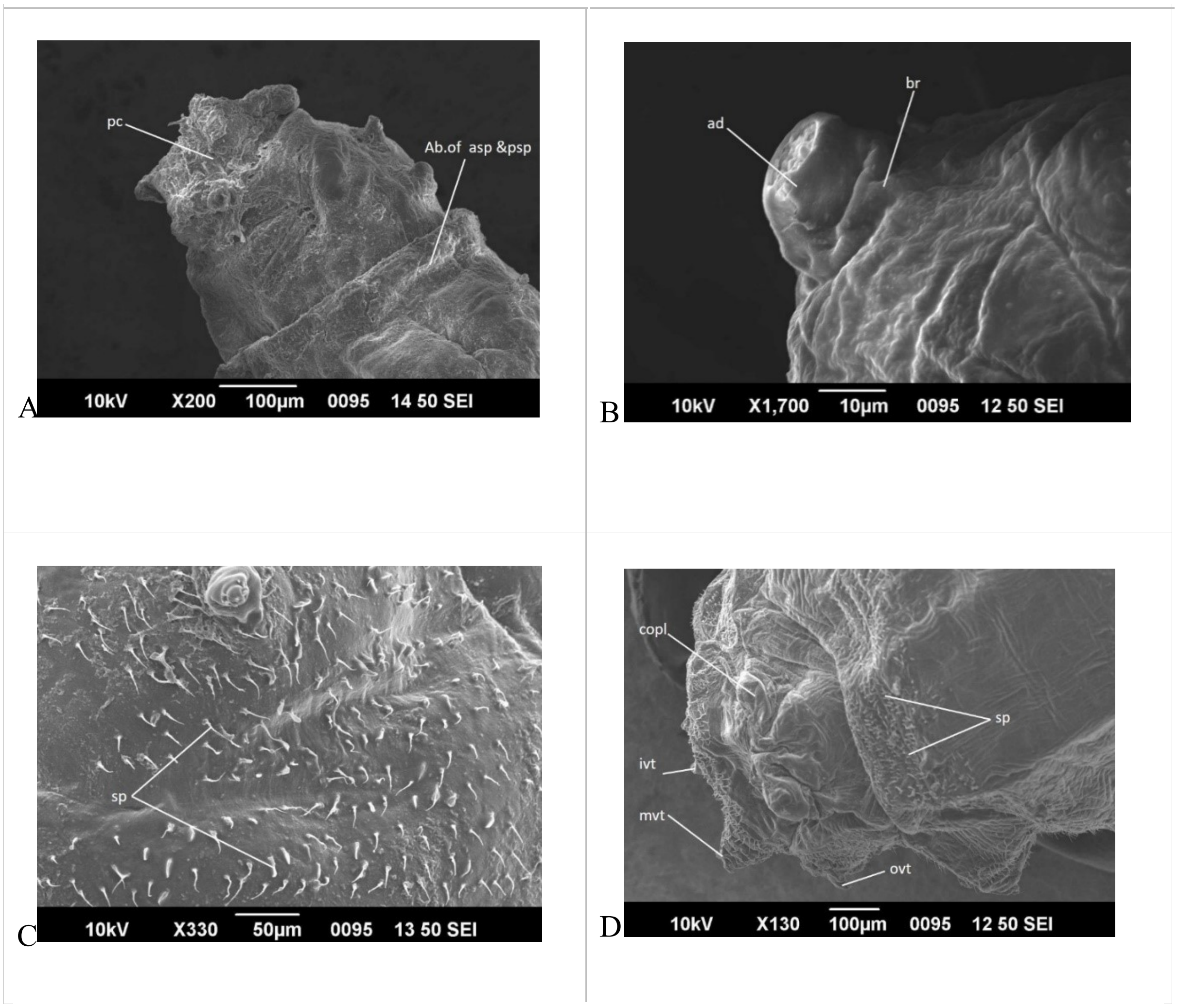

Fig. 4.42. SEM micrographs of post feeding instar larvae of *C.chani*

A) rudimentary anterior and posterior spinous process (asp & psp) B) pseudocephalon showing rudimentary antennal complex (an) C) rudimentary spines on first thoracic segment (sp) D) anal segment displaying inner, medial and outer dorsal tubercles (idt, mdt & odt), postero lateral cones (copl) ,rudimentary spines (sp)

surfaces. Many tubercles present on all the abdominal segments except the caudal segment. The margin of the caudal segment are with pairs of middle, inner and outer dorsal tubercles, middle, inner and outer ventral tubercles and were found to be fully developed in almost equal sizes. (Fig. 4.37. D).

Cephalopharyngeal skeleton is with sclerotized and curved mouth hooks directed downwards. Base of the mouth hooks is broader especially at the posterior aspect. Heavily sclerotized accessory sclerites are seen near the base of the mouth hooks. Base of the mouth hooks are linked to the backwardly directed dental sclerite. Characteristic intermediate sclerite is present. An upwardly curved thin parastomal bar is present. Anterior margin of the parastomal bridge is with the same length as that of dorsal bridge. The dorsal bridge is thin and directed downwards. Intense sclerotization is present on the dorsal and ventral cornua. Ventral cornua is smaller than the dorsal cornua and the pigmentation is uniform. Dorsal cornua is pointed and long and is structurally to the first instar larva but with larger size.Postero dorsal process is projected upwards. (Fig. 4.38. E).

Posterior spiracles with three spiracular slits. A dark pigmented thick sclerotized peritremes were complete in form. Peritreme was seen surrounding the three slits. The button was indistinct (Fig. 4.38. F).

Puparium was ash black in colour (Fig. 4.37. E).

Morphology of larval instars using SEM

First Instars

Shape was vermiform and muscoid. Dorsal organs and terminal organs were present in pairs. Dorsal organ represented by a dome shaped antenna with a flat surface. Height of the antenna was almost equal to the basal ring in the antennal dome. Sensillae basiconium was inconspicuous and was present adjacent to the sensilla coeloconicum. Mouth hooks were branched represented by two to three rows of curved sharp tipped spines. The oral cirri were arranged bilaterally with ten numbers each. The first and second antero lateral rows of spines present near the functional mouth opening were elongated and curved with broad bases (Fig. 4.39).

Second instar

Shape of second instars were similar to that of the first instar. The antennal dome and maxillary palpii were present with no marked differences from the first instar. The labium and mouth hooks were well developed. The labium was trilobed. The sensillae in the terminal organs were present in two separate groups. Ventral organs were curved. Posterior spiracular discs with two spiracular slits were present. The spines present on the abdomen and thorax were similar in nature. The spines present on the anal segment were filiform type. Thoracic spines were with flat triangular base and fine tips (Fig. 4.40).

Third instar

Body size was relatively large. The ultra structural details were similar to that of second instar. The posterior spiracular discs with three slits were positioned in a depression. Three pairs each of dorsal and venral tubercles were present on the anal segment. (Fig. 4.41).

Post feeding stage

Pseudocephalon showed rudimentary antennal complex (an) and rudimentary anterior and posterior spinous process (asp & psp) of the second thoracic segment

.Rudimentary spines with tapering tips were present on the first thoracic segment. Anal segment displayed inner, middle and outer dorsal tubercles (idt, mdt & odt) and postero lateral cones (copl). Rudimentary spines (sp) were present on the anal segment (Fig. 4.42).

Life cycle

Seasonal life cycle data is provided in Appendix III.

Mating

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to the 7th day of emergence. The duration of mating was seen as 10 ± 4 minutes.

Fecundity

The mature female fly laid an average of 240.15 ± 19.6 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small folding, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 8.74 ± 1.26 days after mating. The next batches of eggs were laid after an interval of 4.59 ± 0.31 days. An average of 1667.52 ± 49.78 eggs were laid by the fly during its life span. The fly stopped laying eggs by 41st day. The egg took an average of 21 hrs for hatching (Table 4. 53 - 4. 56).

Development of Larvae and Pupae

First Instar

The average length of the first instar was 2.54 ± 0.30 mm and the average dry weight was 1.49 ± 0.12 mg.The first ecdysis was completed after 18 hours. The cuticle was found to be loosening approximately one to two hours before the ecdysis (Table 4. 57, 4. 62 and 4. 68).

Second Instar

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 6.08 ± 0.33 mm and the average dry weight was 9.82 ± 0.41 mg. The second moulting was completed after 23 hours (Table 4. 58, 4. 63 and 4. 68).

Third Instar

This stage took 36 hours to enter into the post feeding stage. Till then, third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 10.75 ± 0.28 mm and the average dry weight was 30.97 ± 1.09 mg (Table 4. 59, 4. 64 and 4. 68).

Post feeding stage

This non-feeding stage is characterized by shortening of body length. Larva spent 36 hours in this stage before pupation. The average length of the post feeding stage was 10.46 ± 0.35 mm and the average dry weight was 29.90 ± 1.13 mg (Table 4. 60, 4. 65 and 4. 68).

Pupa

The average period of pupation was 119 hours. The colour of the pupae was greyish black. The average length and width of the pupa were 6.7 mm & 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4. 68).

Adult fly

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 73 days where as the males have lesser longevity and live for only 20 days. The average length of the fly was 8-9 mm.

Total life cycle period

The total life cycle period from egg till the emergence of adult fly was found to be 252.89 ± 17.16 hours (Table 4. 68).

Survival

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rates were 75.84 ± 5.53 %, 76.04 ± 5.25 %, 76.45 ± 4.50 %, 68.49 ± 5.19% and 66.69 ± 3.81 % for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *C. chani* was 72.70 ± 6.42 % (Table 4. 69 – 4. 71)

4.3.4. *Hemipyrellia ligurriens*

Morphology of eggs and larval instars

Egg

Creamish white in colour. The caudal end was slightly wider than the anterior end and generally it was oblong (Fig. 4.43. A).

Larvae

In total, there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

First instar

Larvae whitish cream in colour. Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.43. B).

Cephalopharyngeal skeleton is incompletely developed and with no uniform sclerotisation. Dense pigmentation is present on the dorsal cornua and it is long, pointed and slightly curved (Fig. 4.44. A).

Anterior spiracles are not developed completely. Two slits were present on the posterior spiracles and were brown in colour (Fig. 4.44. B).

Second instar

The second instar larvae were muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae are prominent with a broad conical base especially in the outer dorsal and outer ventral papillae. Papillae were surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar (Fig. 4.43. C).

Cephalopharyngeal skeleton pigmentation is uniform. Postero dorsal process projected upwards. Dorsal cornua is pointed and long and is structurally similar to the first instar larva but with larger size. The ventral cornua is shorter than the dorsal cornua. (Fig. 4.44. C).

Anterior spiracles are yellow while the posterior spiracles are deep brown in colour with two spiracular slits (Fig. 4.44. D).

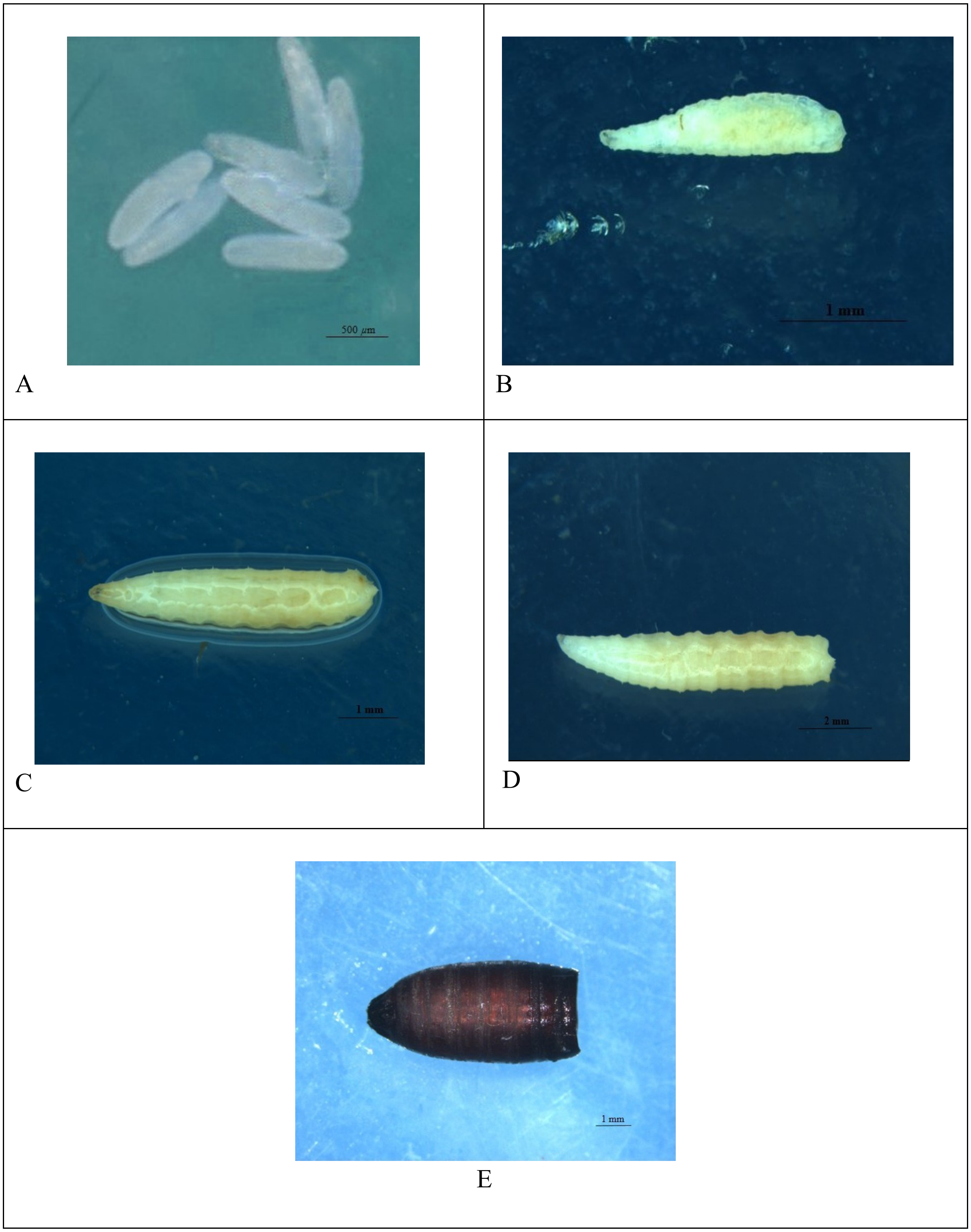

Fig. 4.43. Eggs, different larval instars and pupal case of *H. ligurriens*

(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupal case`

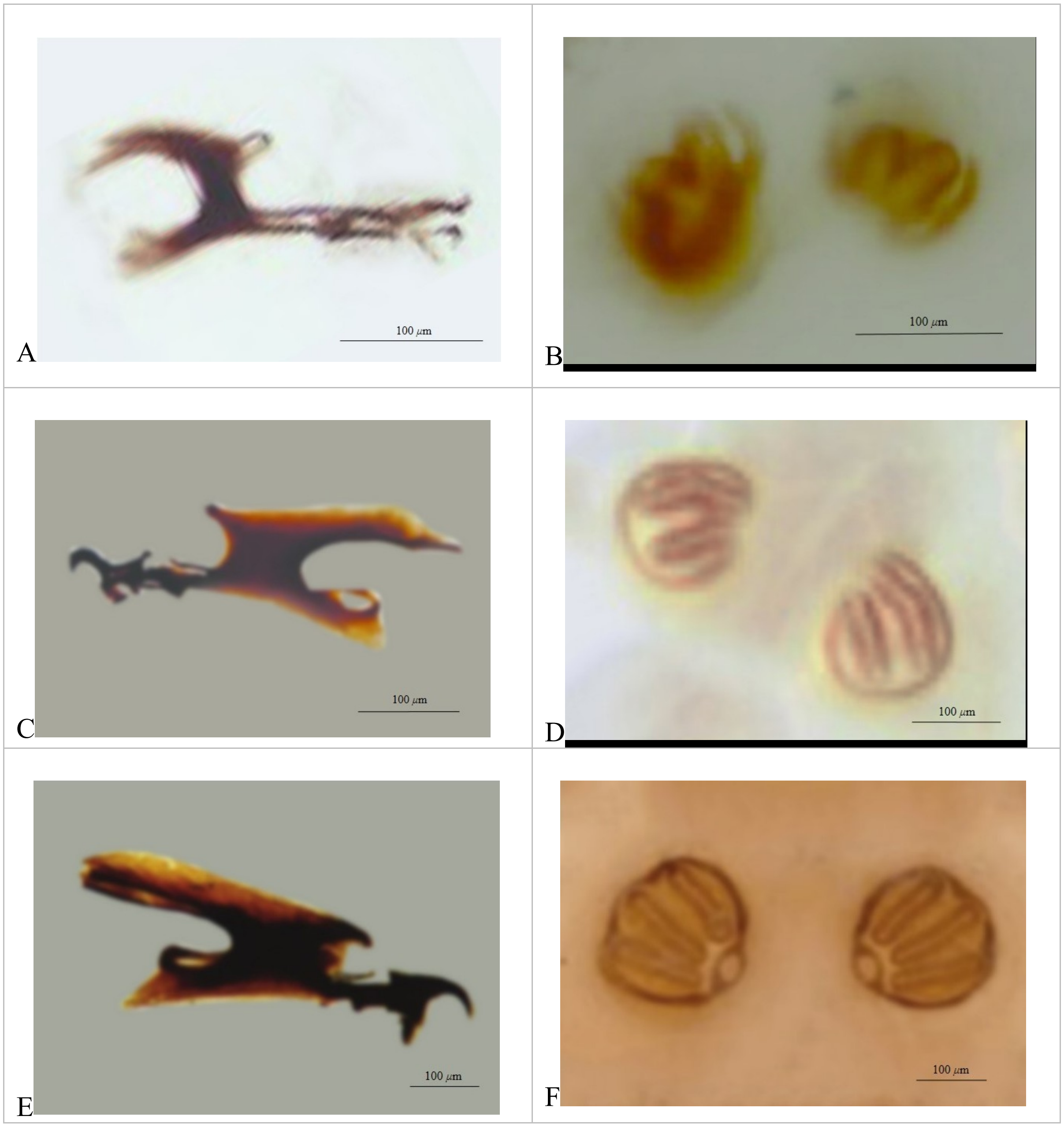

Fig. 4.44. Cephalopharyngeal skeleton and posterior spiracle of *H. ligurriens* larvae

(A, B) 1^{st} instar (C, D) 2nd instar (E, F) 3rd instar

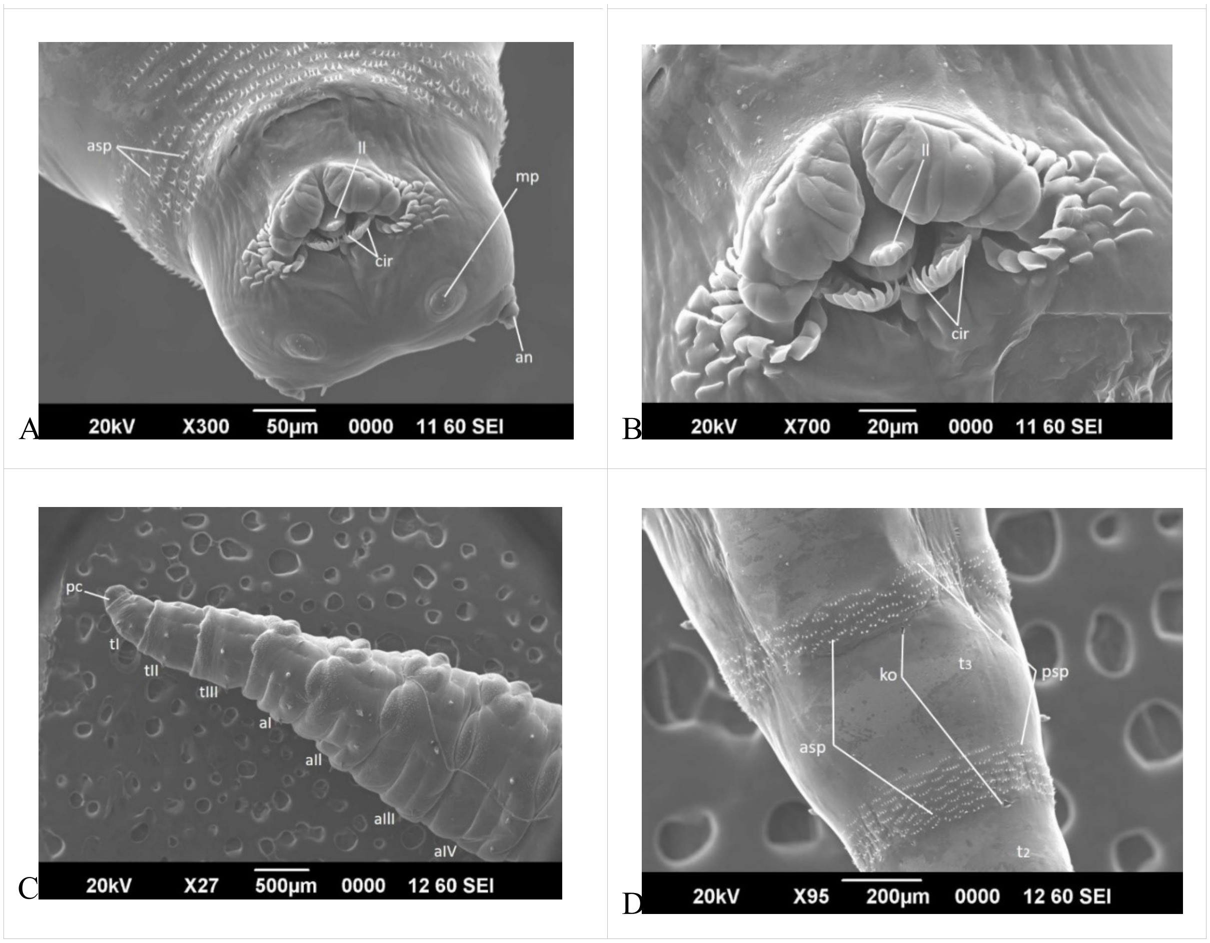

Fig. 4.45. SEM micrographs of first instar of *H. ligurriens.*

A) pseudocephalon showing antennal complex (an), maxillary palpus (mp), cirri (cr), labial lobe (ll) and anterior spinous process of the first thoracic segment (asp) B) cirri (cr), labial lobe C) body segments till fourth abdominal segment (t I - t III & a I – a IV) D) second and third thoracic segments showing anterior spinous processes (asp), posterior spinous process (psp), Keilin's organ (ko)

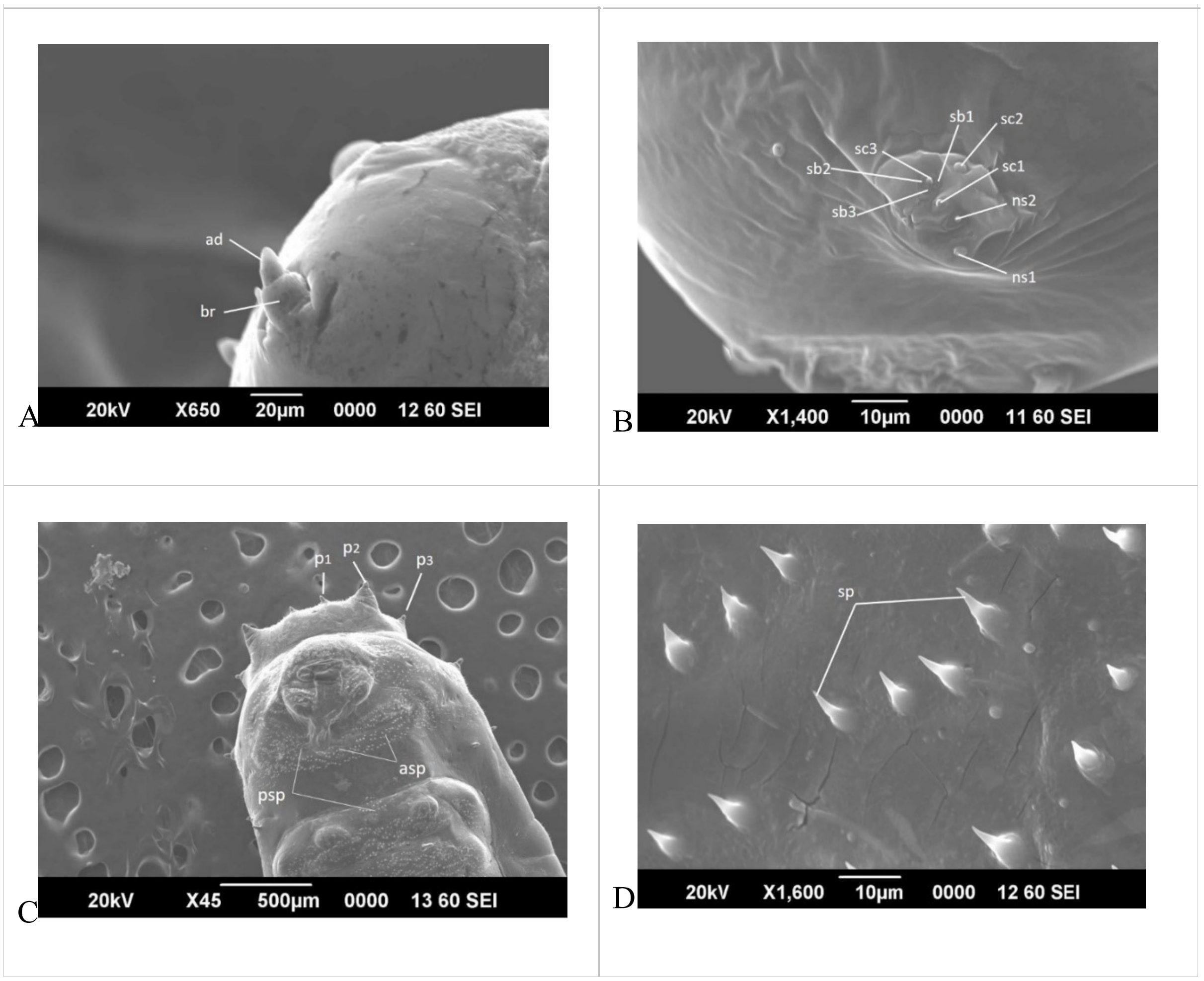

Fig. 4.46. SEM micrographs of second instar of *H. ligurriens.*

A) pseudocephalon showing antennal dome (ad) (B) Maxillary palpus showing eight sensillae (sc1-3; sb1-3; ns1-2) C) anal segment displaying dorsal papillae (p1 p3), D) spines with bulbous base and sharp tips between first and second thoracic segments (sp).

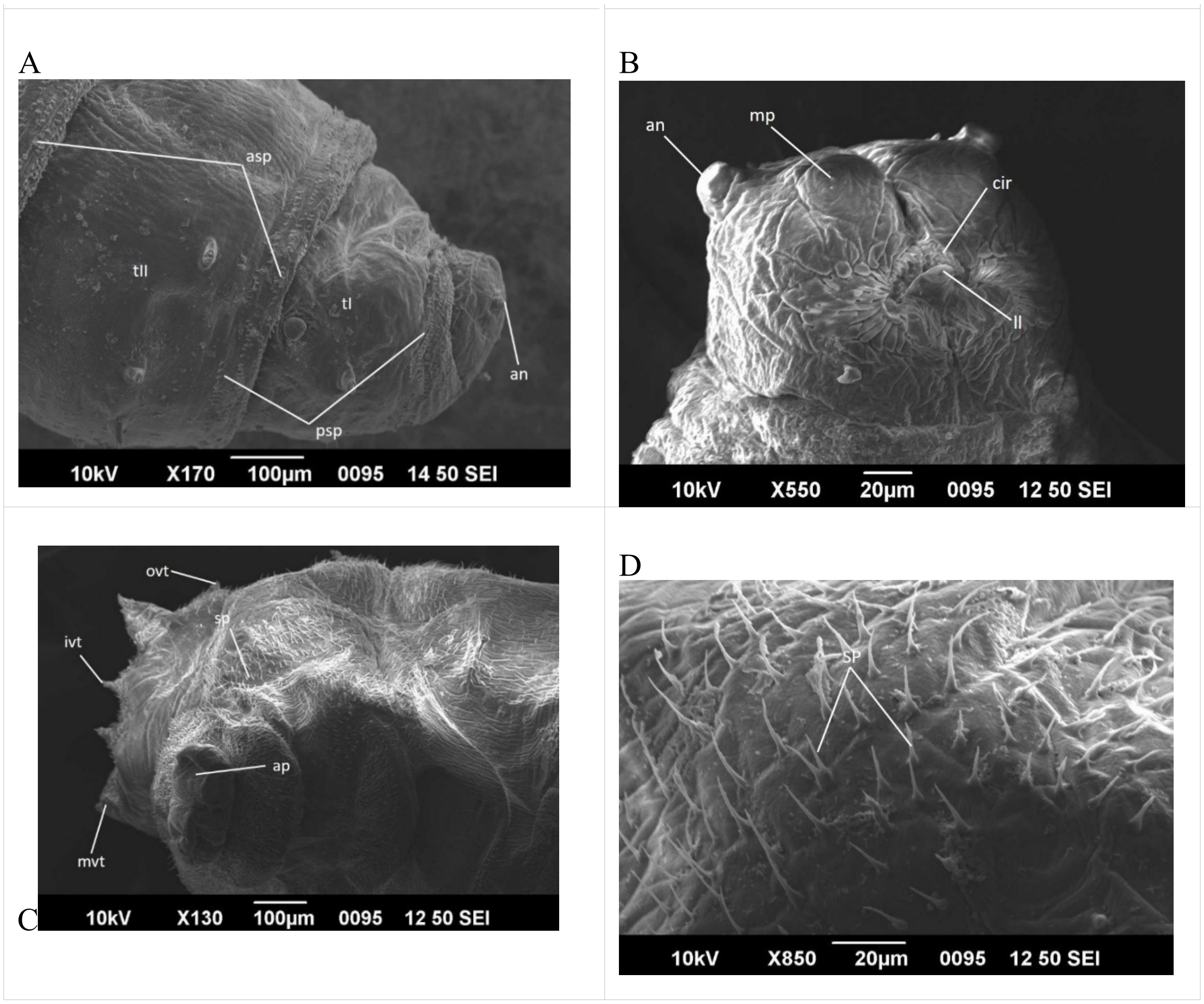

Fig. 4.47. SEM micrographs of third instar of *H. ligurriens.*

A) pseudocephalon showing antennal complex (an), anterior and posterior spinous process (asp & psp) of the first and second thoracic segments B) pseudocephalon showing antennal complex (an), maxillary palpus (mp), cirri (cr), labial lobe (ll). C) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt) D) slender spines with flat base and sharp tips between first and second thoracic segments (sp)

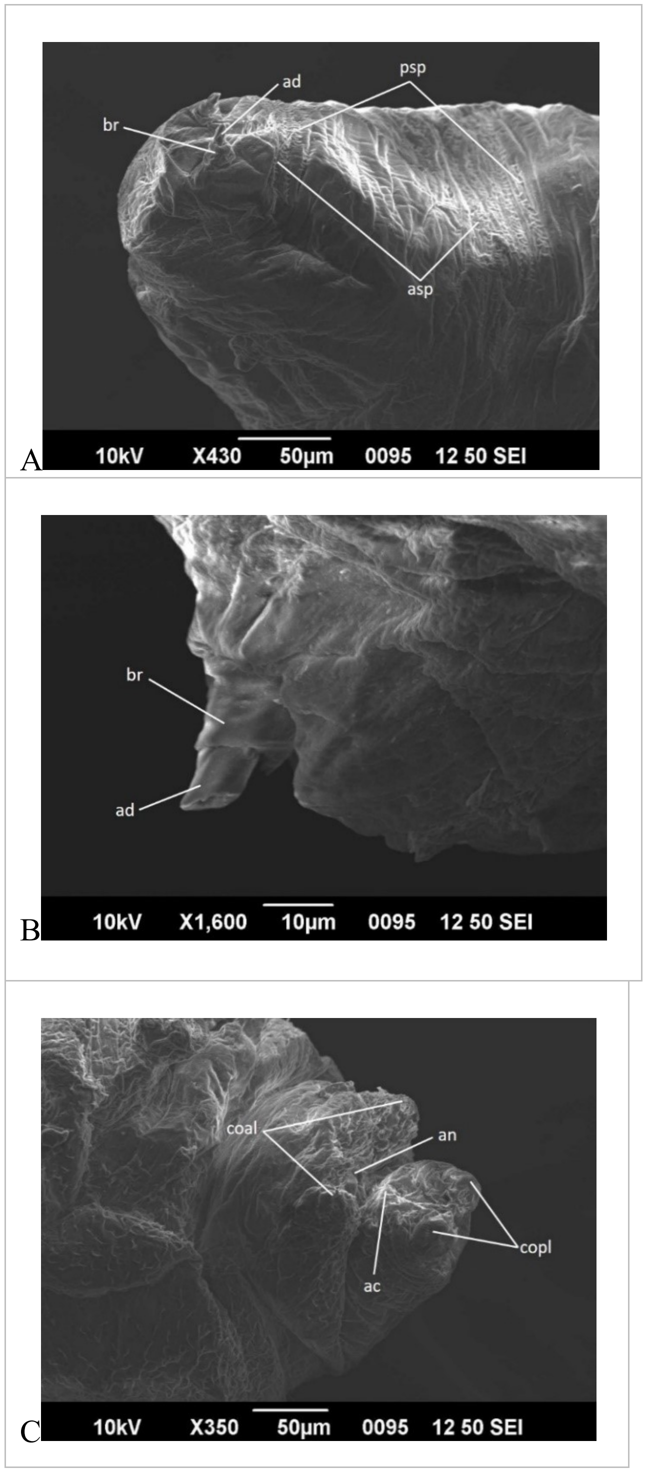

Fig. 4.48. SEM micrographs of post feeding instar stage of *H. ligurriens.*

A) pseudocephalon showing antennal complex having antennae (an) and basal ring (br), anterior and posterior spinous process (asp & psp) of the first and second thoracic segments B) antennal complex having antennae (an) and basal ring (br) C) anal segment displaying anterolateral cones (coal), postero lateral cones (copl), anus, and anal cone (ac)

Third instar

Cream to light yellow in colour. All segments from 2-11 are with spinous bands. The spines acuminate and were arranged singly in rows and have dark points on the tips. The spinous bands were found to be restricted to the lateral and ventral surfaces. The middle dorsal tubercles were moderately sized in comparison to the inner and outer tubercles. The lateral, ventral and dorsal tubercles were large and found to be fully developed (Fig. 4.43. D).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua got reduced in length and is with uniform width. Ventral cornua is equal in length to dorsal cornua (Fig. 4.44. E).

Anterior spiracles were yellowish and the lobes were arranged in a circle. Posterior spiracles were dark brown and size was bigger than that of the second instar. Peritreme is complete. Button is present (Fig. 4.44. F).

Puparium is brown in colour (Fig. 4.43. E).

Morphology of larval instars using SEM

First instar

Shape was muscoid and vermiform. Cephalic region were having terminal organs and dorsal organs in pairs. Mouth parts in the ventral regions were conspicuous with oral cirri and labium. The cirri were in the shape of curved spines. The antero-dorsal side of both pairs of pseudocephalon is occupied by an antenna in the shape of a dome which has a superior cleft and is placed on a ring like base and a circular disc shaped maxillary palpus. The height of dome is shorter than that of the height of basal ring. The diameter of the maxillary palpus is more than the antennal length. Groups of many sensilla were present in the maxillary palpus.

Anterior and posterior spines were present on thorax in 3-4 rows. Characteristic acuminate spines were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Backwardly directed spines have a bulbous base and slender sharp tip. Keilin’s organs which are sensitive to humidity were present on the ventral side of all thoracic segments.

The shape of spines in all abdominal segments was similar to thoracic segments. Filiform spines were present on anal segment Spines were present on the ventral and lateral surfaces of all segments. Anterior spinous bands were 4-5 in number and posterior spinous bands were narrow and 2-3 in number. The spinulation pattern was similar to that of the thoracic segments (Fig. 4.45).

Second Instar

Shape was muscoid and similar to first instar.The antero-dorsal side of both pairs of pseudocephalon is occupied by an antenna in the shape of a dome which has a superior cleft and is placed on a ring like base and a circular disc shaped maxillary palpus. The height of dome is shorter than that of the height of the basal ring. Groups of many sensilla were present in the maxillary palpus. Sensilla coeloconica are three in number and are arranged in a single row with some space in between them. Sensillae basiconicum are also three in number, highly reduced and not visible prominently and are positioned adjacent to the sensilla coeloconicum. Two more sensillae known as ‘first and second additional sensillum coeloconicum’ are seen dorsal to sensilla coeloconicum and basiconicum cluster.

Facial mask is very prominent on the ventral aspect of the pseudocephalon. Numerous well-structured cirri are dominating in the facial mask and are ten in number. They are characteristically arranged bilaterally on each side of the mouth opening and are gently curved medially. Three rows of spine clusters were present dorso-medial to the functional mouth opening. The first and second anterolateral rows of spines were with shapes of elongated pyramids of different sizes having broad bases and flat blunt ends. Third postero-medial row of spines were with broad bases and thin concavo-convex apex. Oral ridges were not prominent. Labial lobe was well developed with fleshy lateral lobes constituting a very distinctively demarcated ventral arch area and a medial small lobe. Rounded sensory structures were seen on lateral lobes.

Backwardly directed acuminate spines with bulbous base and slender sharp tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Keilin's organs which are sensitive to humidity are present on the ventral side of all thoracic segments.

In all the abdominal segments, spines were present on the ventral and lateral surfaces. The shape of spines in all abdominal segments was similar to thoracic segments except the last anal segment which has filiform. Anal papillae are prominent with a broad conical base especially in outer dorsal and outer ventral papillae. These papillae were surrounded by microtrichia. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number. The spinulation pattern was similar to that of the thoracic segments (Fig. 4.46).

Third Instar

The shape was vermiform and muscoid. Well developed antennal dome and terminal organs were present. The dorsal organs were present on the dorsolateral aspect of the terminal organ. The sensillae in the third instar were similar to the second instar. Three to four rows of anterior and posterior spines were seen on the thoracic segments at the inter segmental junctions. Middle ventral, inner ventral, outer ventral, outer dorsal, middle dorsal, and inner dorsal tubercles were present in pairs on the anal segment. (Fig. 4.47).

Post feeding stage

Spines on the thorax, oral cirri, antennal dome and the terminal organs were rudimentary (Fig. 4.48).

Life cycle

Seasonal life cycle data is provided in Appendix III.

Mating

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to the 6th day of emergence. The duration of mating was seen as 10 ± 04 minutes.

Fecundity

The mature female fly laid an average of 215.74 ± 22.29 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small folding, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 9.59 ± 0.89 days after mating. The next batches of eggs were laid after an interval of 3.67 ± 0.37 days. An average of 1451.26 ± 83.71 eggs were laid by the fly during its life span. The fly stopped laying eggs by the 44th day. The eggs took an average of 27 hrs for hatching (Table 4.73 - 4.76).

Development of Larvae and Pupae

First Instar

The average length of the first instar was 2.11 ± 0.23 mm and the average dry weight was 0.87 ± 0.34 mg. The first ecdysis was completed after 17 hours. The cuticle was found to be loosening approximately one to two hours before the ecdysis (Table 4. 77, 4. 82, 4. 88)

Second Instar

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 4.53 ± 0.47mm and the average dry weight was 7.52 ± 1.59 mg. The moulting was completed after 25 hours (Table 4. 78, 4. 83, 4. 88).

Third Instar

This stage took 59 hours to enter into the post feeding stage. Till then third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 7.91 ± 0.37 mm and the average dry weight was 23.35 ± 2mg (Table 4. 79, 4. 84, 4. 88).

Post feeding stage

This non-feeding stage is characterized by shortening of body length. Larva spent 115 hours in this stage before pupation. The average length of the post feeding stage was 7.75 ± 0.55 mm and the average dry weight was 26.35 ± 0.99 mg (Table 4. 80, 4. 85, 4. 88).

Pupa

The average period of pupation was 153 hours. The colour of the pupae was greyish black. The average length and width of the pupa were 6.7 mm & 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4. 88).

Adult fly

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 71 days where as the males have lesser longevity and live for only 19 days. The average length of the fly was 10-11 mm

Total life cycle period

The total life cycle period from egg till the emergence of adult fly was found to be 395.88 ± 35.82 hours (Table 4. 88).

Survival

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rate were 72.45 ±5.94 %,72.57 ± 5.68 % , 70.78 ± 5.81 % , 69.78 ± 6.69 % , 69.77 ± 6.95 % for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *H. ligurriens* was 71.07 ± 6.26% (Table 4.89 – 4. 91).

4.4. Effect of temperature and humidity on the life cycle

4.4.1. *C. megacephala*

Effect on fecundity

Effect of season on the pre-oviposition period were found to be significant ($F = 19.73$, $P = < 0.001$). Effect of year was also found to be significant ($F = 11.545$, $P = < 0.001$). However seasonal variations were same in all years and also the yearly variations were same in all seasons ($F = 0.909$, $P = 0.480$). The pre-oviposition period in *C.*

megacephala was significantly higher in winter (10.33 ± 0.71) in comparison to monsoon (9.33 ± 0.58) and summer 8.44 ± 0.88) (Table 4.13).

Table 4.13. Pre-oviposition period (days) of *C.megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	10.33 ± 0.58	9.33 ± 0.58	10.67 ± 0.58	10.11 ± 0.78^{A}
2020	9.33 ± 0.58	8.33 ± 0.58	10.33 ± 0.58	9.33 ± 1.00^{B}
2021	8.33 ± 0.58	7.67 ± 0.58	10.00 ± 1.00	8.67 ± 1.23^{C}
Overall Season	9.33 ± 1.00^{b}	8.44 ± 0.88^{c}	10.33 ± 0.71^{a}	9.37 ± 1.15
Between year F-value = 11.545**; (P-value = 0.001) Between season F-value = 19.73**; (P-value= < 0.001)) Interaction between season and year F-value =0.909^{ns}; (P-value = 0.480)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect of season on the number of eggs laid in day was found to be significant (F = 74.306, P = < 0.001). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.738, P = 0.578). The number of eggs laid by *C.megacephala* was significantly higher in monsoon in comparison to other seasons (Table 4.14).

Table 4.14. Eggs laid by the *C. megacephala* in a day

Year	Monsoon	Summer	Winter	Overall year
2019	381.00 ± 5.57	356.00 ± 8.19	319.67 ± 6.81	352.22 ± 27.38
2020	374.67 ± 7.10	338.00 ± 14.80	312.33 ± 10.02	341.67 ± 28.78
2021	368.33 ± 9.45	340.33 ± 17.16	319.00 ± 3.00	342.56 ± 23.61
Overall Season	374.67 ± 8.53^{a}	344.78 ± 14.73^{b}	317.00 ± 7.16^{c}	345.48 ± 26.09
Between year F-value = 3.062^{ns}; (P-value = 0.072) Between season F-value = 74.306**; (P-value < 0.001)) Interaction between season and year F-value =0.738^{ns}; (P-value = 0.578)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season on the periodicity of egg laying was found to be significant (F = 6.300, P = < 0.008). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.65, P = 0.205). The periodicity of egg laying in *C. megacephala* was significantly higher in winter (4.67 ± 0.35) in comparison to monsoon (4.33 ± 0.35) and summer (4.17 ± 0.25) (Table 4.15).

Table 4.15. Periodicity of egg laying (days) by *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	4.17 ± 0.29	4.33 ± 0.29	4.33 ± 0.29	4.28 ± 0.26
2020	4.50 ± 0.50	4.17 ± 0.29	4.83 ± 0.29	4.50 ± 0.43
2021	4.33 ± 0.29	4.00 ± 0.00	4.83 ± 0.29	4.39 ± 0.42
Overall Season	4.33 ± 0.35^{b}	4.17 ± 0.25^{b}	4.67 ± 0.35^{a}	4.39 ± 0.38
Between year F-value = 1.200^{ns}; (P-value = 0.324) Between season F-value = 6.300**; (P-value = 0.008)) Interaction between season and year F-value = 1.65^{ns}; (P-value = 0.205)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 323.32, P = < 0.001). Effect of year on the number of eggs laid was also found to be significant (F= 29.859; P = < 0.001). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.130, P = 0.373). The number of eggs laid in *C. megacephala* was significantly higher in monsoon (2796.33 ± 114.39) in comparison to summer (2442.89 ± 80.12) and winter (2217.11 ± 69.91) (Table 4.16).

Table 4.16. Eggs laid by the *C. megacephala* during in its life span

Year	Monsoon	Summer	Winter	Overall year
2019	2919.00 ± 57.72	2533.33 ± 10.97	2285.67 ± 61.27	2579.33 ±279.64^{A}
2020	2791.33 ± 38.28	2426.33 ± 47.9	2204.33 ± 33.31	2474 ± 259.04^{B}
2021	2678.67 ± 64.53	2369.00 ± 48.88	2161.33 ± 52.21	2403 ± 230.55^{C}
Overall Season	2796.33 ± 114.39^{a}	2442.89 ± 80.12^{b}	2217.11 ± 69.91^{c}	2485.44 ± 257.9
Between year F-value = 29.859**; (P-value < 0.001) Between season F-value = 323.32**; (P-value < 0.001) Interaction between season and year F-value = 1.130^{ns} ; (P-value = 0.373)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect on length of larvae

Instar I

Effect of season, year and stage on the length of Ist instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The instar length in *C. megacephala* was significantly higher (1.60 ± 0.05) in monsoon in comparison to summer (1.44 ± 0.09) and winter (1.47 ± 0.05) (Table 4.17).

Table 4.17. Seasonal changes in length (mm) of Ist instar larvae of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	1.65 ± 0.02^{a}	1.41 ± 0.12^{b}	1.44 ± 0.0004^{b}	1.50 ± 0.13^{AB}
2020	1.54 ± 0.04^{a}	1.39 ± 0.03^{b}	1.45 ± 0.05^{ab}	1.46 ± 0.07^{B}
2021	1.61 ± 0.02	1.51 ± 0.08	1.53 ± 0.04	1.55 ± 0.06^{A}
Overall Season	1.60 ± 0.05^{a}	1.44 ± 0.09^{b}	1.47 ± 0.05^{b}	1.5 ± 0.1

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar II

Effect of season, year and stage on the length of IInd instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The instar length in *C. megacephala* was significantly higher in monsoon (6.13 ± 0.65) in comparison to summer (5.65 ± 0.96) and winter (3.65 ± 0.65) (Table 4.18).

Table 4.18. Seasonal changes in length (mm) of IInd instar larvae of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	5.74 ± 0.02^{aB}	4.39 ± 0.09^{bC}	4.47 ± 0.002^{bA}	4.87 ± 0.66^{C}
2020	6.99 ± 0.05^{aA}	6.14 ± 0.18^{bB}	3.00 ± 0.08^{cC}	5.38 ± 1.83^{A}
2021	5.66 ± 0.1^{bB}	6.41 ± 0.09^{aA}	3.49 ± 0.02^{cB}	5.19 ± 1.32^{B}
Overall Season	6.13 ± 0.65^{a}	5.65 ± 0.96^{b}	3.65 ± 0.65^{c}	5.14 ± 1.32

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar III

Effect of season, year and stage on the length of IIIrd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The instar length in *C. megacephala* was significantly higher in summer (11.87 ± 0.35) in comparison to monsoon (11.17 ± 0.59) and winter (9.70 ± 0.40) (Table 4.19).

Table 4.19. Seasonal changes in length (mm) of IIIrd instar larvae of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	10.41 ± 0.07^{aC}	11.72 ± 0.14^{bB}	10.21 ± 0.02^{cA}	10.78 ± 0.72^{B}
2020	11.38 ± 0.09^{bB}	11.57 ± 0.04^{aC}	9.55 ± 0.08^{cB}	10.83 ± 0.97^{B}
2021	11.73 ± 0.01^{bA}	12.31 ± 0.05^{aA}	9.33 ± 0.04^{cC}	11.12 ± 1.37^{A}
Overall Season	11.17 ± 0.59^{b}	11.87 ± 0.35^{a}	9.70 ± 0.40^{c}	10.91 ± 1.02

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Post feeding stage

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The length of post feeding stage in *C. megacephala* was significantly higher in summer (10.98 ± 0.32) in comparison to monsoon (10.62 ± 0.38) and winter (10.2 ± 0.17) (Table 4.20).

Table 4.20. Seasonal changes in length (mm) of post feeding stage of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	10.23 ± 0.07^{bC}	10.68 ± 0.17^{aC}	10.11 ± 0.03^{bB}	10.34 ± 0.28^{C}
2020	10.56 ± 0.05^{bB}	10.92 ± 0.07^{aB}	10.09 ± 0.10^{cB}	10.52 ± 0.37^{B}
2021	11.08 ± 0.07^{bA}	11.34 ± 0.24^{aA}	10.39 ± 0.14^{cA}	10.94 ± 0.45^{A}
Overall Season	10.62 ± 0.38^{b}	10.98 ± 0.32^{a}	10.2 ± 0.17^{c}	10.60 ± 0.44

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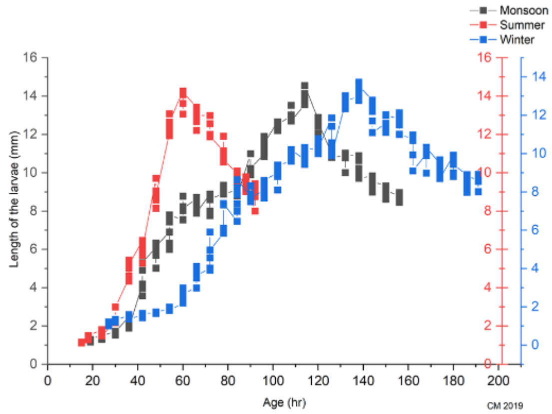
Interaction studies on year, season and stage was found to be significant (F = 115.12; P = < 0.001) indicating that seasonal variations in length of each larval instar were different in different years (Table 4.21).

Table 4.21. ANOVA for comparing the length of larval instars of *C. megacephala*

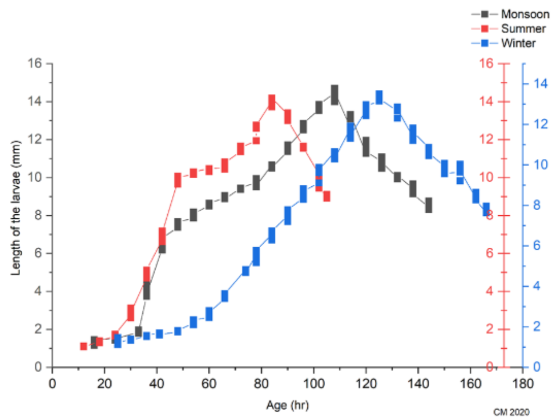
Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	1.95	0.98	126.08**	<0.001
Season	2	33.36	16.68	2155.47**	<0.001
Stage	3	1672.43	557.48	72030.16**	<0.001
Year * Season	4	6.70	1.68	216.48**	<0.001
Year * stage	6	1.59	0.27	34.21**	<0.001
Season * stage	6	22.72	3.79	489.15**	<0.001
Year * Season * Stage	12	10.69	0.89	115.12**	<0.001
Error	72	0.56	0.01		
Total	107	1750.00			

** Significant at 0.01 level

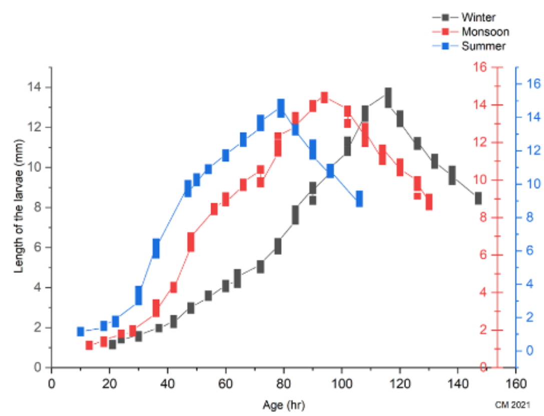
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *C. megacephala* from hatching until pupation during different seasons and years were prepared (Fig. 4.49 and Fig. 4.50).



2019

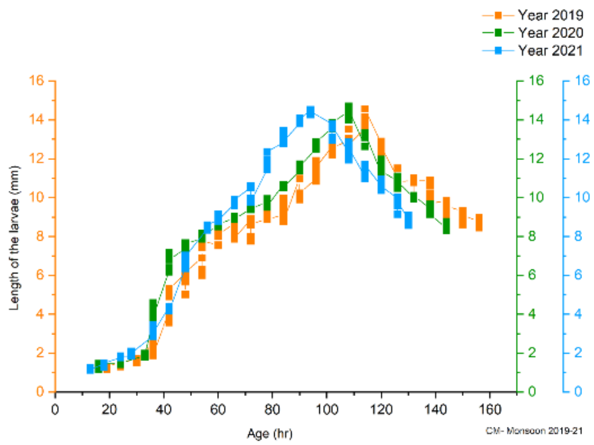


2020

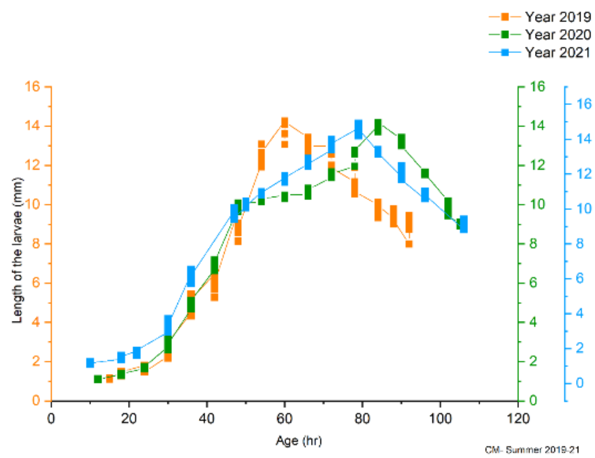


2021

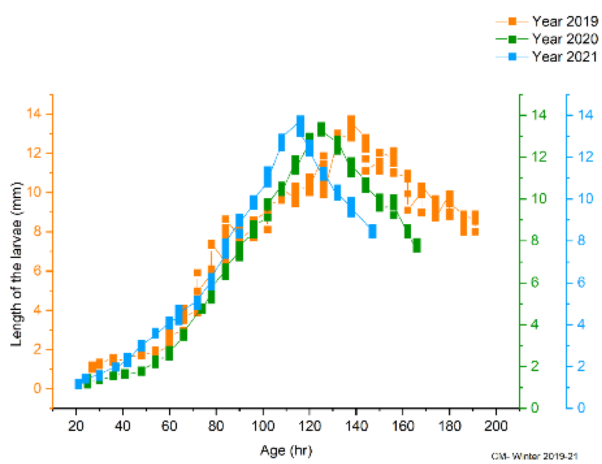
Fig. 4.49. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *C. megacephala* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.50. Developmental rate (Length (mm) Vs. Age (hr) of *C. megacephala* from hatching upto pupation during the study period

Instar I

Effect of season and year on the weight of Ist instar larvae was found to be non significant. The pattern of differences in the weight was similar in all the years and seasons (Table 4. 22).

Table 4. 22. Seasonal changes in weight (mg) of Ist instar larvae of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	1.40 ± 0.05	1.28 ± 0.07	1.50 ± 0.06	1.39 ± 0.11
2020	1.42 ± 0.07	1.44 ± 0.06	1.70 ± 0.08	1.52 ± 0.15
2021	1.46 ± 0.10	1.55 ± 0.06	1.46 ± 0.05	1.49 ± 0.08
Overall Season	1.43 ± 0.07	1.42 ± 0.13	1.55 ± 0.12	1.47 ± 0.12

Means having different small letter as superscript differ significantly within a row
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Instar II

Effect of season, year and stage on the IInd instar weight was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.26). The instar weight in *C. megacephala* was significantly higher in monsoon (10.37 ± 0.42) in comparison to summer (10.33 ± 0.40) and winter (9.88 ± 0.54) (Table 4.23).

Table 4.23. Seasonal changes in weight (mg) of IInd instar larvae of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	10.66 ± 0.30^{aA}	10.60 ± 0.44^{aA}	9.26 ± 0.27^{bB}	10.17 ± 0.75
2020	10.54 ± 0.12^{aA}	10.05 ± 0.33^{bB}	10.24 ± 0.29^{abA}	10.27 ± 0.31
2021	9.93 ± 0.38^{B}	10.35 ± 0.34^{AB}	10.13 ± 0.40^{A}	10.13 ± 0.37
Overall Season	10.37 ± 0.42^{a}	10.33 ± 0.40^{a}	9.88 ± 0.54^{b}	10.19 ± 0.50

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar III

Effect of season, year and stage on the weight of IIIrd instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.26). The instar weight in *C. megacephala* was significantly higher in summer (31.75 ± 1.10) in comparison to monsoon (30.61 ± 0.54) and winter (29.77 ± 0.40) (Table 4.24).

Table 4.24. Seasonal changes in weight (mg) of IIIrd instar larvae of *C. megacephala* during the study period

Year	Monsoon	Summer	Winter	Overall year
2019	30.46 ± 0.18^{bB}	33.05 ± 0.41^{aA}	29.58 ± 0.21^{cB}	31.03 ± 1.58^{A}
2020	31.24 ± 0.30^{aA}	31.62 ± 0.09^{aB}	29.46 ± 0.18^{bB}	30.78 ± 1.01^{B}
2021	30.13 ± 0.28^{bB}	30.58 ± 0.12^{aC}	30.25 ± 0.14^{abA}	30.32 ± 0.26^{C}
Overall Season	30.61 ± 0.54^{b}	31.75 ± 1.10^{a}	29.77 ± 0.40^{c}	30.71 ± 1.09

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Post feeding stage

Effect of season, year and stage on the weight of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.26). The weight of post feeding stage in *C. megacephala* was significantly higher in monsoon (30.03 ± 0.56) in comparison to summer (29.98 ± 0.64) and winter (28.89 ± 0.59) (29.42 ± 0.93) (Table 4.25).

Table 4.25. Seasonal changes in weight (mg) of post feeding stage of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	30.34 ± 0.14^{aA}	30.72 ± 0.48^{aA}	28.94 ± 0.34^{bB}	30.00 ± 0.87^{A}
2020	29.34 ± 0.33^{B}	29.71 ± 0.10^{B}	29.39 ± 0.58^{A}	29.48 ± 0.38^{B}
2021	30.40 ± 0.26^{aA}	29.51 ± 0.32^{bB}	28.35 ± 0.34^{cC}	29.42 ± 0.93^{B}
Overall Season	30.03 ± 0.56^{a}	29.98 ± 0.64^{a}	28.89 ± 0.59^{b}	29.63 ± 0.78

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

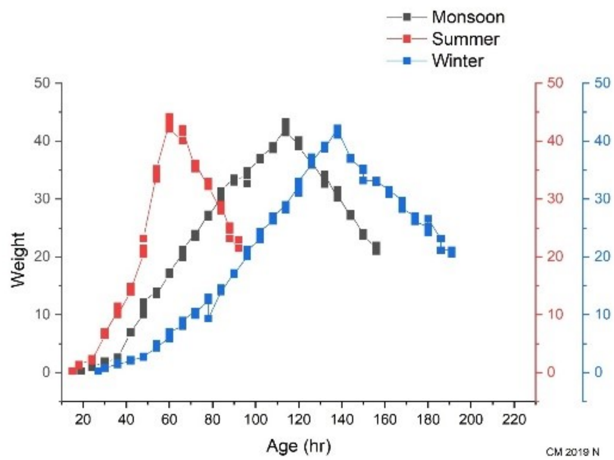
Interaction studies on year, season and stage was found to be significant (F = 14.365; P = < 0.001) indicating that seasonal variations in weight of each stage are different in different years (Table 4.26).

Table 4.26. ANOVA for comparing the weight of larval instars of *C. megacephala*

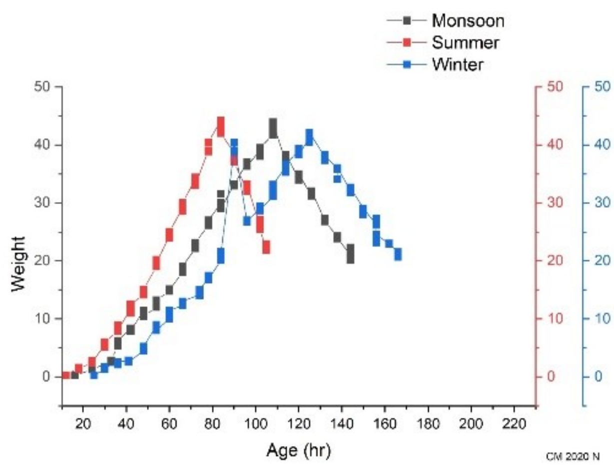
Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	1.70	0.851	11.718**	< 0.001
Season	2	13.60	6.799	93.618**	< 0.001
Stage	3	17041.12	5680.372	78210.86**	< 0.001
Year * Season	4	5.08	1.269	17.473**	< 0.001
Year * stage	6	2.64	0.44	6.061**	< 0.001
Season * stage	6	13.13	2.189	30.135**	< 0.001
Year * Season * Stage	12	12.52	1.043	14.365**	< 0.001
Error	72	5.23	0.073		
Total	107	17095.02			

** Significant at 0.01 level; ns non-Significant

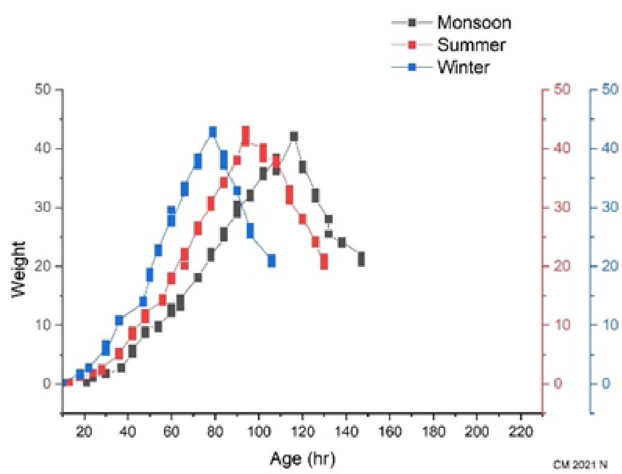
The growth curve representing the developmental rate (Weight (mg) Vs. Age (hr) of *C. megacephala* from hatching until pupation during different seasons and years were prepared (Fig. 4.51 and Fig. 4.52).



2019

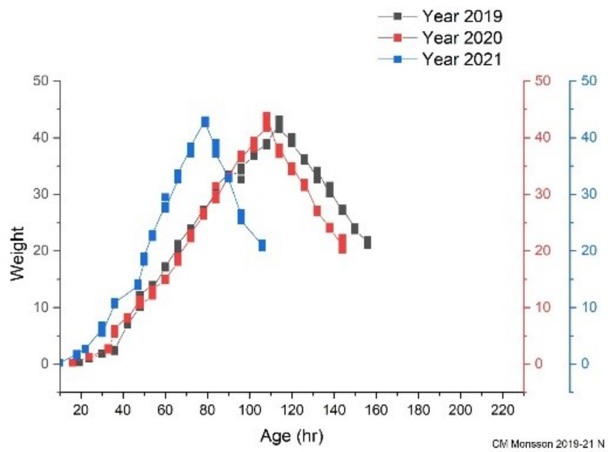


2020

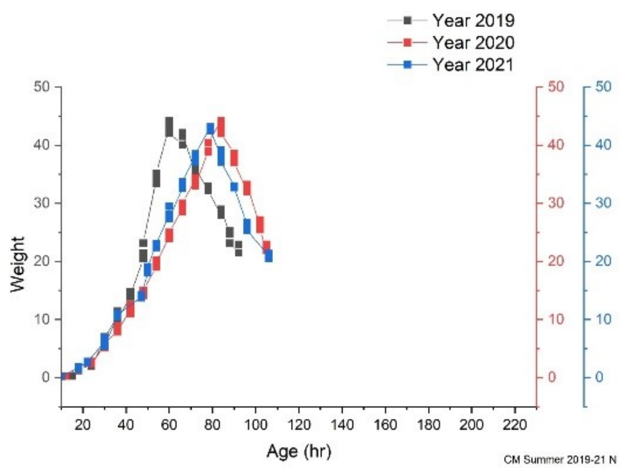


2021

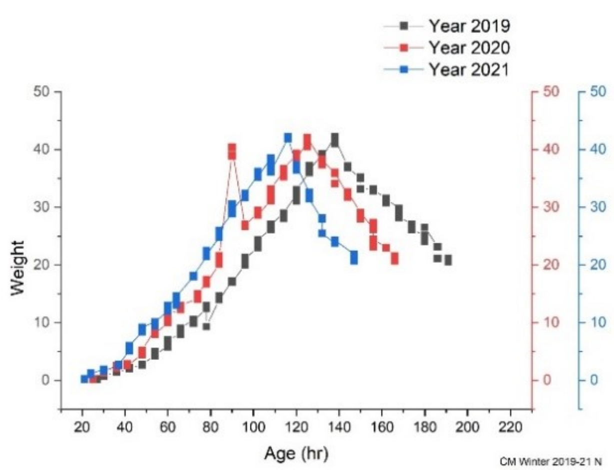
Fig. 4.51. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *C. megacephala* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.52. Developmental rate (Weight (mg) Vs Age (hr) of *C. megacephala* from hatching upto pupation during the study period

Effect on life cycle duration

Significantly higher duration (hr) was observed during winter for *C. megacephala* for incubation (24.00 ± 2.65) ($F = 408.40$; $P = <0.001$), duration of IInd larval instar (28.00 ± 5.29) ($F = 8.555$; $P = 0.036$), post feeding stage (36.33 ± 10.69) ($F = 8.704$; $P = 0.035$), pupation (125.67 ± 7.37) ($F = 480.82$; $P = <0.001$), and total life cycle (286.00 ± 23.26) ($F = 50.49$; $P = <0.001$) (Table 4.27).

Table 4.27. Seasonal changes in duration (hrs) of life cycle of *C. megacephala*

Stages	Monsoon	Summer	Winter	F-value (P-value)
Incubation	15.67 ± 2.52^{b}	12.00 ± 2.00^{c}	24.00 ± 2.65^{a}	408.40** (<0.001)
Instar I	16.67 ± 1.53	12.00 ± 0	23.00 ± 6.08	6.802^{ns} (0.052)
Instar II	22.33 ± 2.08^{ab}	17.00 ± 1.00^{b}	28.00 ± 5.29^{a}	8.555* (0.036)
Instar III	45.33 ± 13.32	27.00 ± 4.36	49.00 ± 6.25	5.417^{ns} (0.073)
Post feeding stage	32.00 ± 5.29^{ab}	23.00 ± 4.58^{b}	36.33 ± 10.69^{a}	8.704* (0.035)
Pupation	95.00 ± 3.61^{b}	77.00 ± 5.57^{c}	125.67 ± 7.37^{a}	480.82** (<0.001)
Total time taken from egg stage till emergence	227.00 ± 22.52^{b}	168.00 ± 5.29^{c}	286.00 ± 23.26^{a}	50.49** (<0.001)

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
Means having different letter as super script differ significantly between seasons (within a row)

Significantly higher duration (hrs) was observed during 2019 for *C. megacephala* for incubation (19.33 ± 6.11) ($F = 60.40$; $P = < 0.001$) and post feeding stage (38.00 ± 10.00) ($F = 8.244$; $P = 0.038$). The duration of pupation stage was significantly higher in 2020 (105.33 ± 26.08) ($F = 24.029$; $P = 0.006$) (Table 4.28).

Table 4.28. Changes in duration (hrs) of life cycle of *C. megacephala*

Stages	2019	2020	2021	F-value (P-value)
Incubation	19.33 ± 6.11^{a}	17.67 ± 6.66^{b}	14.67 ± 5.69^{c}	60.40** (< 0.001)
Instar I	19.00 ± 7.55	15.00 ± 2.65	17.67 ± 7.37	0.926^{ns} (0.467)
Instar II	24.33 ± 9.07	22.67 ± 4.16	20.33 ± 3.51	1.141^{ns} (0.405)
Instar III	46.00 ± 20.88	39.67 ± 8.74	35.67 ± 7.64	1.058^{ns} (0.428)
Post feeding stage	38.00 ± 10.00^{a}	27.67 ± 7.77^{b}	25.67 ± 3.22^{b}	8.244* (0.038)
Pupation	94.67 ± 24.11^{b}	105.33 ± 26.08^{a}	97.67 ± 23.71^{b}	24.029** (0.006)
Total time taken from egg stage till emergence	241.33 ± 73.8	228.00 ± 54	211.67 ± 49.94	3.202^{ns} (0.148)

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
Means having different letter as super script differ significantly between years (within a row)

Effect on survival rate

Effect of season on the survival rate (%) of *C. megacephala* was found to be significant (Table 4.32).The survival rate (%) in *C. megacephala* was significantly higher in monsoon (80.73 ± 9.94) in comparison to summer (77.9 ± 10.38) and winter (72.48 ± 6.39) and significantly higher in 2021 (78.06 ± 7.08) compared to 2019 and 2020 (Table 4.29).

Table 4.29. Seasonal changes in survival rate (%) of *C. megacephala*

Year	Monsoon	Summer	Winter	Overall year
2019	81.33 ± 11.64	77.41 ± 13.11	72.05 ± 8.30	76.93 ± 11.59^{AB}
2020	79.87 ± 10.57	77.25 ± 10.81	71.23 ± 5.82	76.12 ± 9.84^{B}
2021	80.98 ± 7.89	79.03 ± 6.91	74.16 ± 4.57	78.06 ± 7.08^{A}
Overall Season	80.73 ± 9.94^{a}	77.9 ± 10.38^{b}	72.48 ± 6.39^{c}	77.04 ± 9.65

Means having different small letter as superscript differ significantly within a row
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Effect of year and stage on the survival rate was found to be significant (Table 4.32) The survival rate of egg was significantly higher in 2019 (90.76 ± 4.94) compared to 2020 (86.68 ± 5.97) and 2021 (81.53 ± 5.39). However, the survival rate in 2nd Instar (77.96 ± 7.05) and 3rd Instar (72.74 ± 4.14) was significantly higher in 2021. Similarly the survival rate of pupation was also significantly higher in 2021 (72.74 ± 4.12) (Table 4.30).

Table 4.30. Survival rate (%) of life cycle stages of *C.megacephala*

Stage	2019	2020	2021	Overall stage
Egg	90.76 ± 4.94^{aA}	86.68 ± 5.97^{bA}	81.53 ± 5.39^{cB}	86.32 ± 6.50^{A}
Ist Instar	84.70 ± 8.58^{B}	82.64 ± 8.25^{B}	85.32 ± 5.08^{A}	84.22 ± 7.27^{B}
IInd Instar	74.84 ± 9.11^{bC}	75.15 ± 8.37^{bC}	77.96 ± 7.05^{aC}	75.98 ± 8.03^{C}
IIIrd Instar	67.54 ± 4.68^{bD}	67.49 ± 4.01^{bD}	72.74 ± 4.14^{aD}	69.26 ± 4.82^{D}
Pupa	66.81 ± 5.86^{bD}	68.64 ± 4.71^{bD}	72.74 ± 4.12^{aD}	69.4 ± 5.38^{D}
Overall year	76.93 ± 11.59^{ab}	76.12 ± 9.84^{b}	78.06 ± 7.08^{a}	77.04 ± 9.65

Means having different small letter as superscript differ significantly within a row
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Effect of season and stage on the survival rate was found to be significant (Table 4.32). The survival of egg (89.70 ± 4.78), Ist Instar (89.29 ± 2.61), IInd Instar (84.65 ± 2.72), IIIrd Instar larvae (73.83 ± 3.69) was significantly higher in monsoon compared to other seasons. Survival of pupa of *C. megacephala* was significantly higher in winter (73.85 ± 3.07) compared to monsoon (66.18 ± 3.31) and summer (68.17 ± 6.17) (Table 4.31).

Table 4.31. Seasonal changes in survival rate (%) of life cycle stages of *C. megacephala*

Stage	Monsoon	Summer	Winter	Overall stage
Egg	89.70 ± 4.78^{aA}	89.39 ± 4.12^{aA}	79.88 ± 5.23^{bA}	86.32 ± 6.50^{A}
Ist Instar	89.29 ± 2.61^{aA}	88.33 ± 2.39^{aA}	75.04 ± 4.09^{bB}	84.22 ± 7.27^{B}
IInd Instar	84.65 ± 2.72^{aB}	76.52 ± 2.23^{bB}	66.77 ± 4.09^{cD}	75.98 ± 8.03^{C}
IIIrd Instar	73.83 ± 3.69^{aC}	67.09 ± 3.93^{bC}	66.85 ± 3.32^{bD}	69.26 ± 4.82^{D}
Pupa	66.18 ± 3.31^{bD}	68.17 ± 6.17^{bC}	73.85 ± 3.07^{aC}	69.40 ± 5.38^{D}
Overall Season	80.73 ± 9.94^{a}	77.9 ± 10.38^{b}	72.48 ± 6.39^{c}	77.04 ± 9.65

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between year and stage (F=10.06; P=<0.001) as well as season and stage F = 29.87; P = <0.001) (Table 4.32).

Table 4.32. ANOVA for comparing the survival rate of *C. megacephala*

Source	df	Sum of Squares	Mean Square	F-value	P-value
Year	2	85.34	42.67	4.79*	0.011
Season	2	1581.27	790.64	88.68**	<0.001
Stage	4	6963.08	1740.77	195.25**	<0.001
Year * season	4	29.40	7.35	0.82^{ns}	0.513
Year * Stage	8	717.24	89.66	10.06**	<0.001
season * Stage	8	2130.54	266.32	29.87**	<0.001
Year * season * Stage	16	159.34	9.96	1.12^{ns}	0.352
Error	90	802.40	8.92		
Corrected Total	134	12468.60			

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

4.4.2. *C. rufifacies*

Effect on fecundity

Effect of season on the pre-oviposition period were found to be significant (F = 23.444, P = < 0.001). Effect of year on the pre-oviposition period were found to be significant (F = 2.111, P = 0.150) However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.778, P = 0.177). The pre-oviposition period (days) in *C.rufifacies was* significantly higher in winter (9.22 ± 0.67) in comparison to monsoon (7.56 ± 0.53) and summer (7.67 ± 0.71) (Table 4.33).

Table 4.33. Pre-oviposition period (days) of *C.rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	7.33 ± 0.58	8.33 ± 0.58	9.67 ± 0.58	8.44 ± 1.13
2020	7.67 ± 0.58	7.33 ± 0.58	9.33 ± 0.58	8.11 ± 1.05
2021	7.67 ± 0.58	7.33 ± 0.58	8.67 ± 0.58	7.89 ± 0.78
Overall Season	7.56 ± 0.53^{b}	7.67 ± 0.71^{b}	9.22 ± 0.67^{a}	8.15 ± 0.99
Between year F-value = 2.111^{ns}; (P-value = 0.150) Between season F-value = 23.444**; (P-value < 0.001)) Interaction between season and year F-value =1.778^{ns}; (P-value = 0.177)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season on the number of eggs laid in a day was found to be significant (F = 223.63, P = < 0.001). Effect of year on the oviposition was also found to be significant (F = 3.67, P = 0.046). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 2.91, P = 0.051). The number of eggs laid by *C. rufifacies* was significantly higher in monsoon (281.00 ± 5.45) in comparison to summer (246.89 ± 11.68) and winter (215.33 ± 5.87) (Table 4.34).

Table 4.34. Eggs laid by the *C. rufifacies* in a day

Year	Monsoon	Summer	Winter	Overall year
019	278.33 ± 2.08	254.67 ± 9.07	217.00 ± 8.19	250.00 ± 27.5^{A}
2020	282.67 ± 4.16	252.00 ± 4.58	216.33 ± 4.73	250.33 ± 29.01^{A}
2021	282.00 ± 9.00	234.00 ± 7.94	212.67 ± 5.69	242.89 ± 31.46^{B}
Overall Season	281.00 ± 5.45^{a}	246.89 ± 11.68^{b}	215.33 ± 5.87^{c}	247.74 ± 28.43
Between year F-value = 3.67*; (P-value = 0.046) Between season F-value = 223.63**; (P-value < 0.001)) Interaction between season and year F-value =2.91^{ns}; (P-value = 0.051)				

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect of season on the periodicity of egg laying was found to be significant (F = 5.727, P = 0.012). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.091; P = 0.391). The periodicity of egg laying in

C. rufifacies was significantly higher in winter (4.67 ± 0.35) in comparison to monsoon (4.50 ± 0.35) and summer (4.17 ± 0.25) (Table 4.35).

Table 4.35. Periodicity of egg laying (days) by *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	4.83 ± 0.29	4.17 ± 0.29	4.67 ± 0.29	4.56 ± 0.39
2020	4.33 ± 0.29	4.17 ± 0.29	4.50 ± 0.50	4.33 ± 0.35
2021	4.33 ± 0.29	4.17 ± 0.29	4.83 ± 0.29	4.44 ± 0.39
Overall Season	4.50 ± 0.35^{a}	4.17 ± 0.25^{b}	4.67 ± 0.35^{a}	4.44 ± 0.38
Between year F-value = 1.091^{ns}; (P-value = 0.357) Between season F-value = 5.727*; (P-value = 0.012)) Interaction between season and year F-value =1.091^{ns}; (P-value = 0.391)				

Means having different small letter as superscript differ significantly within a row

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 835.8, P =< 0.001). However, seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 3.28, P = 0.035). The number of eggs laid by *C. rufifacies* was significantly higher in monsoon (1953.89 ± 22.70) in comparison to summer (1849.11 ± 26.05) and winter (1723.78 ± 12.18) (Table 4.36).

Table 4.36. Eggs laid by the *C. rufifacies* during in its life span

Year	Monsoon	Summer	Winter	Overall year
2019	1975.67 ± 14.19^{aA}	1877.67 ± 11.59^{bA}	1734.00 ± 11.14^{c}	1862.44 ± 105.81^{A}
2020	1953.67 ± 10.26^{aB}	1848.33 ± 13.32^{bB}	1714.33 ± 8.33^{c}	1838.78 ± 104.31^{B}
2021	1932.33 ± 18.58^{aC}	1821.33 ± 4.51^{bC}	1723.00 ± 10.44^{c}	1825.56 ± 91.35^{C}
Overall Season	1953.89 ± 22.70^{a}	1849.11 ± 26.05^{b}	1723.78 ± 12.18^{c}	1842.26 ± 97.99
Between year F-value = 22.00; (P-value < 0.001) Between season F-value = 835.8**; (P-value < 0.001)) Interaction between season and year F-value =3.28*; (P-value = 0.035)				

** Significant at 0.01 level; * *significant at 0.05 level*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect on length of larvae

Instar I

Effect of season, year and stage on the length of Ist instar was found to be significant. Season wise variations were not same in all years and also the year wise variations were not same in all seasons (Table 4.41). The instar length in C. *rufifacies* was significantly higher in monsoon (1.79 ± 0.16) in comparison to summer (1.64 ± 0.26) and winter (1.56 ± 0.02). It was significantly higher (1.84 ± 0.22) in 2021 compared to 2019 (1.54 ± 0.10) and 2020 (1.61 ± 0.10) (Table 4.37).

Table 4.37. Seasonal changes in length (mm) of Ist instar larvae of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	1.64 ± 0.01^{aB}	1.41 ± 0.02^{bB}	1.56 ± 0.01^{a}	1.54 ± 0.10^{C}
2020	1.74 ± 0.02^{aB}	1.53 ± 0.05^{bB}	1.56 ± 0.01^{b}	1.61 ± 0.10^{B}
2021	1.99 ± 0.06^{aA}	1.97 ± 0.02^{aA}	1.55 ± 0.05^{b}	1.84 ± 0.22^{A}
Overall Season	1.79 ± 0.16^{a}	1.64 ± 0.26^{b}	1.56 ± 0.02^{c}	1.66 ± 0.20

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar II

Effect of season, year and stage on the length of IInd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.41). The instar length in *C. rufifacies* was significantly higher in monsoon (5.83 ± 0.07) in comparison to summer (5.63 ± 0.08) and winter (4.76 ± 0.41). It was significantly higher (5.56 ± 0.28) in 2021 compared to 2019 (4.50 ± 1.51) and 2020 (5.08 ± 0.53) (Table 4.38).

Table 4.38. Seasonal changes in length (mm) of IInd instar larvae of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	6.34 ± 0.12^{aA}	2.88 ± 0.02^{cC}	4.29 ± 0.10^{bC}	4.50 ± 1.51^{C}
2020	5.77 ± 0.09^{aB}	4.69 ± 0.18^{bB}	4.77 ± 0.07^{bB}	5.08 ± 0.53^{B}
2021	5.83 ± 0.07^{aB}	5.63 ± 0.08^{bA}	5.22 ± 0.07^{cA}	5.56 ± 0.28^{A}
Overall Season	5.98 ± 0.28^{a}	4.40 ± 1.22^{c}	4.76 ± 0.41^{b}	5.05 ± 1

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar III

Effect of season, year and stage on the length of IIIrd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.41). The instar length in *C. rufifacies* was significantly higher in monsoon (10.94 ± 0.10) in comparison to summer (10.29 ± 0.77) and winter (10.61 ± 0.13). It was significantly higher (10.87 ± 0.29) in 2021 compared to 2019 (10.26 ± 0.71) and 2020 (10.71 ± 0.19) (Table 4.39).

Table 4.39. Seasonal changes in length (mm) of IIIrd instar larvae of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	10.88 ± 0.08^{aB}	9.33 ± 0.1^{cC}	10.58 ± 0.07^{bB}	10.26 ± 0.71^{C}
2020	10.87 ± 0.04^{aB}	10.49 ± 0.17^{bB}	10.76 ± 0.04^{aA}	10.71 ± 0.19^{B}
2021	11.06 ± 0.04^{aA}	11.06 ± 0.08^{aA}	10.5 ± 0.04^{bB}	10.87 ± 0.29^{A}
Overall Season	10.94 ± 0.10^{a}	10.29 ± 0.77^{c}	10.61 ± 0.13^{b}	10.61 ± 0.51

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Post feeding stage

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.41). The length of post feeding stage in *C. rufifacies* was significantly higher in monsoon (10.81 ± 0.52) in comparison to summer (10.37 ±

0.22) and winter (10.66 ± 0.41). It was significantly higher (10.80 ± 0.46) in 2021 compared to 2019 (10.31 ± 0.25) and 2020 (10.73 ± 0.40) (Table 4.40).

Table 4.40. Seasonal changes in length (mm) of post feeding stage of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	10.15 ± 0.05^{bB}	10.65 ± 0.05^{aA}	10.14 ± 0.02^{bC}	10.31 ± 0.25^{B}
2020	10.98 ± 0.07^{aA}	10.21 ± 0.08^{bB}	11.00 ± 0.14^{aA}	10.73 ± 0.40^{A}
2021	11.30 ± 0.09^{aA}	10.26 ± 0.09^{cB}	10.85 ± 0.09^{bB}	10.80 ± 0.46^{A}
Overall Season	10.81 ± 0.52^{a}	10.37 ± 0.22^{c}	10.66 ± 0.41^{b}	10.61 ± 0.43

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

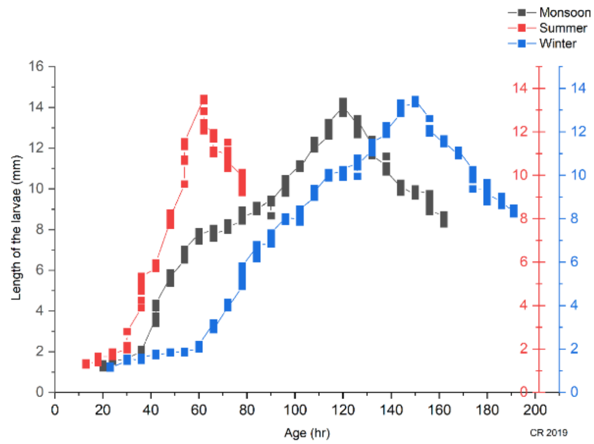
Interaction studies on year, season and stage was found to be significant (F = 0.95; P = < 0.001) indicating that seasonal variations in length of each larval instar stage were different in different years (Table 4.41).

Table 4.41. ANOVA for comparing length of larval instars of *C. rufifacies*

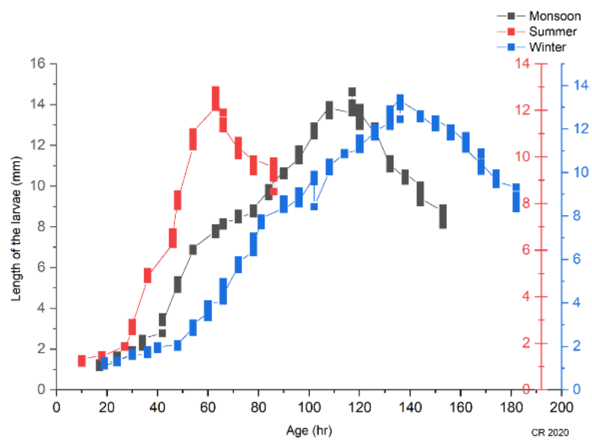
Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	6.91	3.45	571.01**	<0.001
Season	2	9.31	4.66	769.84**	<0.001
Stage	3	1577.29	525.76	86910.80**	<0.001
Year * Season	4	2.97	0.74	122.62**	<0.001
Year * stage	6	1.62	0.27	44.65**	<0.001
Season * stage	6	6.06	1.01	167.04**	<0.001
Year * Season * Stage	12	11.39	0.95	156.91**	<0.001
Error	72	0.44	0.01		
Total	107	1615.99			

** Significant at 0.01 level

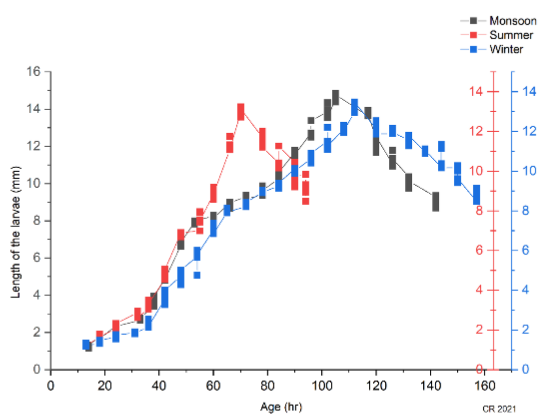
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *C. rufifacies* from hatching until pupation during different seasons and years were prepared (Fig. 4.53 & Fig. 4.54).



2019

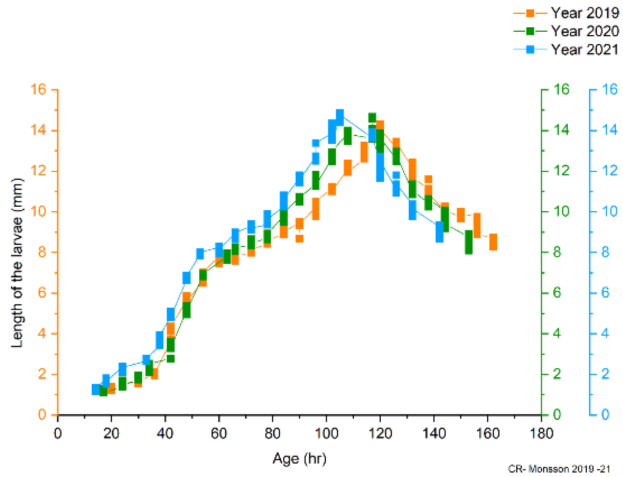


2020

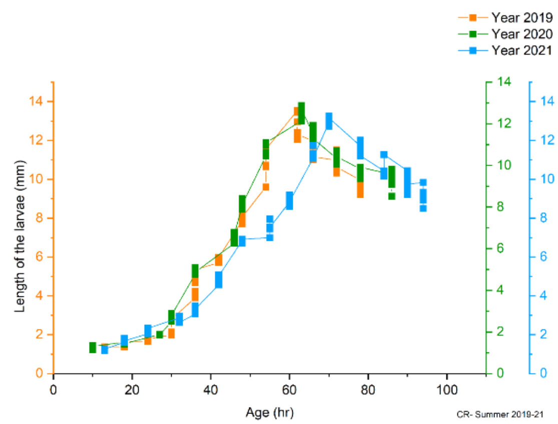


2021

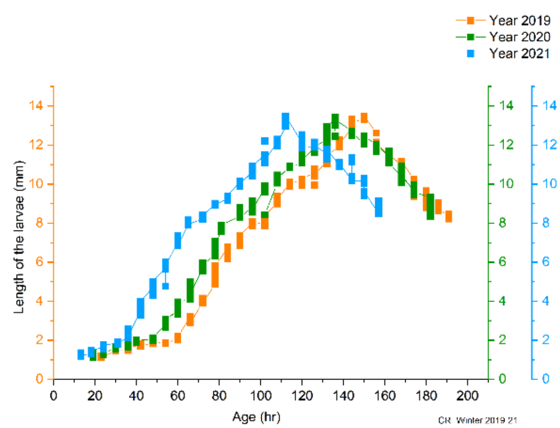
Fig. 4.53. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *C. rufifacies* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.54. Developmental rate (Length (mm) Vs. Age (hr.) of *C. rufifacies* from hatching up to pupation during the study period

Effect on weight of larvae

Instar I

Effect of season and year on the weight of Ist instar was found to be not significant. The pattern of differences in the weight was similar in all the years and seasons (Table 4.42).

Table 4.42. Seasonal changes in weight (mg) of Ist instar larvae of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	1.54 ± 0.04	1.54 ± 0.07	1.52 ± 0.04	1.53 ± 0.05
2020	1.53 ± 0.04	1.47 ± 0.07	1.61 ± 0.05	1.54 ± 0.08
2021	1.54 ± 0.04	1.43 ± 0.06	1.58 ± 0.04	1.52 ± 0.08
Overall Season	1.53 ± 0.03	1.48 ± 0.08	1.57 ± 0.06	1.53 ± 0.07

Instar II

Effect of season, year and stage on the weight of IInd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table. 4.46). The instar weight in *C. rufifacies* was significantly higher in monsoon (10.59 ± 1.06) in comparison to summer (10.55 ± 0.57) and winter (9.53 ± 0.21) (Table 4.43).

Table 4.43. Seasonal changes in weight (mg) of IInd instar larvae of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	9.25 ± 0.35^{bB}	10.70 ± 0.23^{aA}	9.45 ± 0.22^{b}	9.80 ± 0.72^{C}
2020	11.25 ± 0.28^{aA}	11.08 ± 0.29^{aA}	9.61 ± 0.29^{b}	10.65 ± 0.82^{A}
2021	11.28 ± 0.48^{aA}	9.88 ± 0.08^{bB}	9.52 ± 0.18^{b}	10.23 ± 0.84^{B}
Overall Season	10.59 ± 1.06^{a}	10.55 ± 0.57^{a}	9.53 ± 0.21^{b}	10.22 ± 0.84

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar III

Effect of season, year and stage on the weight of IIIrd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table. 4.46). The instar weight in *C. rufifacies* was significantly higher in summer (32.47 ± 1.34) in comparison to monsoon (31.86 ± 0.31) and winter (30.13 ± 0.46) (Table 4.44).

Table 4.44. Seasonal changes in weight (mg) of IIIrd instar larvae of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	32.19 ± 0.27^{a}	30.72 ± 0.28^{bB}	30.60 ± 0.13^{bA}	31.17 ± 0.79^{B}
2020	31.71 ± 0.15^{b}	33.56 ± 0.19^{aA}	29.88 ± 0.34^{cB}	31.72 ± 1.61^{A}
2021	31.69 ± 0.22^{b}	33.12 ± 0.29^{aA}	29.91 ± 0.43^{cB}	31.57 ± 1.42^{A}
Overall Season	31.86 ± 0.31^{b}	32.47 ± 1.34^{a}	30.13 ± 0.46^{c}	31.49 ± 1.29

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Post feeding stage

Effect of season, year and stage on the weight of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table. 4.46). The weight of post feeding stage in *C. rufifacies* was significantly higher in monsoon (30.93 ± 0.96) in comparison to summer (29.16 ± 0.69) and winter (29.79 ± 0.36) (Table 4.45).

Table 4.45. Seasonal changes in weight (mg) post feeding stage of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	30.64 ± 0.37^{aB}	28.53 ± 0.29^{bB}	30.17 ± 0.29^{a}	29.78 ± 1.00
2020	30.85 ± 0.32^{aAB}	29.90 ± 0.59^{bA}	29.61 ± 0.09^{b}	30.12 ± 0.66
2021	31.31 ± 1.75^{aA}	29.04 ± 0.13^{bB}	29.59 ± 0.32^{b}	29.98 ± 1.36
Overall Season	30.93 ± 0.96^{a}	29.16 ± 0.69^{c}	29.79 ± 0.36^{b}	29.96 ± 1.01

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

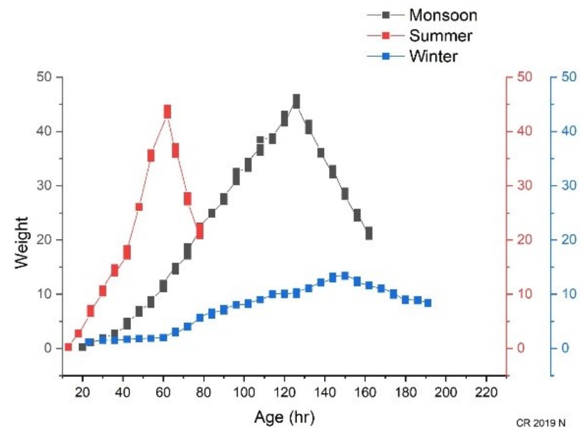
Interaction studies on year, season and stage was found to be significant (F-value = 10.095; P-value <0.001) indicating that seasonal variations in weight of each stage are different in different years (Table 4.46).

Table 4.46. ANOVA for comparing the weight of larval instars of *C. rufifacies*

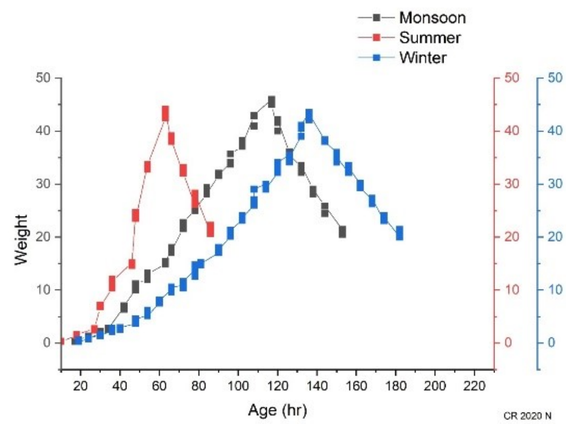
Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	3.43	1.71	11.425**	< 0.001
Season	2	17.87	8.94	59.586**	< 0.001
Stage	3	17721.43	5907.14	39388.42**	< 0.001
Year * Season	4	6.86	1.72	11.439**	< 0.001
Year * stage	6	1.77	0.30	1.97**	< 0.001
Season * stage	6	29.77	4.96	33.081**	< 0.001
Year * Season * Stage	12	18.17	1.51	10.095**	< 0.001
Error	72	10.80	0.15		
Total	107	17810.1			

** Significant at 0.01 level

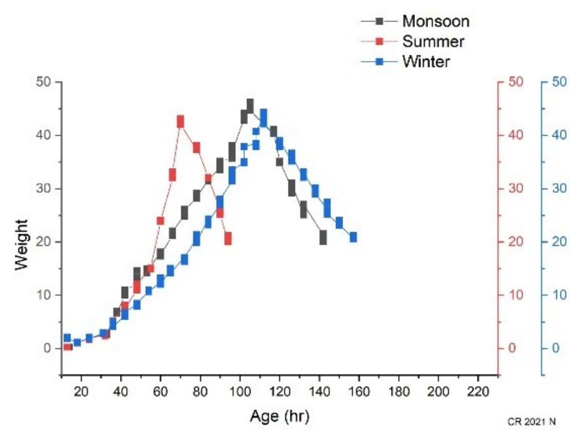
The growth curve representing the developmental rate (Weight (mg) Vs. Age (hr) of *C. rufifacies* from hatching until pupation during different seasons and years were prepared (Fig. 4.55 & Fig. 4.56).



2019

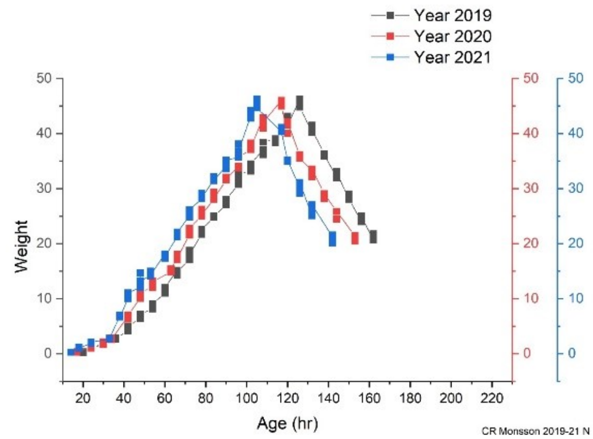


2020

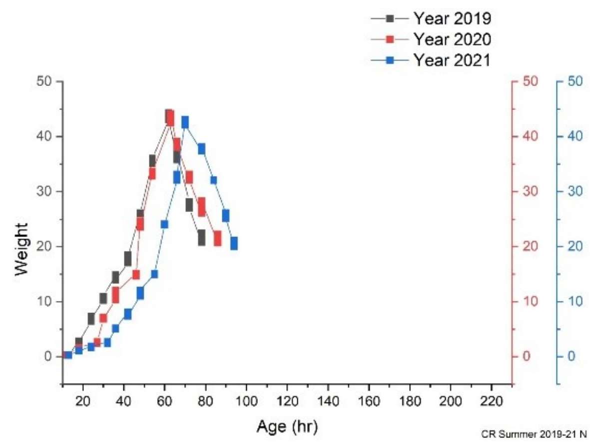


2021

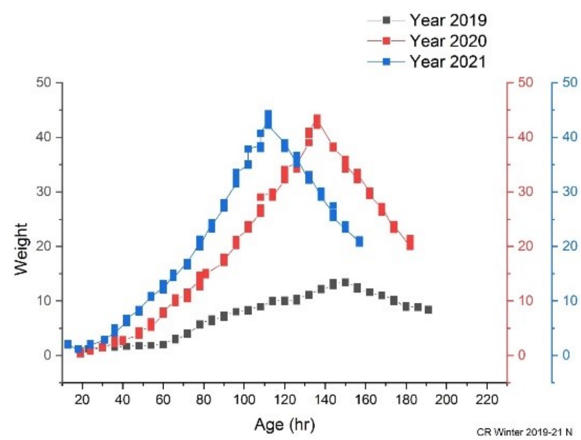
Fig. 4.55. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *C. rufifacies* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.56. Developmental rate (Weight (mg) Vs. Age (hr) of *C.rufifacies* from hatching upto pupation during the study period

Effect on life cycle duration

Significantly higher duration (hr) was observed for *C.rufifacies* during winter for IInd larval instar (32.67 ± 3.51) (F = 12.250; P = 0.020), post feeding stage (36.67 ± 1.53) (F = 45.953; P = 0.002), pupation (111.33 ± 3.79) (F = 115.71; P = <0.001), and total life cycle (267.00 ± 18.68) (F = 65.588; P = < 0.001). The duration of IIIrd Instar larvae was significantly higher in monsoon (50.00 ± 4.58) (F = 491.46; P = <0.001) (Table 4.47).

Table 4.47. Seasonal changes in duration (hrs) of life cycle of *C.rufifacies*

Stages	Monsoon	Summer	Winter	F-value (P-value)
Incubation	17.00 ± 3.00	12.00 ± 1.73	18.33 ± 5.03	4.300^{ns} (0.101)
Instar I	17.67 ± 2.08	16.67 ± 2.52	21.33 ± 3.51	1.606^{ns} (0.308)
Instar II	20.00 ± 4.58^{b}	15.67 ± 3.51^{b}	32.67 ± 3.51^{a}	12.250* (0.020)
Instar III	50.00 ± 4.58^{a}	15.00 ± 5.00^{b}	46.67 ± 7.02^{a}	491.46** (<0.001)
Post feeding stage	29.33 ± 4.04^{b}	16.00 ± 4.00^{c}	36.67 ± 1.53^{a}	45.953** (0.002)
Pupation	89.00 ± 1.00^{b}	73.00 ± 2.65^{c}	111.33 ± 3.79^{a}	115.71** (<0.001)
Total time taken from egg stage till emergence	223.00 ± 13.45^{b}	148.33 ± 6.43^{c}	267.00 ± 18.68^{a}	65.588** (0.001)

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
Means having different letter as super script differ significantly between seasons (within a row)

Significantly higher duration (hrs) was observed during 2019 for *C.rufifacies* for IIIrd larval instar (42.67 ± 19.63) compared to 2020 (37.33 ± 19.5) and 2021 (31.67 ± 18.93) (F = 39.854; P = 0.002) (Table 4.48).

Table 4.48. Changes in duration (hrs) of life cycle of *C. rufifacies*

Stages	2019	2020	2021	F-value (P-value)
Incubation	18.67 ± 5.13	15.33 ± 4.73	13.33 ± 0.58	2.800 (0.174)
Instar I	18.33 ± 5.86	18.33 ± 2.31	19.00 ± 1.00	0.039^{ns} (0.962)
Instar II	24.00 ± 12.00	23.33 ± 8.74	21.00 ± 7.21	0.390^{ns} (0.701)
Instar III	42.67 ± 19.63^{a}	37.33 ± 19.5^{b}	31.67 ± 18.93^{c}	39.854** (0.002)
Post feeding stage	25.67 ± 12.10	30.33 ± 9.29	26.00 ± 10.54	2.837^{ns} (0.171)
Pupation	91.33 ± 22.03	90.00 ± 16.09	92.00 ± 19.67	0.324^{ns} (0.741)
Total time taken from egg stage till emergence	220.67 ± 73.91	214.67 ± 56.52	203.00 ± 49.69	1.471^{ns} (0.332)

*** Significant at 0.01 level; ns non-significant*
Means having different letter as super script differ significantly between years (within a row)

Effect on survival rate

Effect of season on the survival rate (%) of *C. rufifacies* was found to be significant (Table 4.52). The survival rate (%) in *C. rufifacies* was significantly higher in monsoon (79.09 ± 7.56) in comparison to summer (76.70 ± 7.51) and winter (75.22 ± 5.93) (Table 4.49).

Table 4.49. Seasonal changes in survival rate (%) of *C. rufifacies*

Year	Monsoon	Summer	Winter	Overall year
2019	81.34 ± 8.52	76.07 ± 9.66	73.22 ± 4.89	76.88 ± 8.48
2020	77.14 ± 8.43	76.90 ± 8.21	75.50 ± 5.94	76.51 ± 7.47
2021	78.79 ± 5.14	77.12 ± 3.99	76.94 ± 6.61	77.61 ± 5.30
Overall Season	79.09 ± 7.56^{a}	76.70 ± 7.51^{b}	75.22 ± 5.93^{b}	77.00 ± 7.17

Means having different small letter as superscript differ significantly within a row
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Effect of year and stage on the survival rate of developmental stages was found to be significant (Table 4.52). The survival rate of I[st] larvar instar was significantly higher (84.76 ± 7.64) in 2019 compared to 2020 (80.66 ± 6.21) and 2021 (80.27 ± 3.54). However, the survival rate in pupation stage was significantly higher in 2021 (76.74 ± 4.37) compared to 2019 (69.96 ± 4.47) and 2020 (70.30 ± 7.12) (Table 4.50).

Table 4.50. Survival rate (%) of life cycle of *C. rufifacies* during the study period

Stage	2019	2020	2021	Overall stage
Egg	82.60 ± 6.69	84.39 ± 4.42	80.43 ± 4.84	82.47 ± 5.45^{A}
I[st] Instar	84.76 ± 7.64^{a}	80.66 ± 6.21^{ab}	80.27 ± 3.54^{b}	81.90 ± 6.16^{A}
II[nd] Instar	76.64 ± 5.70	74.70 ± 3.57	76.75 ± 4.72	76.03 ± 4.66^{B}
III[rd] Instar	70.42 ± 5.74	72.50 ± 5.57	73.90 ± 6.55	72.27 ± 5.92^{C}
Pupa	69.96 ± 4.47^{b}	70.30 ± 7.12^{b}	76.74 ± 4.37^{a}	72.33 ± 6.14^{C}
Overall year	76.88 ± 8.48	76.51 ± 7.47	77.61 ± 5.30	77.00 ± 7.17

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect of season and stage on the survival rate of developmental stages was found to be significant (Table 4.52). The survival rate of developmental stages in *C. rufifacies* was significantly higher in monsoon (79.09 ± 7.56) in comparison to summer (76.70 ± 7.51) and winter (75.22 ± 5.93) (Table 4.51).

Table 4.51. Seasonal changes in survival rate (%) of life cycle stages of *C. rufifacies*

Stage	Monsoon	Summer	Winter	Overall stage
Egg	84.23 ± 6.46^{A}	83.31 ± 3.52^{A}	79.89 ± 5.54^{A}	82.47 ± 5.45^{A}
I[st] Instar	85.29 ± 5.15^{A}	82.79 ± 6.72^{A}	77.61 ± 4.14^{A}	81.90 ± 6.16^{A}
II[nd] Instar	78.95 ± 5.29^{B}	75.49 ± 3.42^{B}	73.65 ± 3.81^{AB}	76.03 ± 4.66^{B}
III[rd] Instar	76.76 ± 4.63^{C}	72.11 ± 6.07^{B}	67.96 ± 3.44^{B}	72.27 ± 5.92^{C}
Pupa	70.22 ± 5.41^{C}	69.81 ± 5.87^{C}	76.98 ± 4.73^{AB}	72.33 ± 6.14^{C}
Overall Season	79.09 ± 7.56^{a}	76.70 ± 7.51^{b}	75.22 ± 5.93^{b}	77.00 ± 7.17

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between season and stage ($F = 4.965$; $P = <$

0.001), year and stage (F = 3.082; P = 0.004), as well as year and season (F = 2.751; P = 0.033) (Table 4.52).

Table 4.52. ANOVA for comparing the survival rate of *C. rufifacies*

Source	df	Sum of Squares	Mean Square	F-value	P-value
Year	2	28.40	14.20	0.707^{ns}	0.496
Season	2	343.43	171.71	8.553**	< 0.001
Stage	4	2672.32	668.08	33.275**	< 0.001
Year * season	4	220.97	55.24	2.751*	0.033
Year * Stage	8	494.95	61.87	3.082**	0.004
season * Stage	8	797.50	99.69	4.965**	< 0.001
Year * season * Stage	16	521.86	32.62	1.625^{ns}	0.078
Error	90	1806.98	20.08		
Corrected Total	134	6886.414			

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

4.4.3. *C. chani*

Effect on fecundity

Effect of season on the pre-oviposition were found to be significant (F = 40.111, P = < 0.001). Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 4.778, P = 0.008). The pre-oviposition period in *C chani* was significantly higher in winter (10.11 ± 0.60) in comparison to monsoon (7.78 ± 0.67) and summer (8.33 ± 1.00) (Table 4.53).

Table 4.53. Pre-oviposition period (days) of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	7.67 ± 0.58^{cAB}	9.33 ± 0.58^{bA}	10.33 ± 0.58^{a}	9.11 ± 1.27
2020	7.33 ± 0.58^{cB}	8.33 ± 0.58^{bB}	9.67 ± 0.58^{a}	8.44 ± 1.13
2021	8.33 ± 0.58^{bA}	7.33 ± 0.58^{cC}	10.33 ± 0.58^{a}	8.67 ± 1.41
Overall Season	7.78 ± 0.67^{b}	8.33 ± 1.00^{b}	10.11 ± 0.60^{a}	8.74 ± 1.26
Between year F-value = 3.111^{ns}; (P-value = 0.069) Between season F-value = 40.111**; (P-value < 0.001)) Interaction between season and year F-value =4.778^{**}; (P-value = 0.008)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect of season on the number of eggs laid in a day was found to be significant (F = 133.56, P = < 0.001). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.91, P = 0.477). The number of eggs laid by *C chani* was significantly higher (265.00 ± 6.27) in monsoon in comparison to summer (234.44 ± 5.75) and winter (221.00 ± 5.72) (Table 4.54).

Table 4.54. Eggs laid by the *C. chani* in a day

Year	Monsoon	Summer	Winter	Overall year
2019	263.33 ± 7.51	240.00 ± 5.57	224.33 ± 7.02	242.56 ± 17.97
2020	264.33 ± 7.57	230.67 ± 2.52	218.67 ± 6.81	237.89 ± 21.16
2021	267.33 ± 5.51	232.67 ± 4.73	220.00 ± 3.00	240.00 ± 21.58
Overall Season	265.00 ± 6.27^{a}	234.44 ± 5.75^{b}	221.00 ± 5.72^{c}	240.15 ± 19.6
Between year F-value = 1.44^{ns}; (P-value = 0.264) Between season F-value = 133.56**; (P-value < 0.001)) Interaction between season and year F-value =0.91^{ns}; (P-value = 0.477)				

** Significant at 0.01 level; *ns non-significant*

Means having different small letter as superscript differ significantly within a row

Effect of season (F = 3.111; P = 0.069), effect of year (F = 0.111; P-value = 0.895), and the interaction between year and season (F = 1.444; P-value = 0.260) were found to be non-significant on the periodicity of egg laying in *C. chani*. The above results showed that there were no significant variations in the periodicity of egg laying between seasons and between years (Table 4.55).

Table 4.55. Periodicity of egg laying (Days) by *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	4.67 ± 0.29	4.33 ± 0.29	4.83 ± 0.29	4.61 ± 0.33
2020	4.33 ± 0.29	4.67 ± 0.29	4.83 ± 0.29	4.61 ± 0.33
2021	4.67 ± 0.29	4.33 ± 0.29	4.67 ± 0.29	4.56 ± 0.33
Overall Season	4.56 ± 0.33	4.44 ± 0.33	4.78 ± 0.26	4.59 ± 0.31
Between year F-value = 0.111^{ns}; (P-value = 0.895) Between season F-value = 3.111^{ns}; (P-value = 0.069) Interaction between season and year F-value = 1.444^{ns}; (P-value = 0.260)				

ns – non significant

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 901.52, P = < 0.001). Effect of year on the number of eggs laid was also found to be significant (F = 12.54, P = < 0.001). Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 3.65, P = 0.024). The number of eggs laid in *C.chani* was significantly higher in monsoon (1735.11 ± 12.62) in comparison to summer (1640.22 ± 5.40) and winter (1627.22 ± 7.82) (Table 4.56).

Table 4.56. Eggs laid by the *C. chani* during in its life span

Year	Monsoon	Summer	Winter	Overall year
2019	1740.67 ± 5.13^{aA}	1638.67 ± 4.51^{b}	1632.67 ± 10.02^{b}	1670.67 ± 52.91^{A}
2020	1719.67 ± 4.16^{aB}	1637.33 ± 5.51^{b}	1621.67 ± 6.66^{c}	1659.56 ± 45.84^{B}
2021	1745.00 ± 6.56^{aA}	1644.67 ± 4.51^{b}	1627.33 ± 3.06^{c}	1672.33 ± 55.18^{A}
Overall Season	1735.11 ± 12.62^{a}	1640.22 ± 5.40^{b}	1627.22 ± 7.82^{c}	1667.52 ± 49.78
Between year F-value = 12.54**; (P-value < 0.001) Between season F-value = 901.52**; (P-value < 0.001) Interaction between season and year F-value = 3.65*; (P-value = 0.024)				

** Significant at 0.01 level; * *significant at 0.05 level*
Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Effect on length of larvae

Instar I

Effect of season, year and stage on the length of Ist instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The instar length in *C. Chani* was significantly lower in winter (2.65 ± 0.11) in comparison to monsoon (2.48 ± 0.17) and summer (2.49 ± 0.48) (Table. 4.57).

Table 4.57. Seasonal changes in length (mm) of Ist instar larvae of *C. Chani*

Year	Monsoon	Summer	Winter	Overall year
2019	2.59 ± 0.01^{aA}	1.88 ± 0.02^{bC}	2.68 ± 0.03^{aAB}	2.38 ± 0.38^{B}
2020	2.59 ± 0.03^{A}	2.66 ± 0.12^{B}	2.57 ± 0.05^{B}	2.61 ± 0.08^{A}
2021	2.25 ± 0.04^{cB}	2.94 ± 0.10^{aA}	2.71 ± 0.18^{bA}	2.64 ± 0.32^{A}
Overall Season	2.48 ± 0.17^{b}	2.49 ± 0.48^{b}	2.65 ± 0.11^{a}	2.54 ± 0.30

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar II

Effect of season, year and stage on the length of IInd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The instar length in *C. chani* was significantly higher in summer (6.40 ± 0.22) in comparison to monsoon (5.79 ± 0.23) and winter (6.04 ± 0.19) (Table 4.58).

Table 4.58. Seasonal changes in length (mm) of IInd instar larvae of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	5.63 ± 0.12^{cB}	6.66 ± 0.03^{aA}	6.05 ± 0.06^{bB}	6.11 ± 0.46^{A}
2020	6.08 ± 0.12^{bA}	6.39 ± 0.07^{aB}	5.81 ± 0.01^{cC}	6.09 ± 0.26^{AB}
2021	5.67 ± 0.04^{bB}	6.16 ± 0.05^{aC}	6.25 ± 0.03^{aA}	6.03 ± 0.27^{B}
Overall Season	5.79 ± 0.23^{c}	6.40 ± 0.22^{a}	6.04 ± 0.19^{b}	6.08 ± 0.33

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar III

Effect of season, year and stage on the length IIIrd instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The instar length in *C. chani* was significantly higher in summer (11.04 ± 0.23) in comparison to monsoon (10.72 ± 0.06) and winter (10.49 ± 0.19). (Table 4.59).

Table 4.59. Seasonal changes in length (mm) of IIIrd instar larvae of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	10.74 ± 0.04^{b}	11.32 ± 0.03^{aA}	10.32 ± 0.05^{cB}	10.79 ± 0.43^{A}
2020	10.71 ± 0.09^{b}	10.94 ± 0.07^{aB}	10.43 ± 0.07^{cB}	10.69 ± 0.23^{B}
2021	10.7 ± 0.07^{b}	10.85 ± 0.13^{aB}	10.71 ± 0.12^{abA}	10.76 ± 0.12^{AB}
Overall Season	10.72 ± 0.06^{b}	11.04 ± 0.23^{a}	10.49 ± 0.19^{c}	10.75 ± 0.28

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Post feeding stage

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The length of post feeding stage in *C. chani* was significantly higher in summer (10.61 ± 0.30) in comparison to monsoon (10.50 ± 0.19) and winter (10.27 ± 0.46) (Table 4.60).

Table 4.60. Seasonal changes in length (mm) of post feeding stage of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	10.31 ± 0.03^{bC}	10.89 ± 0.04^{aA}	9.68 ± 0.03^{cC}	10.29 ± 0.52^{C}
2020	10.70 ± 0.08^{bA}	10.27 ± 0.16^{aC}	10.46 ± 0.07^{cB}	10.48 ± 0.21^{B}
2021	10.50 ± 0.15^{bB}	10.66 ± 0.19^{aB}	10.66 ± 0.15^{aA}	10.61 ± 0.16^{A}
Overall Season	10.50 ± 0.19^{b}	10.61 ± 0.30^{a}	10.27 ± 0.46^{c}	10.46 ± 0.35

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

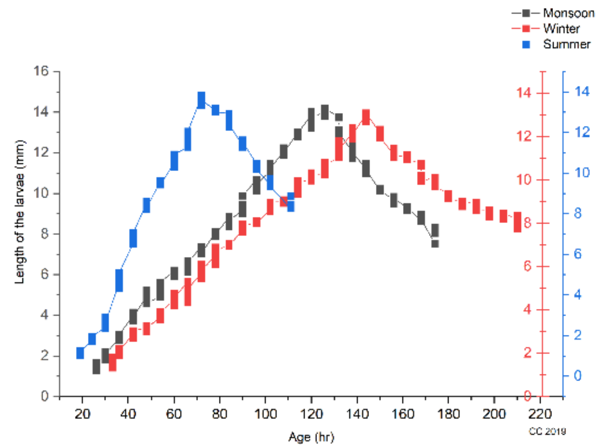
Interaction studies on year, season and stage was found to be significant (F = 0.335; P = <0.001) indicating that seasonal variations in length of each stage are different in different years (Table 4.61).

Table 4.61. ANOVA for comparing length of larval instars of *C. Chani*

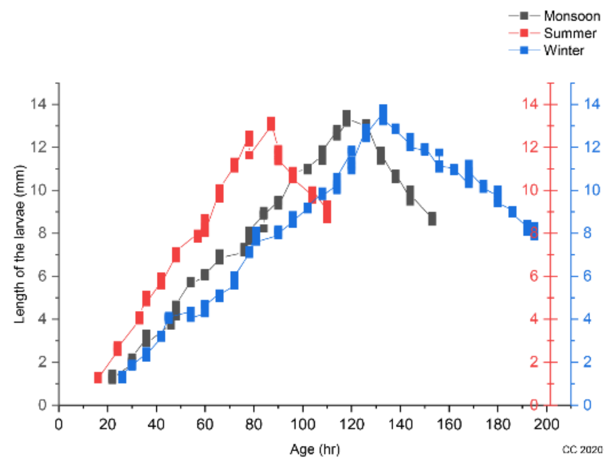
Source	Df	Sum of Squares	Mean Square	F-value	P-value
Year	2	0.223	0.111	14.249**	<0.001
Season	2	1.722	0.861	110.150**	<0.001
Stage	3	1239.390	413.130	52853.036**	<0.001
Year * Season	4	1.287	0.322	41.157**	<0.001
Year * stage	6	0.647	0.108	13.788**	<0.001
Season * stage	6	2.057	0.343	43.862**	<0.001
Year * Season * Stage	12	4.018	0.335	42.832**	<0.001
Error	72	0.563	0.008		
Total	107	1249.906			

** Significant at 0.01 level

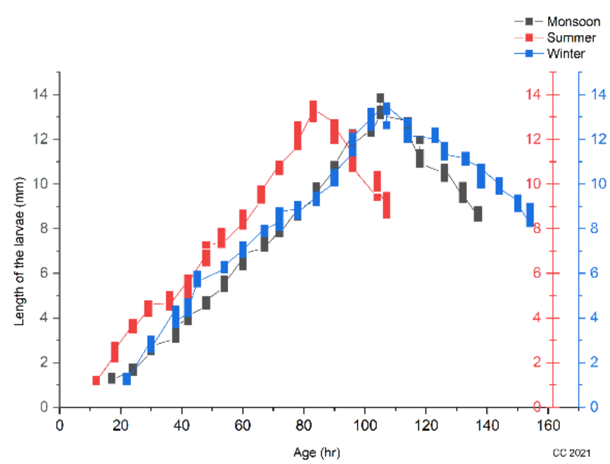
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *C. Chani* from hatching until pupation during different seasons and years were prepared (Fig. 4.57 and Fig. 4.58).



2019

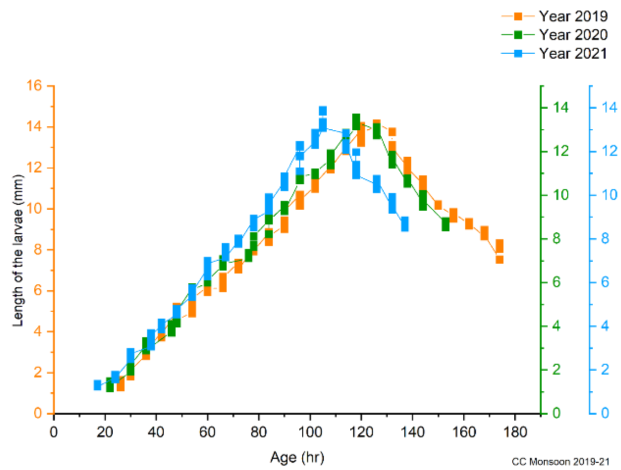


2020

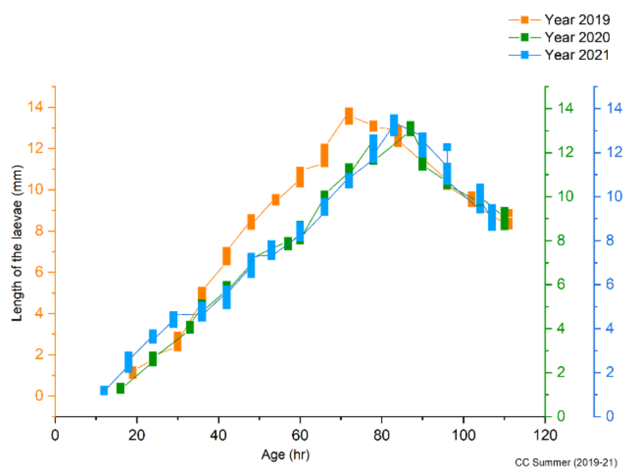


2021

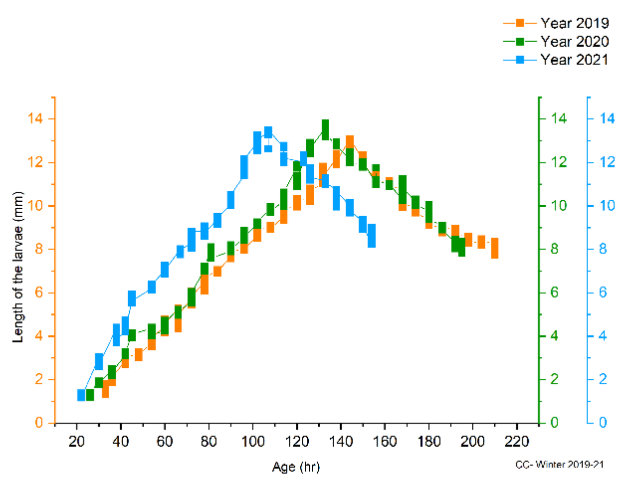
Fig. 4.57. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *C. chani* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.58. Developmental rate (Length (mm) Vs. Age (hr) of *C. chani* from hatching upto pupation during the study period

Effect on weight of larvae

Instar I

Effect of season and year on the Ist instar weight was found to be not significant. The pattern of differences in the weight was similar in all the years and seasons (Table 4.62).

Table 4.62. Seasonal changes in weight (mg) of Ist instar larvae of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	1.41 ± 0.07	1.45 ± 0.12	1.60 ± 0.08	1.48 ± 0.12
2020	1.39 ± 0.06	1.40 ± 0.13	1.62 ± 0.10	1.47 ± 0.14
2021	1.40 ± 0.06	1.60 ± 0.03	1.55 ± 0.03	1.52 ± 0.1
Overall Season	1.40 ± 0.06	1.49 ± 0.13	1.59 ± 0.07	1.49 ± 0.12

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar II

Effect of season, year and stage on the weight of IInd instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.66). The instar weight in *C. chani* was significantly higher in summer (10.08 ± 0.33) in comparison to monsoon (9.87 ± 0.39) and winter (9.52 ± 0.33) (Table 4.63).

Table 4.63. Seasonal changes in weight (mg) of IInd instar larvae of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	9.98 ± 0.40^{bA}	10.5 ± 0.12^{aA}	9.49 ± 0.32^{c}	9.99 ± 0.51^{A}
2020	10.16 ± 0.13^{aA}	9.91 ± 0.05^{abB}	9.56 ± 0.32^{b}	9.87 ± 0.31^{A}
2021	9.46 ± 0.22^{B}	9.84 ± 0.03^{B}	9.52 ± 0.49	9.61 ± 0.32^{B}
Overall Season	9.87 ± 0.39^{a}	10.08 ± 0.33^{a}	9.52 ± 0.33^{b}	9.82 ± 0.41

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Instar III

Effect of season, year and stage on the weight of IIIrd instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.66). The instar weight in *C. chani* was significantly higher in summer (32.26 ± 0.70) in comparison to monsoon (30.49 ± 0.47) and winter (30.16 ± 0.53) (Table.4.64).

Table 4.64. Seasonal changes in weight (mg) of IIIrd instar larvae of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	30.23 ± 0.27^{bB}	32.87 ± 0.20^{aA}	29.66 ± 0.38^{cB}	30.92 ± 1.51
2020	31.05 ± 0.23^{bA}	31.53 ± 0.62^{aC}	30.60 ± 0.22^{cA}	31.06 ± 0.53
2021	30.2 ± 0.20^{bB}	32.38 ± 0.36^{aB}	30.21 ± 0.51^{bA}	30.93 ± 1.14
Overall Season	30.49 ± 0.47^{b}	32.26 ± 0.70^{a}	30.16 ± 0.53^{c}	30.97 ± 1.09

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

Post feeding stage

Effect of season, year and stage on the weight of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the year wise variations were not same in all seasons (Table 4.66). The weight of post feeding stage in *C. chani* was significantly higher in summer (30.14 ± 0.82) in comparison to monsoon (29.77 ± 1.35) and winter (29.80 ± 1.25) (Table 4.65).

Table 4.65. Seasonal changes in weight (mg) of post feeding stage of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	30.83 ± 0.29^{aA}	31.15 ± 0.12^{abA}	30.48 ± 0.25^{bA}	30.82 ± 0.35^{A}
2020	30.44 ± 0.25^{aA}	29.46 ± 0.14^{bB}	30.76 ± 0.29^{aA}	30.22 ± 0.62^{A}
2021	28.03 ± 0.49^{bB}	29.80 ± 0.49^{aB}	28.16 ± 0.12^{bB}	28.67 ± 0.93^{B}
Overall Season	29.77 ± 1.35^{b}	30.14 ± 0.82^{a}	29.80 ± 1.25^{b}	29.90 ± 1.13

Means having different small letter as superscript differ significantly within a row
Means having different capital letter as superscript differ significantly within a column

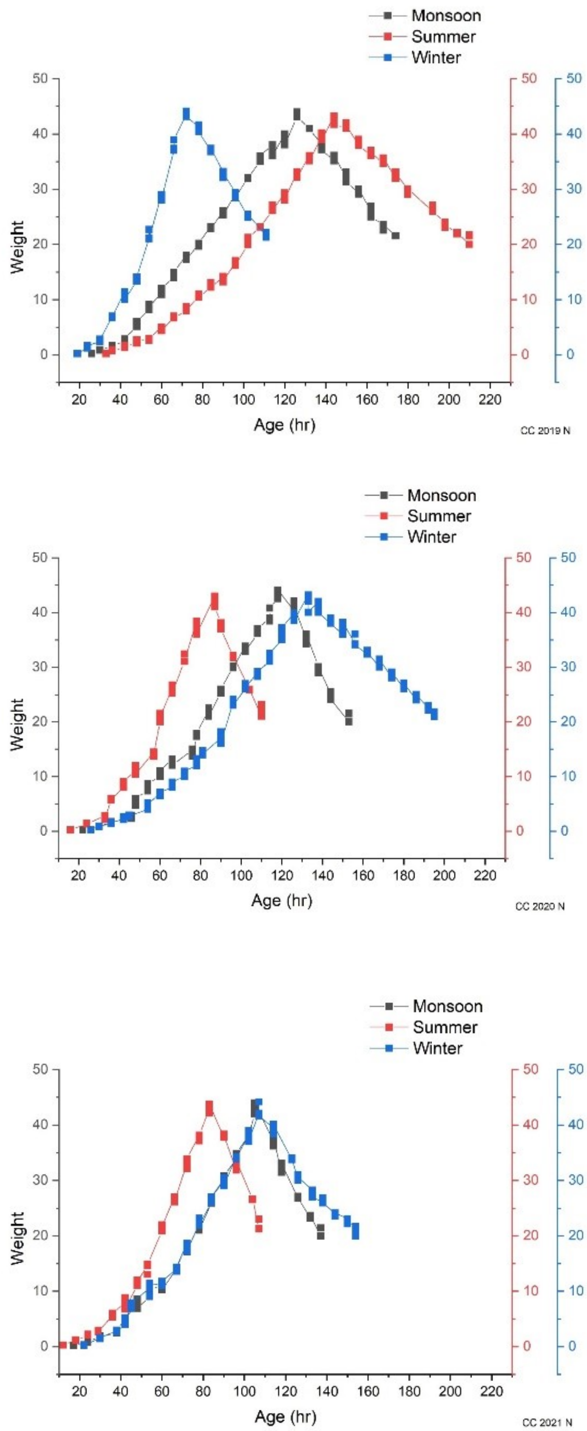
Interaction studies on year, season and stage was found to be significant (F = 14.365; P = <0.001) indicating that seasonal variations in weight of each stage are different in different years (Table 4.66).

Table 4.66 ANOVA for comparing weight of larval instars of *C. chani*

Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	7.62	3.81	50.271**	< 0.001
Season	2	10.92	5.46	71.999**	< 0.001
Stage	3	17528.77	5842.92	77063.61**	< 0.001
Year * Season	4	7.95	1.99	26.22**	< 0.001
Year * stage	6	15.40	2.57	33.858**	< 0.001
Season * stage	6	14.44	2.41	31.739**	< 0.001
Year * Season * Stage	12	6.97	0.58	7.656**	< 0.001
Error	72	5.46	0.08		
Total	107	17597.53			

** Significant at 0.01 level; ns non-Significant

The growth curve representing the developmental rate (Weight (mg) Vs. Age (hr) of *C. chani* from hatching until pupation during different seasons and years were prepared (Fig. 4.59 and Fig.4.60).

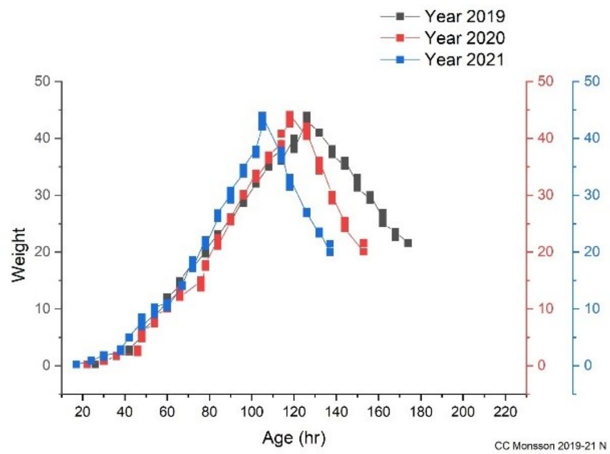


2019

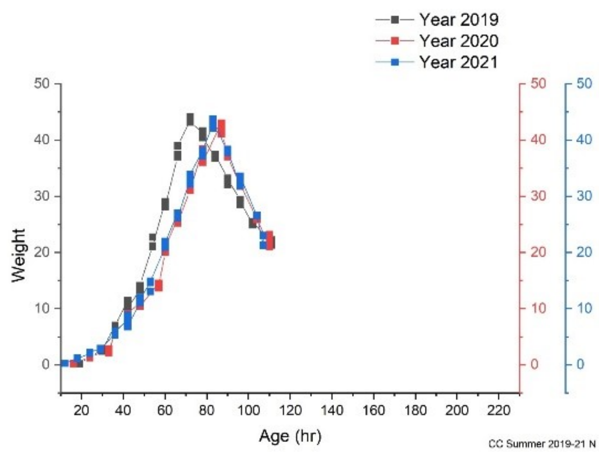
2020

2021

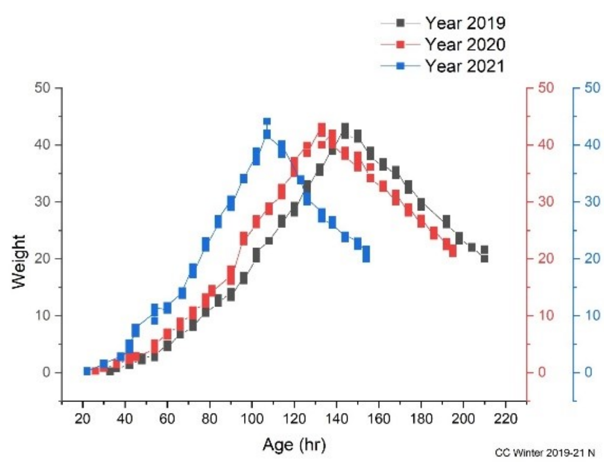
Fig. 4.59. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *C. chani* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.60. Developmental rate (Weight (mg) Vs. Age (hr) of *C. chani* from hatching upto pupation during the study period

Effect on the life cycle duration

Significantly higher duration (hr) was observed for *C. chani* during winter for incubation (27.00 ± 5.57) (F = 66.77; P = 0.001), II[nd] larval instar (27.33 ± 2.52) (F = 5.59 ; P = 0.070), post feeding stage (52.00 ± 10.58) (F =29.726 ; P =0.004), pupation (153.33 ± 5.51) (F = 367.82; P = <0.001), and total life cycle (320.33 ± 24.03) (F = 152.50; P = <0.001) (Table 4.67).

Table 4.67. Seasonal changes in duration (hrs) of life cycle of *C. chani*

Stages	Monsoon	Summer	Winter	F-value (P-value)
Incubation	21.67 ± 4.51^{b}	15.67 ± 3.51^{c}	27.00 ± 5.57^{a}	66.77** (0.001)
Instar I	20.33 ± 4.04	15.33 ± 2.89	18.67 ± 2.52	1.862^{ns} (0.268)
Instar II	23.67 ± 5.13^{ab}	16.67 ± 4.51^{b}	27.33 ± 2.52^{a}	5.59* (0.070)
Instar III	42.00 ± 11.14	22.67 ± 4.51	42.00 ± 6.56	6.824^{ns} (0.051)
Post feeding stage	30.33 ± 10.21^{b}	25.33 ± 11.93^{b}	52.00 ± 10.58^{a}	29.726** (0.004)
Pupation	108.00 ± 3.61^{b}	96.67 ± 1.53^{c}	153.33 ± 5.51^{a}	367.82** (<0.001)
Total time taken from egg stage till emergence	246 ± 20.08^{b}	192.33 ± 8.15^{c}	320.33 ± 24.03^{a}	152.50** (<0.001)

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
Means having different letter as super script differ significantly between seasons (within a row)

Significantly higher duration (hr) was observed for *C. chani* during 2019 for incubation (26.00 ± 7.00) (F = 42.08; P = 0.002) and post feeding stage (47.00 ± 11.36) (F = 15.69; P =0.013). However total life cycle duration was significantly higher (253.00 ± 61.99) in 2020 (F = 10.88; P = 0.024) compared to other years (Table 4.68).

Table 4.68. Changes in duration (hrs) of life cycle of *C. chani*

Stages	2019	2020	2021	F-value (P-value)
Incubation	26.00 ± 7.00^{a}	21.33 ± 5.03^{b}	17.00 ± 5.00^{c}	42.08** (0.002)
Instar I	16.33 ± 4.51	20.00 ± 3.61	18.00 ± 2.65	0.968^{ns} (0.454)
Instar II	20.00 ± 9.17	25.33 ± 3.79	22.33 ± 4.62	1.359^{ns} (0.354)
Instar III	40.00 ± 19.29	36.67 ± 8.51	30.00 ± 6.25	1.420^{ns} (0.342)
Post feeding stage	47.00 ± 11.36^{a}	34.00 ± 19.29^{b}	26.67 ± 11.93^{b}	15.69* (0.013)
Pupation	120.67 ± 28.75	115.67 ± 28.36	121.67 ± 32.88	4.227^{ns} (0.103)
Total time taken from egg stage till emergence	270.00 ± 73.55^{a}	253.00 ± 61.99^{ab}	235.67 ± 57.49^{b}	10.88* (0.024)

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
Means having different letter as super script differ significantly between years (within a row)

Effect on survival rate

Effect of season on the survival rate (%) of *C. chani* was found to be significant (Table 4.72). During 2019, the survival rate (%) in *C. chani* was significantly higher in monsoon (73.51 ± 4.18) in comparison to summer (71.66 ± 3.39) and winter (69.75 ± 3.94). However, during 2021, the survival rate was significantly higher in winter (75.45 ± 9.72) compared to monsoon (73.00 ± 8.59) and summer (72.21 ± 6.95) (Table 4.69).

Table 4.69. Seasonal changes in survival rate (%) of *C. chani*

Year	Monsoon	Summer	Winter	Overall year
2019	73.51 ± 4.18^{a}	71.66 ± 3.39^{ab}	69.75 ± 3.94^{bC}	71.64 ± 4.07^{B}
2020	73.57 ± 6.19	72.73 ± 5.40	72.44 ± 6.69^{B}	72.92 ± 6.00^{AB}
2021	73.00 ± 8.59^{ab}	72.21 ± 6.95^{b}	75.45 ± 9.72^{aA}	73.55 ± 8.42^{A}
Overall Season	73.36 ± 6.43	72.20 ± 5.34	72.55 ± 7.40	72.70 ± 6.42

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Effect of year and stage on the survival rate of developmental stages of *C. chani* was found to be significant (Table 4.72). The survival rate of developmental stages in *C. C.chani* was significantly higher in 2021 (73.55 ± 8.42) in comparison to 2019 (71.64 ± 4.07) and 2020 (72.92 ± 6) (Table 4.70).

Table 4.70. Survival rate (%) of life cycle of *C. chani* during the study period

Stage	2019	2020	2021	Overall stage
Egg	72.27 ± 3.7^{bAB}	76.36 ± 4.45^{aA}	78.90 ± 6.38^{aA}	75.84 ± 5.53^{A}
Ist Instar	73.14 ± 4.4^{bA}	76.77 ± 4.51^{aA}	78.22 ± 5.88^{aA}	76.04 ± 5.25^{A}
IInd Instar	74.09 ± 3.54^{bA}	75.28 ± 4.44^{bA}	79.97 ± 3.41^{aA}	76.45 ± 4.50^{A}
IIIrd Instar	69.45 ± 4.17^{B}	68.65 ± 5.73^{B}	67.37 ± 5.90^{B}	68.49 ± 5.19^{B}
Pupa	69.25 ± 2.57^{aB}	67.51 ± 3.92^{aB}	63.31 ± 2.05^{bC}	66.69 ± 3.81^{B}
Overall year	71.64 ± 4.07^{b}	72.92 ± 6^{ab}	73.55 ± 8.42^{a}	72.70 ± 6.42

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Effect of season and stage on the survival rate of developmental stages of *C.chani* was found to be significant (Table 4.72). The survival rate of egg (78.12 ± 3.95) and Ist Instar larvae (78.67 ± 4.02) were significantly higher in monsoon compared to other seasons. Survival rate of IIIrd Instar larvae was significantly higher in summer (73.30 ± 4.11) compared to monsoon (66.69 ± 4.46) and winter (65.48 ± 3.30) (Table 4.71).

Table 4.71. Seasonal changes in survival rate (%) of life cycle stages of *C. chani*

Stage	Monsoon	Summer	Winter	Overall stage
Egg	78.12 ± 3.95^{aA}	72.06 ± 3.02^{bB}	77.35 ± 7.08^{aA}	75.84 ± 5.53^{A}
I[st] Instar	78.67 ± 4.02^{aA}	72.12 ± 2.66^{bB}	77.35 ± 6.28^{aA}	76.04 ± 5.25^{A}
II[nd] Instar	75.41 ± 4.05^{A}	77.66 ± 4.74^{A}	76.26 ± 4.89^{A}	76.45 ± 4.50^{A}
III[rd] Instar	66.69 ± 4.46^{bB}	73.30 ± 4.11^{aB}	65.48 ± 3.30^{bB}	68.49 ± 5.19^{B}
Pupa	67.91 ± 3.73^{B}	65.87 ± 4.60^{C}	66.30 ± 3.07^{B}	66.69 ± 3.81^{B}
Overall Season	73.36 ± 6.43	72.20 ± 5.34	72.55 ± 7.4	72.70 ± 6.42

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Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between season and stage (F = 6.93; P= <0.001), year and stage (F = 5.59; P = <0.001), as well as year and season (F = 3.17; P = 0.018) (Table 4.72).

Table 4.72. ANOVA for comparing survival of *C. chani*

Source	df	Sum of Squares	Mean Square	F-value	P-value
Year	2	85.41	42.70	3.19*	0.046
Season	2	31.77	15.88	1.19^{ns}	0.311
Stage	4	2400.67	600.17	44.76**	< 0.001
Year * season	4	169.79	42.45	3.17*	0.018
Year * Stage	8	600.00	75.00	5.59**	< 0.001
season * Stage	8	743.48	92.94	6.93**	< 0.001
Year * season * Stage	16	277.33	17.33	1.29^{ns}	0.219
Error	90	1206.81	13.41		
Corrected Total	134	5515.25			

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

4.4.4. *Hemipyrellia ligurriens*

Effect on fecundity

Effect of season on the pre-oviposition period were found to be significant (F = 13.727, P = < 0.001). Seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.363, P = 0.831). The pre-oviposition period (days) in *H.ligurriens* was significantly higher in winter (10.44 ± 0.53) in comparison to monsoon (9.44 ± 0.53) and summer (8.89 ± 0.78) (Table 4.73).

Table 4.73. Pre-oviposition period (days) of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	9.67 ± 0.58	9.33 ± 0.58	10.67 ± 0.58	9.89 ± 0.78
2020	9.33 ± 0.58	8.33 ± 0.58	10.33 ± 0.58	9.33 ± 1.00
2021	9.33 ± 0.58	9.00 ± 1.00	10.33 ± 0.58	9.56 ± 0.88
Overall Season	9.44 ± 0.53^{b}	8.89 ± 0.78^{b}	10.44 ± 0.53^{a}	9.59 ± 0.89
Between year F-value = 1.727^{ns}; (P-value = 0206) Between season F-value = 13.727**; (P-value < 0.001)) Interaction between season and year F-value =0.363^{ns}; (P-value = 0.831)				

** Significant at 0.01 level; *ns non-significant*
Means having different small letter as superscript differ significantly within a row

Effect of season on the number of eggs laid in a day was found to be significant (F = 417.585, P = < 0.001). Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 3.562, P = 0.026). The number of eggs laid by *H. ligurriens was* significantly higher in monsoon (238.78 ± 3.96) in comparison to summer (221.33 ± 4.98) and winter (187.11 ± 4.46) (Table 4.74).

Table 4.74. Eggs laid by the *H. ligurriens* in a day

Year	Monsoon	Summer	Winter	Overall year
2019	237.00 ± 2.00^{a}	221.00 ± 1.00^{bAB}	190.00 ± 3.00^{c}	216.00 ± 20.78
2020	237.67 ± 4.16^{a}	226.00 ± 3.00^{bA}	184.00 ± 3.61^{c}	215.89 ± 24.65
2021	241.67 ± 4.73^{a}	217.00 ± 5.29^{bB}	187.33 ± 5.51^{c}	215.33 ± 23.98
Overall Season	238.78 ± 3.96^{a}	221.33 ± 4.98^{b}	187.11 ± 4.46^{c}	215.74 ± 22.29
Between year F-value = 0.077^{ns}; (P-value = 0.926) Between season F-value = 417.585**; (P-value < 0.001)) Interaction between season and year F-value = 3.562*; (P-value = 0.026)				

** Significant at 0.01 level; * Significant at 0.05 level; *ns non-significant*
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Effect of season (F=0.750; P =0.487), effect of year (F =0.563; P-value =0.579), and the interaction between year and season (F = 0.750; P-value = 0.571) were found to be non-significant on the on the periodicity of egg laying in *H. ligurriens*. There were no significant variations in the periodicity of egg laying between seasons and between years (Table 4.75).

Table 4.75. Periodicity of egg laying (days) by *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	3.50 ± 0.50	3.83 ± 0.58	3.50 ± 0.50	3.61 ± 0.49
2020	3.83 ± 0.29	3.67 ± 0.29	3.33 ± 0.29	3.61 ± 0.33
2021	3.67 ± 0.29	3.83 ± 0.29	3.83 ± 0.29	3.78 ± 0.26
Overall Season	3.67 ± 0.35	3.78 ± 0.36	3.56 ± 0.39	3.67 ± 0.37
Between year F-value = 0.563^{ns}; (P-value = 0.579) Between season F-value = 0.750^{ns}; (P-value = 0.487)) Interaction between season and year F-value = 0.750^{ns}; (P-value = 0.571)				

ns non-significant

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 576.86, P = < 0.001). Effect of year on the number of eggs laid was also found to be significant (F = 5.199, P = 0.017). However, seasonal variations were same in all years and also the year wise variations were same in all seasons (F = 1.192, P = 0.150). The number of eggs laid by *H. ligurriens* was significantly higher in monsoon (1531.56 ± 16.01) in comparison to summer (1481.67 ± 9.99) and winter (1340.56 ± 18.3). (Table 4.76).

Table 4.76. Eggs laid by the *H. ligurriens* during in its life span

Year	Monsoon	Summer	Winter	Overall year
2019	1539.00 ± 7.94	1487.67 ± 3.06	1354.33 ± 9.45	1460.33 ± 82.79^{A}
2020	1522.67 ± 7.37	1486.67 ± 7.23	1346.33 ± 4.16	1451.89 ± 80.88^{AB}
2021	1533.00 ± 26.51	1470.67 ± 8.02	1321.00 ± 18.03	1441.56 ± 95.80^{B}
Overall Season	1531.56 ± 16.01^{a}	1481.67 ± 9.99^{b}	1340.56 ± 18.3^{c}	1451.26 ± 83.71
Between year F-value = 5.199*; (P-value = 0.017) Between season F-value = 576.86**; (P-value < 0.001) Interaction between season and year F-value = 1.192^{ns}; (P-value = 0.150)				

** Significant at 0.01 level; *ns non-significant*
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Effect on length of larvae

Instar I

Effect of season, year and stage on the length of Ist instar larvae was found to be significant. Seasonal variations were same in all years and also the yearly variations were same in all seasons (Table. 4.81). The instar length in *H. ligurriens* was significantly higher in winter (2.28 ± 0.09) in comparison to monsoon (2.00 ± 0.28) and summer (2.06 ± 0.19). (Table 4.77).

Table 4.77. Seasonal changes in length (mm) of Ist instar larvae of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	2.15 ± 0.03^{A}	2.15 ± 0.01	2.21 ± 0.04	2.17 ± 0.04^{AB}
2020	2.21 ± 0.02^{A}	2.21 ± 0.02	2.28 ± 0.09	2.23 ± 0.06^{A}
2021	1.63 ± 0.03^{bB}	1.81 ± 0.02^{b}	2.36 ± 0.07^{a}	1.93 ± 0.33^{B}
Overall Season	2.00 ± 0.28^{b}	2.06 ± 0.19^{ab}	2.28 ± 0.09^{a}	2.11 ± 0.23

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Instar II

Effect of season, year and stage on the length of IInd instar larvae was found to be significant. Seasonal variations were same in all years and also the yearly variations were same in all seasons (Table. 4.81). The instar length in *H. ligurriens* was significantly higher in winter (4.79 ± 0.27) in comparison to monsoon (4.55 ± 0.44) and summer (4.25 ± 0.53). (Table 4.78).

Table 4.78. Seasonal changes in length (mm) of IInd instar larvae of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	4.70 ± 0.02^{A}	4.6 ± 0.04^{A}	4.69 ± 0.03^{AB}	4.66 ± 0.06^{A}
2020	4.95 ± 0.02^{abA}	4.61 ± 0.03^{bA}	5.13 ± 0.04^{aA}	4.90 ± 0.23^{A}
2021	3.99 ± 0.09^{bB}	3.54 ± 0.11^{bB}	4.54 ± 0.01^{aB}	4.02 ± 0.44^{B}
Overall Season	4.55 ± 0.44^{a}	4.25 ± 0.53^{b}	4.79 ± 0.27^{a}	4.53 ± 0.47

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Instar III

Effect of season, year and stage on the length of IIIrd instar larvae was found to be significant. Seasonal variations were same in all years and also the year wise variations were same in all seasons (Table. 4.81). The instar length in *H. ligurriens* was significantly higher in winter (8.18 ± 0.33) in comparison to monsoon (8.06 ± 0.20) and summer (7.51 ± 0.08). (Table 4.79).

Table 4.79. Seasonal changes in length (mm) of IIIrd instar larvae of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	7.85 ± 0.03	7.55 ± 0.02	7.81 ± 0.03^{B}	7.74 ± 0.14^{B}
2020	8.30 ± 0.02^{a}	7.58 ± 0.01^{b}	8.56 ± 0.003^{aA}	8.15 ± 0.44^{A}
2021	8.02 ± 0.02^{a}	7.41 ± 0.04^{b}	8.16 ± 0.01^{aAB}	7.86 ± 0.35^{B}
Overall Season	8.06 ± 0.20^{b}	7.51 ± 0.08^{b}	8.18 ± 0.33^{a}	7.91 ± 0.37

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Post feeding stage

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were same in all years and also the yearly variations were same in all seasons (Table. 4.81). The length of post feeding stage in *H. ligurriens* was significantly higher in summer (7.97 ± 0.05) in comparison to monsoon (7.80 ± 0.89) and winter (7.48 ± 0.19) (Table 4.80).

Table 4.80. Seasonal changes in length (mm) of post feeding stage of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	7.36 ± 0.01^{bB}	8.02 ± 0.03^{a}	7.26 ± 0.01^{b}	7.55 ± 0.36^{B}
2020	8.20 ± 1.62^{aA}	7.95 ± 0.03^{a}	7.46 ± 0.02^{b}	7.87 ± 0.87^{A}
2021	7.83 ± 0.03^{aA}	7.94 ± 0.03^{a}	7.70 ± 0.02^{b}	7.82 ± 0.11^{A}
Overall Season	7.80 ± 0.89^{a}	7.97 ± 0.05^{a}	7.48 ± 0.19^{b}	7.75 ± 0.55

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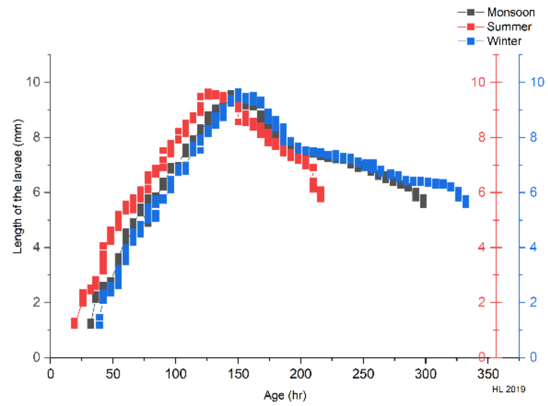
Three way ANOVA indicated that interaction between year and season (F = 4.60, P = 0.002), year and stage (F = 6.32, P = < 0.001) and season and stage (F = 9.15, P < 0.001) were found to be significant (Table 4.81).

Table 4.81 ANOVA for comparing length of larval instars of *H. ligurriens*

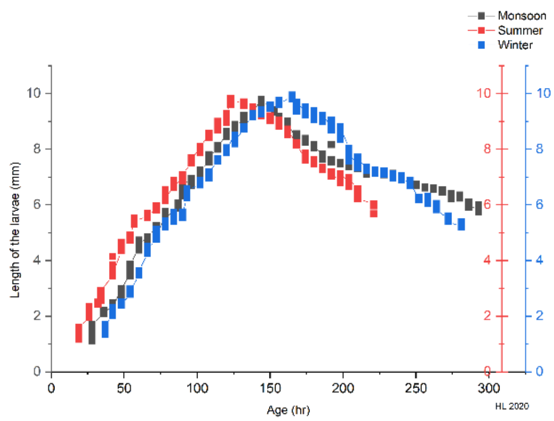
Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	2.69	1.34	18.05**	< 0.001
Season	2	1.01	0.51	6.79**	0.002
Stage	3	628.61	209.54	2813.60**	< 0.001
Year * Season	4	1.40	0.35	4.69**	0.002
Year * stage	6	2.82	0.47	6.32**	< 0.001
Season * stage	6	4.09	0.68	9.15**	< 0.001
Year * Season * Stage	12	0.93	0.08	1.04^{ns}	0.422
Error	72	5.36	0.07		
Total	107	646.91			

** Significant at 0.01 level; ns non-Sgnificant

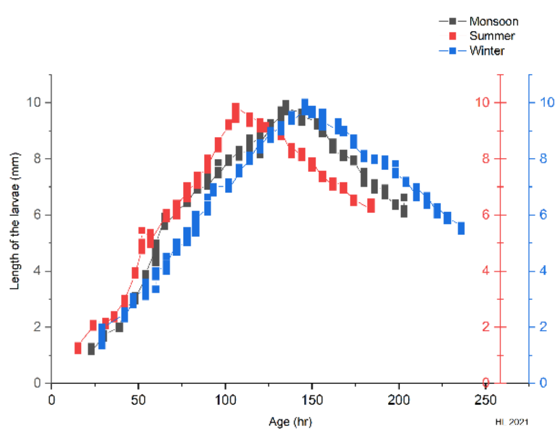
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching until pupation during different seasons and years were prepared (Fig. 4.61 & Fig. 4.62).



2019

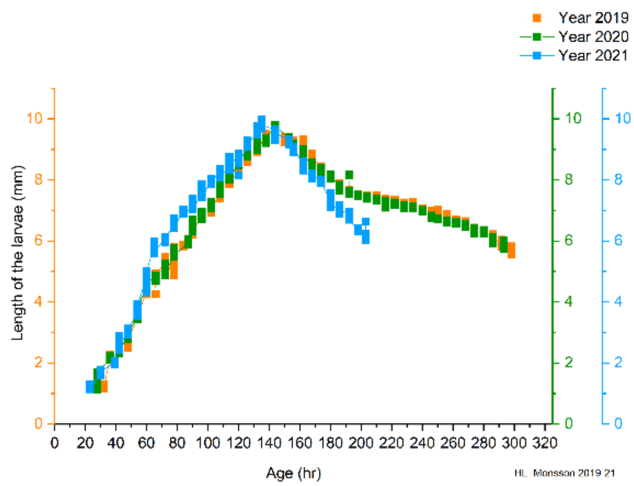


2020

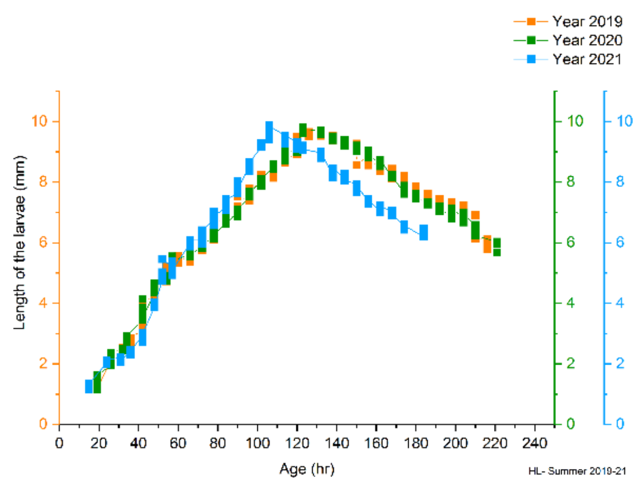


2021

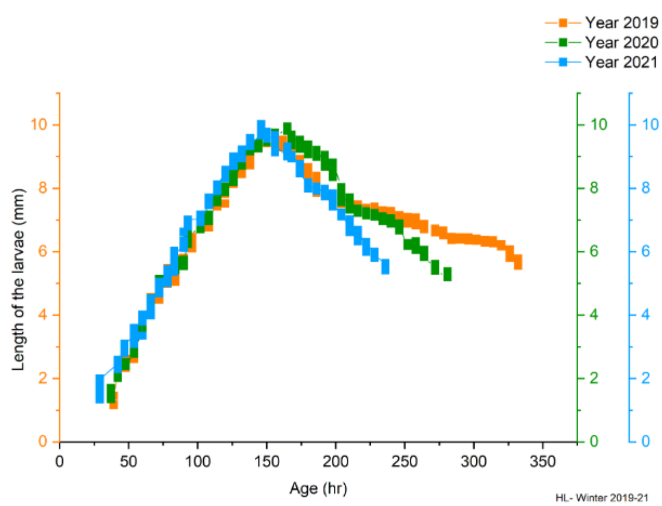
Fig. 4.61. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.62. Developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation during the study period

Effect on weight of larvae

Instar I

Effect of season, year and stage on the weight of Ist instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The instar weight in *H. ligurriens* was significantly higher in summer (1.06 ± 0.52) in comparison to monsoon (0.84 ± 0.17) and winter (0.71 ± 0.11 (Table 4.82).

Table 4.82. Seasonal changes in weight (mg) of Ist instar larvae of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	0.65 ± 0.14^{b}	1.34 ± 0.03^{aA}	0.64 ± 0.03^{b}	0.88 ± 0.36^{B}
2020	0.94 ± 0.04^{b}	1.47 ± 0.06^{aA}	0.65 ± 0.01^{b}	1.02 ± 0.36^{AB}
2021	0.94 ± 0.04^{a}	0.38 ± 0.005^{bB}	0.85 ± 0.07^{a}	0.72 ± 0.26^{B}
Overall Season	0.84 ± 0.17^{b}	1.06 ± 0.52^{a}	0.71 ± 0.11^{b}	0.87 ± 0.34

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Instar II

Effect of season, year and stage on the weight of IInd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The instar weight in *H. ligurriens* was significantly higher in monsoon (7.99 ± 0.43) in comparison to summer (7.28 ± 2.72) and winter (7.29 ± 0.47) (Table 4.83).

Table 4.83. Seasonal changes in weight (mg) of IInd instar larvae of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	7.72 ± 0.37^{bB}	9.23 ± 0.20^{aA}	7.82 ± 0.13^{bA}	8.26 ± 0.76^{A}
2020	8.49 ± 0.15^{bA}	8.93 ± 0.15^{aA}	6.74 ± 0.03^{cC}	8.05 ± 1.01^{A}
2021	7.77 ± 0.2^{aB}	3.67 ± 0.30^{cB}	7.30 ± 0.02^{bB}	6.25 ± 1.95^{B}
Overall Season	7.99 ± 0.43^{a}	7.28 ± 2.72^{b}	7.29 ± 0.47^{b}	7.52 ± 1.59

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Instar III

Effect of season, year and stage on the weight of IIIrd instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The instar weight in *H. ligurriens* was significantly higher in summer (24.06 ± 0.3) in comparison to monsoon (23.23 ± 2.7) and winter (22.77 ± 2.16) (Table 4.84).

Table 4.84. Seasonal changes in weight (mg) of IIIrd instar larvae of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	19.65 ± 0.46^{bB}	23.99 ± 0.10^{a}	19.89 ± 0.08^{bB}	21.18 ± 2.13^{B}
2020	24.98 ± 0.08^{aA}	23.92 ± 0.46^{b}	24.08 ± 0.05^{bA}	24.33 ± 0.55^{A}
2021	25.07 ± 0.04^{aA}	24.27 ± 0.20^{b}	24.34 ± 0.18^{bA}	24.56 ± 0.41^{A}
Overall Season	23.23 ± 2.7^{b}	24.06 ± 0.3^{a}	22.77 ± 2.16^{a}	23.35 ± 2

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Post feeding stage

Effect of season, year and stage on the post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The weight of post feeding stage in *H. ligurriens* was significantly higher in winter (27.19 ± 0.58) in comparison to monsoon (25.62 ± 1.14) and summer (26.24 ± 0.35) (Table 4.85).

Table 4.85. Seasonal changes in weight (mg) of post feeding stage of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	24.33 ± 0.85^{cB}	26.66 ± 0.13^{bA}	27.22 ± 0.05^{aB}	26.07 ± 1.40^{B}
2020	25.77 ± 0.01^{cA}	26.15 ± 0.17^{bA}	27.82 ± 0.14^{aA}	26.58 ± 0.95^{A}
2021	26.76 ± 0.06^{aA}	25.93 ± 0.11^{bB}	26.54 ± 0.34^{aC}	26.41 ± 0.42^{A}
Overall Season	25.62 ± 1.14^{c}	26.24 ± 0.35^{b}	27.19 ± 0.58^{a}	26.35 ± 0.99

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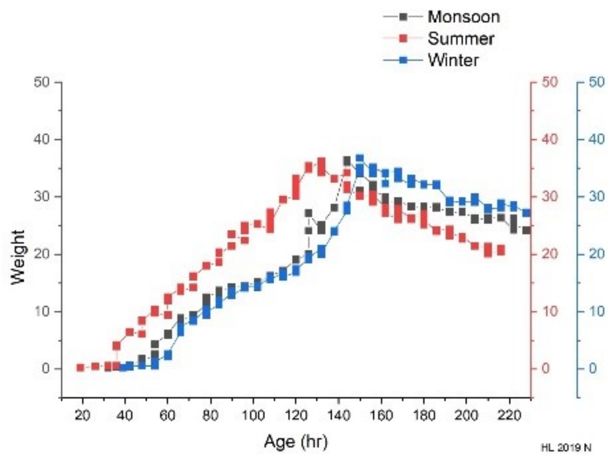
Interaction studies on year, season and stage was found to be significant (F = 14.365; P = < 0.001) indicating that seasonal variations in length of each stage were different in different years (Table 4.86).

Table 4.86. ANOVA for comparing weight of larval instars of *H. ligurriens*

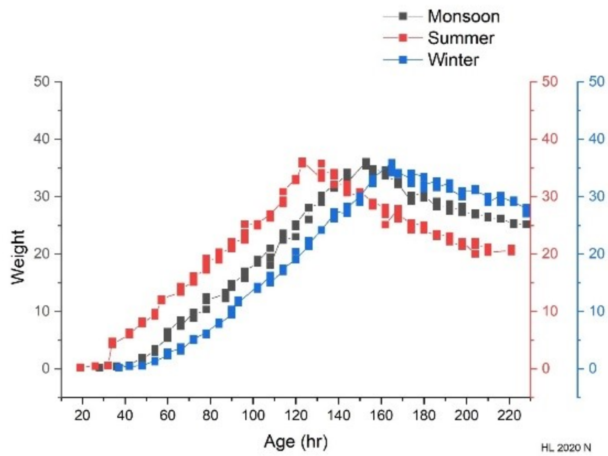
Source	df	Sum of Squares	Mean Square	F-Value	P-value
Year	2	14.69	7.35	142.786**	< 0.001
Season	2	1.10	0.55	10.679**	< 0.001
Stage	3	12238.69	4079.56	79292.09**	< 0.001
Year * Season	4	46.02	11.51	223.612**	< 0.001
Year * stage	6	73.29	12.22	237.413**	< 0.001
Season * stage	6	21.52	3.59	69.713**	< 0.001
Year * Season * Stage	12	37.74	3.15	61.121**	< 0.001
Error	72	3.70	0.05		
Total	107	12436.75			

** Significant at 0.01 level; ns non-Significant

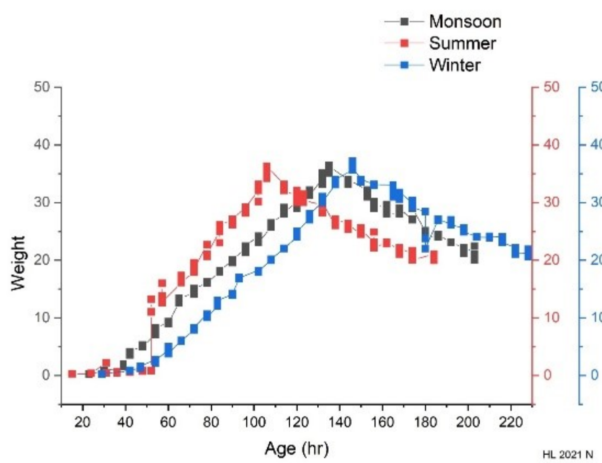
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching until pupation during different seasons and years were prepared (Fig. 4.63 & Fig. 4.64).



2019

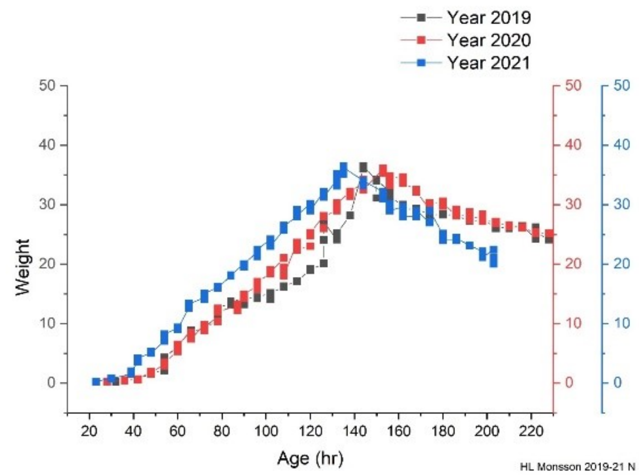


2020

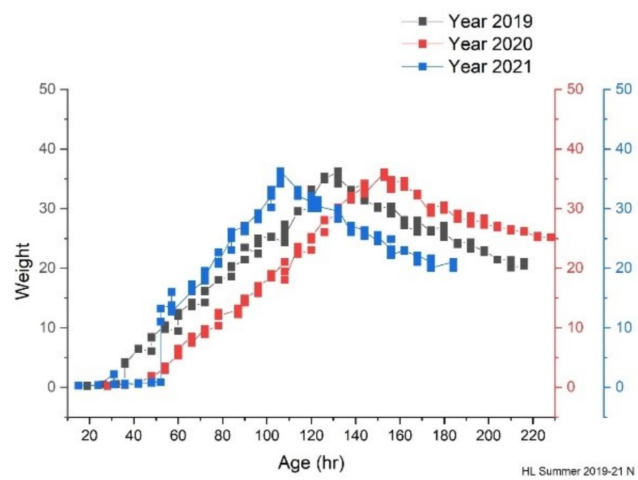


2021

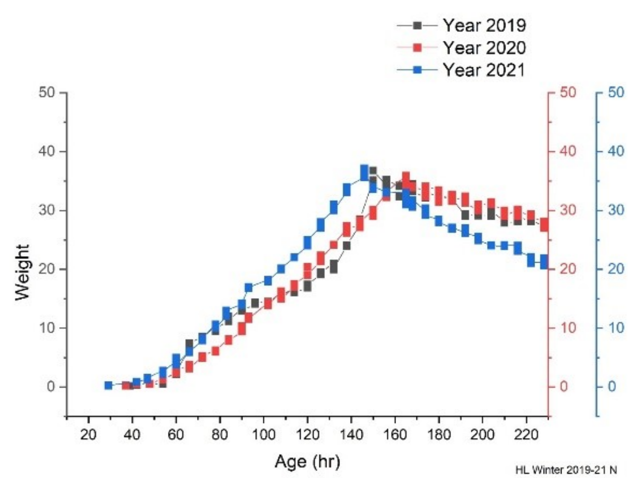
Fig. 4.63. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation



Monsoon



Summer



Winter

Fig. 4.64. Developmental rate (Weight (mg) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation during the study period

Effect on life cycle duration

Significantly higher duration (hr) was observed for *H. ligurriens* during winter for incubation (35.00 ± 5.29) (F = 84.88; P = < 0.001), pupation (192.33 ± 9.61) (F = 128.58; P = < 0.001) and total life cycle (457.33 ± 54.31) (F = 56.409; P = < 0.001). However significantly higher duration was observed during monsoon for IInd larval instar (29.33 ± 5.51) (F = 8.269; P = 0.038) (Table 4.87).

Table 4.87. Seasonal changes in duration (hrs) of life cycle of *H. ligurriens*

Stages	Monsoon	Summer	Winter	F-value (P-value)
Incubation	27.33 ± 4.04^{b}	17.67 ± 2.31^{c}	35.00 ± 5.29^{a}	84.88** (<0.001)
Instar I	18.00 ± 2.00	16.00 ± 1.00	16.67 ± 1.53	0.903^{ns} (0.475)
Instar II	29.33 ± 5.51^{a}	16.33 ± 1.53^{b}	28.67 ± 4.51^{a}	8.269* (0.038)
Instar III	62.00 ± 1.73	55.33 ± 5.69	59.67 ± 3.51	2.575^{ns} (0.191)
Post feeding stage	139.33 ± 14.64	81.00 ± 9.85	125 ± 46.18	5.774^{ns} (0.066)
Pupation	154.33 ± 10.6^{b}	113.67 ± 8.15^{c}	192.33 ± 9.61^{a}	128.58** (<0.001)
Total time taken from egg stage till emergence	430.33 ± 33.5^{a}	300.00 ± 23.07^{b}	457.33 ± 54.31^{a}	56.409** (0.001)

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

Means having different letter as super script differ significantly between seasons (within a row)

Significantly higher duration (hr) was observed for *H. ligurriens* during 2019 for incubation (29.67 ± 10.07) (F = 16.63; P =0.012) and total life cycle (425.33 ± 104.41) (F = 10.229; P = 0.027) (Table 4.88).

Table 4.88. Changes in duration (hrs) of life cycle of *H. ligurriens*

Stages	2019	2020	2021	F-value (P-value)
Incubation	29.67 ± 10.07^{a}	28.00 ± 9.00^{a}	22.33 ± 7.02^{b}	16.63* (0.012)
Instar I	16.67 ± 1.53	17.33 ± 2.52	16.67 ± 1.16	0.129^{ns} (0.882)
Instar II	24.67 ± 7.02	27.00 ± 10.39	22.67 ± 6.51	0.726^{ns} (0.538)
Instar III	60.00 ± 0.00	61.00 ± 3.46	56.00 ± 7.00	1.575^{ns} (0.313)
Post feeding stage	138.33 ± 48.21	113.00 ± 24.00	94.00 ± 28.84	3.091^{ns} (0.154)
Pupation	156.00 ± 42.57	160.00 ± 39.15	144.33 ± 37.02	5.505^{ns} (0.071)
Total time taken from egg stage till emergence	425.33 ± 104.41^{a}	406.33 ± 77.68^{a}	356.00 ± 71.08^{b}	10.229* (0.027)

** Significant at 0.05 level; ns non-significant*
Means having different letter as super script differ significantly between Years (within a row)

Effect on survival rate

Effect of season on the survival rate (%) of *H. ligurriens* was found to be significant (Table 4.92). The survival rate (%) in *H. ligurriens* was significantly higher in monsoon (75.08 ± 5.18) in comparison to summer (73.20 ± 3.93) and winter (64.94 ± 4.21). (Table 4.89).

Table 4.89. Seasonal changes in the survival rate (%) of *H. ligurriens*

Year	Monsoon	Summer	Winter	Overall year
2019	78.30 ± 2.94^{aA}	74.50 ± 3.19^{bA}	65.32 ± 2.50^{c}	72.71 ± 6.19^{A}
2020	77.33 ± 3.80^{aA}	74.62 ± 4.21^{bA}	65.10 ± 3.46^{c}	72.35 ± 6.49^{A}
2021	69.61 ± 3.55^{aB}	70.49 ± 2.95^{aB}	64.39 ± 6.09^{b}	68.16 ± 5.09^{B}
Overall Season	75.08 ± 5.18^{a}	73.20 ± 3.93^{b}	64.94 ± 4.21^{c}	71.07 ± 6.26

Means having different small letter as superscript differ significantly within a row
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Effect of year and stage on the survival rate of developmental stages of *H. ligurriens* was found to be significant (Table 4.92). The survival rate of I^{st} larvar instar larvae was significantly higher in 2020 (75.97 ± 6.6) compared to other years. However, the survival rate for II^nd^ (68.99 ± 4.41) and III^rd^ instar larvae (64.71 ± 4.56) was significantly lower in 2021. Survival rate for pupation was significantly higher in 2019 (73.00 ±6.68) compared to 2020 (70.88 ± 6.58) and 2021 (65.43 ± 5.90) (Table 4.90).

Table 4.90. Survival rate (%) of life cycle of *H. ligurriens*

Stage	2019	2020	2021	Overall stage
Egg	72.76 ± 7.13	72.33 ± 6.63	72.27 ± 4.42	72.45 ± 5.94^{A}
Ist Instar	72.34 ± 5.69^{b}	75.97 ± 6.6^{a}	69.41 ± 2.18^{b}	72.57 ± 5.68^{A}
IInd Instar	72.91 ± 7.03^{a}	70.45 ± 5.64^{ab}	68.99 ± 4.41^{b}	70.78 ± 5.81^{AB}
IIIrd Instar	72.52 ± 5.75^{a}	72.12 ± 6.93^{a}	64.71 ± 4.56^{b}	69.78 ± 6.69^{B}
Pupa	73.00 ± 6.68^{a}	70.88 ± 6.58^{a}	65.43 ± 5.90^{b}	69.77 ± 6.95^{B}
Overall year	72.71 ± 6.19^{a}	72.35 ± 6.49^{a}	68.16 ± 5.09^{b}	71.07 ± 6.26

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Effect of season and stage on the survival rate of developmental stages was found to be significant (Table 4.92).Survival rate of developmental stages of *H. ligurriens* was significantly higher in monsoon (75.08 + 5.18) compared to summer (73.20 + 3.93) and winter (64.94 + 4.21) (Table 4.91).

Table 4.91. Seasonal changes in survival rate (%) of life cycle stages of *H. ligurriens*

Stage	Monsoon	Summer	Winter	Overall stage
Egg	76.55 + 3.77	74.15 + 3.12	66.66 + 5.54	$72.45 + 5.94^{A}$
IIst Instar	76.17 + 6.30	73.30 + 4.65	68.24 + 2.71	$72.57 + 5.68^{A}$
IInd Instar	75.04 + 4.43	73.27 + 2.80	64.04 + 1.89	$70.78 + 5.81^{AB}$
IIIrd Instar	73.25 + 5.63	72.55 + 5.72	63.55 + 3.89	$69.78 + 6.69^{B}$
Pupa	74.38 + 5.83	72.73 + 3.33	62.20 + 3.71	$69.77 + 6.95^{B}$
Overall Season	$75.08 + 5.18^{a}$	$73.20 + 3.93^{b}$	$64.94 + 4.21^{c}$	71.07 + 6.26

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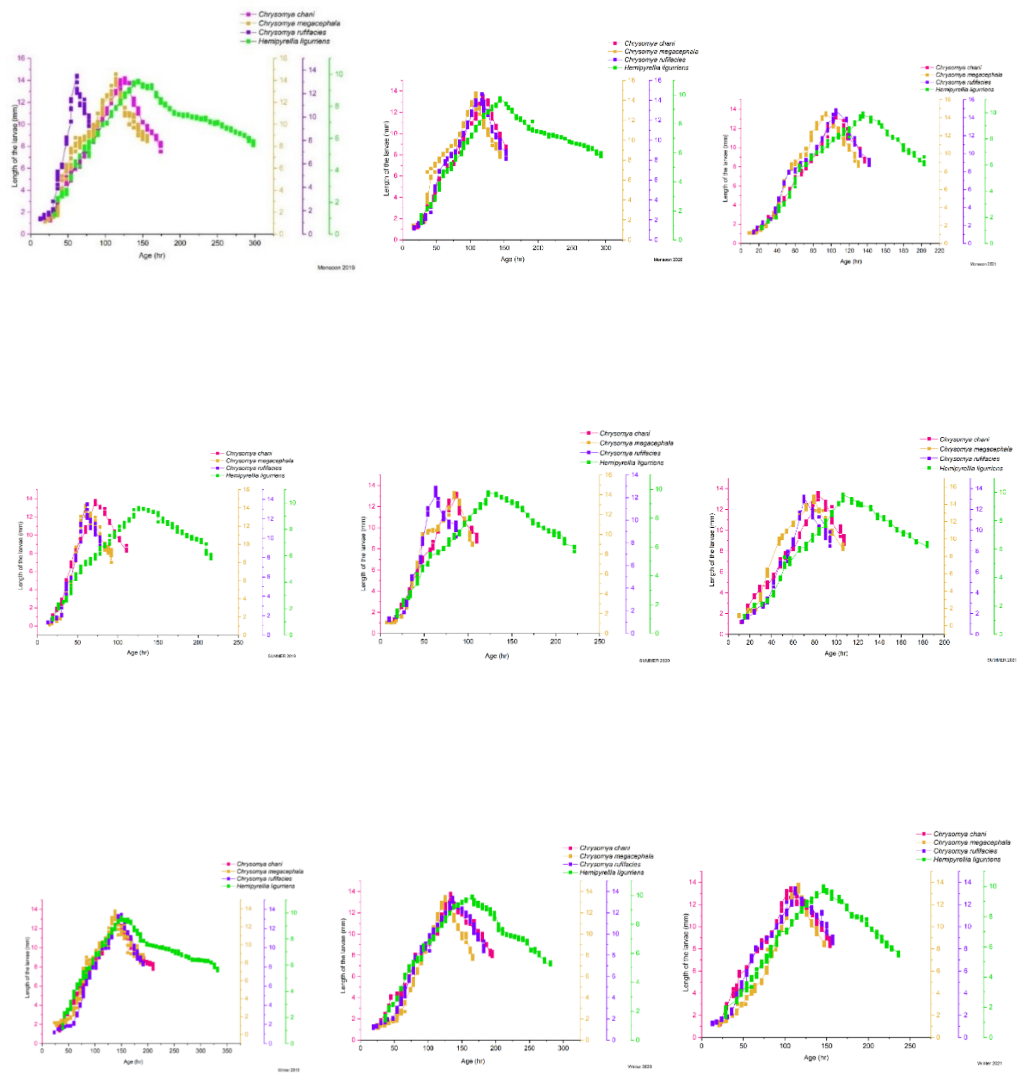
Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between year and stage (F = 3.30; P = < 0.002), as well as year and season (F = 5.85; P = < 0.001) (Table 4.92).

Table 4.92. ANOVA for comparing survival of *H. ligurriens*

Source	df	Sum of Squares	Mean Square	F-value	P-value
Year	2	574.49	287.24	24.12**	< 0.001
Season	2	2619.17	1309.59	109.97**	< 0.001
Stage	4	205.09	51.27	4.31**	0.003
Year * season	4	278.62	69.66	5.85**	< 0.001
Year * Stage	8	314.34	39.29	3.30**	0.002
Season * Stage	8	91.02	11.38	0.955^{ns}	0.476
Year * season * Stage	16	103.04	6.44	0.541^{ns}	0.918
Error	90	1071.79	11.91		
Corrected Total	134	5257.55			

*** Significant at 0.01 level; ns non-significant*

2019 2020 2021



Monsoon

Summer

Winter

■ *C. chani* ■ *C. megacephala* ■ *C. rufifacies* ■ *H. ligurriens*

Fig.4.65. Developmental rate (Length (mm) Vs. Age (hr) *C. chani, C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation

4.4.5 Laboratory rearing

C. megacephala

Length

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F = 56.98; P = < 0.001). The length of Instar III was found to be significantly higher (11.69 ± 0.04) in Group II while the length of Instar II was significantly lower (6.23 ± 0.04) in Group II compared to other groups. The length of post feeding stage was significantly higher in Group III (11.12 ± 0.02) compared to Group I (10.66 ± 0.06) and Group II (10.44 ± 0.01) (Table 4.93) (Fig. 4.66 and Fig.4.74).

Table 4.93. Length (mm) of larval instars of *C. megacephala* under laboratory conditions

Stage	Group			Overall stage
	I.	II.	III.	
Instar I	2.54 ± 0.02^{D}	2.53 ± 0.02^{D}	2.51 ± 0.08^{D}	2.53 ± 0.04^{D}
Instar II	6.41 ± 0.04^{bC}	6.23 ± 0.04^{aC}	6.46 ± 0.08^{bC}	6.37 ± 0.12^{C}
Instar III	11.45 ± 0.02^{bA}	11.69 ± 0.04^{aA}	11.51 ± 0.03^{bA}	11.55 ± 0.11^{A}
Post feeding stage	10.66 ± 0.06^{bB}	10.44 ± 0.01^{cB}	11.12 ± 0.02^{aB}	10.74 ± 0.3^{B}
Between stage F-value = 78852.68**; (P-value < 0.001) Between group F-value = 51.30**; (P-value < 0.001) Interaction between stage and group F-value =56.98**; (P-value < 0.001)				

** Significant at 0.01 level

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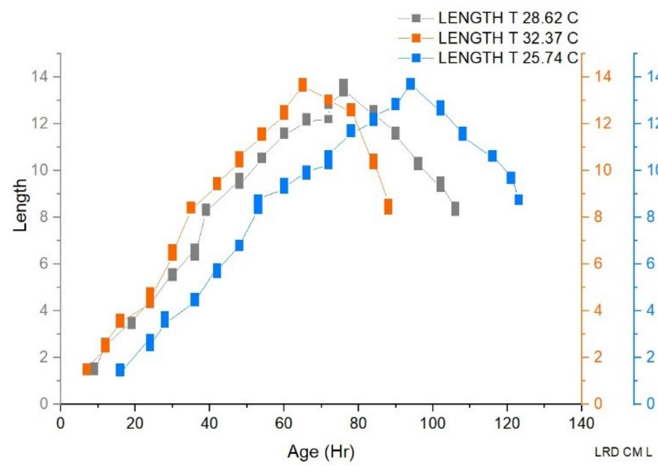

Fig. 4.66. Developmental rate (Length (mm) Vs. Age (hrs.) of *C. megacephala* from hatching upto pupation under laboratory conditions

Weight

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F = 11.83; P = < 0.001). The weight of instar II (11.68 ± 0.25), instar III (33.70 ± 0.47) and post feeding stage (30.21 ± 0.5) was significantly higher in Group III compared to other groups (Table 4.94) (Fig. 4.67 and Fig.4.75).

Table 4.94. Weight (mg) of larval instars of *C. megacephala* under laboratory conditions

Stage	Group			Overall stage
	I.	II.	III.	
Instar I	1.56 ± 0.19^{C}	1.64 ± 0.16^{D}	1.77 ± 0.04^{D}	1.66 ± 0.16^{D}
Instar II	10.02 ± 0.34^{bB}	10.25 ± 0.11^{bC}	11.68 ± 0.25^{aC}	10.65 ± 0.81^{C}
Instar III	29.50 ± 0.83^{cA}	30.37 ± 0.86^{bA}	33.70 ± 0.47^{aA}	31.19 ± 2.02^{A}
Post feeding stage	29.53 ± 0.24^{aA}	28.28 ± 0.82^{bB}	30.21 ± 0.5^{aB}	29.34 ± 0.98^{B}
Between stage F-value = 7790.18**; (P-value < 0.001) Between group F-value = 47.91**; (P-value < 0.001) Interaction between stage and group F-value = 11.83**; (P-value < 0.001)				

** Significant at 0.01 level

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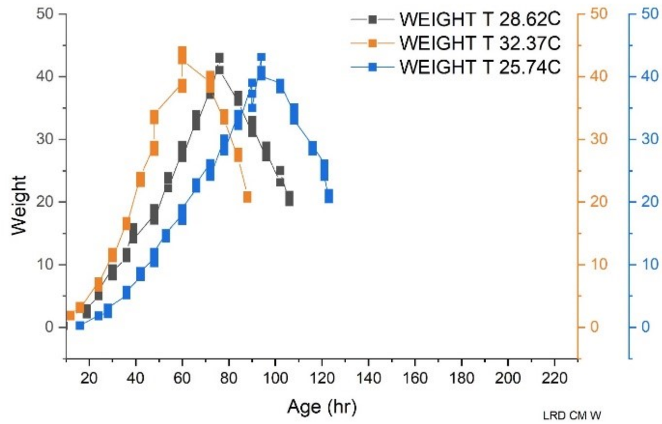

Fig. 4.67. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. megacephala* from hatching upto pupation under laboratory conditions

Developmental duration

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were 16 ± 1.73, 12 ± 2.69, 17 ± 3.75, 34 ± 4.18, 21 ± 3.29, 112 ± 11.03 respectively. The total duration (hrs) for the completion of the life cycle was 212 ± 28.43h.

In group II, the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 8 ± 2..93, 10 ± 3.36, 15 ± 2.71, 28 ± 3.90, 22 ± 4.17, 85 ± 7.92 respectively. The total duration (hrs) for the completion of the life cycle was 168 ± 17.

In group III, the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 6 ± 3.66, 9 ± 2.78, 11 ± 3.19, 23 ± 3.63, 16 ± 3.89, 66 ± 8.03 respectively. The total duration (hrs) for the completion of the life cycle was 131 ± 13.03 (Table 4.95).

Table 4.95. Duration (hrs) of life cycle of *C. megacephala* under laboratory conditions

Group	Duration of different life cycle stages(hrs)						Total time taken from egg stage till emergence
	Incubation period of eggs	I instar	II instar	III instar	Post feeding Stage	Pupation stage	
I	16 ± 1.73	12 ± 2.69	17 ± 3.75	34 ± 4.18	21 ± 3.29	112 ± 11.03	212 ± 28.43
II	8 ± 2..93	10 ± 3.36	15 ± 2.71	28 ± 3.90	22 ± 4.17	85 ± 7.92	168 ± 17.55
III	6 ± 3.66	9 ± 2.78	11 ± 3.19	23 ± 3.63	16 ± 3.89	66 ± 8.03	131 ± 13.03

The laboratory rearing data was compared with the outdoor rearing data for validation. In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing groups. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *C. megacephala* was 212 ± 28.43 (Group-I), 168 ± 17.55 (Group-II) and 131 ± 13.03h (Group-III) respectively **(**Table 4.95).This was significantly shorter than the outdoor rearing results obtained for winter (286.00 ± 23.26), monsoon (227.00 ± 22.52) and summer (168.00 ± 5.29) respectively.

C. rufifacies

Length

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F=109.56; P=< 0.001).The length of instar I (1.75 ± 0.03) was found to be significantly higher in Group III, while the length of instar III (11.32 ± 0.03) was significantly higher in Group II compared to other groups. The length of post feeding stage (10.71 ± 0.03) was found to be significantly higher in Group I compared to other groups (Table.4.96) (Fig. 4.68 and Fig.4.74).

Table 4.96. Length (mm) of larval instars of *C. rufifacies* under laboratory conditions

Stage	Group			Overall stage
	I.	II.	III.	
Instar I	1.59 ± 0.03^{cD}	2.32 ± 0.04^{aD}	1.75 ± 0.03^{bD}	1.89 ± 0.33^{D}
Instar II	5.37 ± 0.001^{C}	5.36 ± 0.04^{C}	5.36 ± 0.02^{C}	5.37 ± 0.02^{C}
Instar III	10.85 ± 0.01^{aA}	11.32 ± 0.03^{cA}	10.77 ± 0.03^{bA}	10.98 ± 0.26^{A}
Post feeding stage	10.71 ± 0.03^{aB}	10.53 ± 0.09^{bB}	10.20 ± 0.05^{cB}	10.48 ± 0.23^{B}
Between stage F-value = 113693.27**; (P-value < 0.001) Between group F-value = 282.34**; (P-value < 0.001) Interaction between stage and group F-value = 109.56**; (P-value < 0.001)				

** Significant at 0.01 level

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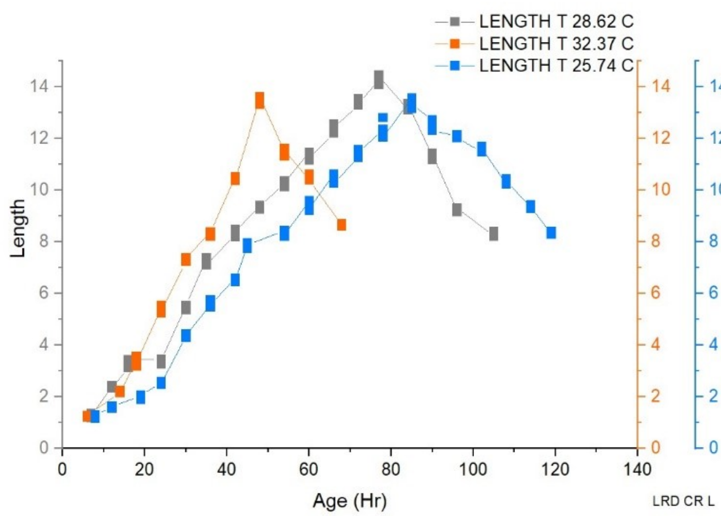

Fig. 4.68. Developmental rate (Length (mm) Vs. Age (hrs) of *C. rufifacies* from hatching upto pupation under laboratory conditions

Weight

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F = 16.31; P = < 0.001). The weight of instar II (11.81 ± 0.56) and instar III was significantly higher (33.48 ± 0.36) in Group III compared to other groups, while the weight of post feeding stage (29.25 ± 0.41) was significantly higher in Group I compared to Group II (28.91 ± 0.36) and Group III (27.45 ± 0.6) (Table 4.97) (Fig. 4.69 and Fig.4.75).

Table 4.97. Weight (mg) of larval instars of *C. rufifacies* under laboratory conditions

Stage	Group			Overall stage
	I.	II.	III.	
Instar I	1.58 ± 0.02^{C}	1.5 ± 0.05^{D}	1.54 ± 0.14^{D}	1.54 ± 0.08^{D}
Instar II	10.49 ± 0.46^{bB}	11.75 ± 0.29^{aC}	11.81 ± 0.56^{aC}	11.35 ± 0.76^{C}
Instar III	31.34 ± 0.34^{bA}	32.96 ± 0.39^{aA}	33.48 ± 0.36^{aA}	32.59 ± 1.02^{A}
Post feeding stage	29.25 ± 0.41^{aA}	28.91 ± 0.36^{aB}	27.45 ± 0.6^{bB}	28.54 ± 0.92^{B}
Between stage F-value = 13439.64**; (P-value < 0.001) Between group F-value = 8.32**; (P-value < 0.001) Interaction between stage and group F-value = 16.31**; (P-value < 0.001)				

** Significant at 0.01 level

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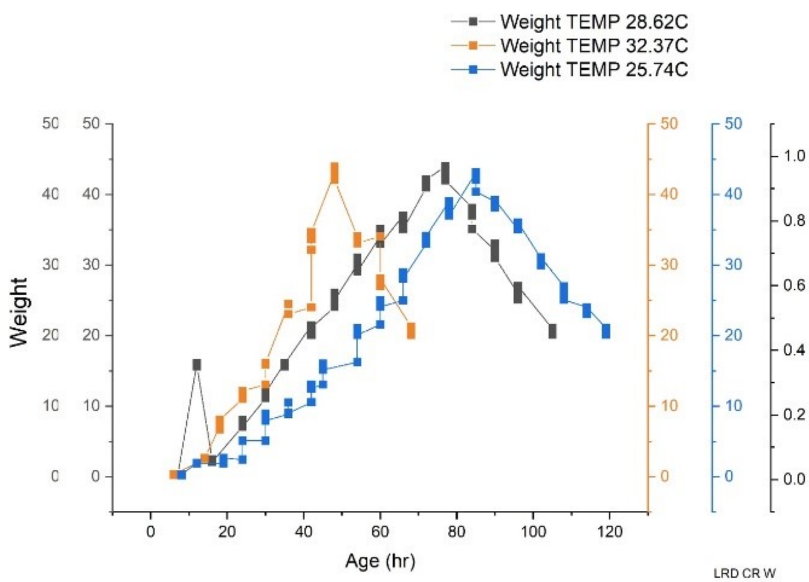

Fig. 4.69. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. rufifacies* from hatching upto pupation under laboratory conditions

Developmental duration

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation in *C.*

rufifacies were, 7± 2.81, 11 ± 3.11, 21 ± 4.16, 31 ± 3.83, 29 ± 4.41 and 98 ± 7.72 respectively. The total duration (hrs) for the completion of the life cycle was 197 ± 18.18 h.

In group II, the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 6 ± 3.72, 9 ± 1.93, 11 ± 3.81, 35 ± 4.73, 21 ± 3.18, 78 ± 9.18 respectively. The total duration (hrs) for the completion of the life cycle was 160 ± 21.03.

In group III, the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 5 ± 1.13, 8 ± 3.03, 12 ± 2.17, 29 ± 3.14, 14 ± 4.14, 62 ± 5.47 respectively. The total duration (hrs) for the completion of the life cycle was130 ± 26.77 (Table 4.98).

Table 4.98. Duration (hrs) of life cycle of *C. rufifacies* under laboratory conditions

Group	Duration of different life cycle stages(hrs)						Total time taken from egg stage till emergence
	Incubation period of eggs	I instar	II instar	III instar	Post feeding Stage	Pupation stage	
I	7± 2.81	11 ± 3.11	21 ± 4.16	31 ± 3.83	29 ± 4.41	98 ± 7.72	197 ± 18.18
II	6 ± 3.72	9 ± 1.93	11 ± 3.81	35 ± 4.73	21 ± 3.18	78 ± 9.18	160 ± 21.03
III	5 ± 1.13	8 ± 3.03	12 ± 2.17	29 ± 3.14	14 ± 4.14	62 ± 5.47	130 ± 26.77

The laboratory rearing data was compared with the outdoor rearing data for validation .In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *C. rufifacies* was 197 ± 18.18 (Group-I), 160 ± 21.03 (Group-II) and 130 ± 26.77 (Group-III) respectively (Table 4.98). . This was significantly shorter than the outdoor rearing results obtained for winter (267.00 ± 18.68), monsoon (223.00 ± 13.45) and summer (148.33 ± 6.43) respectively.

C. chani

Length

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F=92.660; P = < 0.001). The length of instar I (2.81 ± 0.07) was significantly higher in Group III, while that of instar II was significantly lower (5.56 ± 0.03) in Group II compared to other groups. The length of instar III (11.22 ± 0.01) and post feeding stage (10.98 ± 0.06) was significantly higher in Group I compared to other groups. (Table.4.99) (Fig. 4.70 and Fig.4.74).

Table 4.99. Length (mm) of larval instars of *C. chani* under laboratory conditions

Stage	Group			Overall stage
	I.	II.	III.	
Instar I	2.59 ± 0.03^{bD}	2.35 ± 0.01^{cC}	2.81 ± 0.07^{aD}	2.58 ± 0.20^{D}
Instar II	6.54 ± 0.06^{aC}	5.56 ± 0.03^{bB}	6.49 ± 0.06^{aC}	6.2 ± 0.48^{C}
Instar III	11.22 ± 0.01^{aA}	10.44 ± 0.01^{cA}	10.86 ± 0.05^{bA}	10.84 ± 0.34^{A}
Post feeding stage	10.98 ± 0.06^{aB}	10.44 ± 0.01^{bA}	10.47 ± 0.03^{bB}	10.63 ± 0.27^{B}
Between stage F-value = 76347.37**; (P-value < 0.001) Between group F-value = 701.69**; (P-value < 0.001) Interaction between stage and group F-value =92.660**; (P-value < 0.001)				

** Significant at 0.01 level
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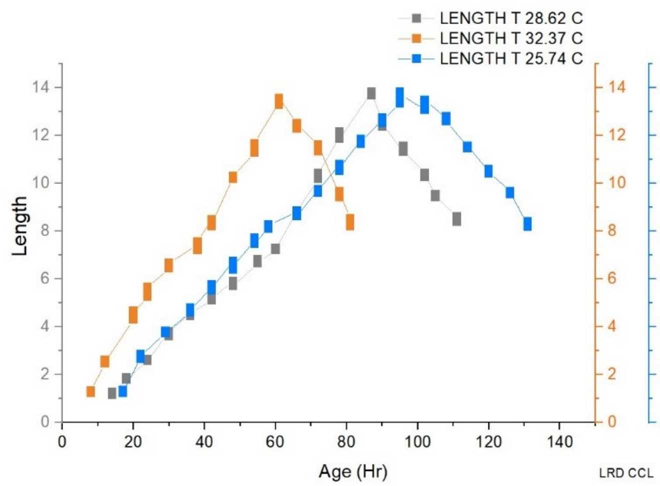

Fig.4.70. Developmental rate (Length (mm) Vs. Age (hrs) of *C. chani* from hatching upto pupation under laboratory controlled conditions

Weight

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F = 6.621; P = < 0.001). The weight of instar II (11.83 ± 0.71) and instar III (31.95 ± 0.52) was significantly higher in Group III compared to other groups, while the weight of post feeding stage (28.29 ± 0.62) was significantly lower in Group I compared to other groups (Table 4.99) (Fig. 4.71 and Fig.4.75).

Table 4.99. Weight (mg) of larval instars of *C. chani* under laboratory conditions

Stage	Group			
	I.	II.	III.	Overall stage
Instar I	1.54 ± 0.13^{C}	1.4 ± 0.10^{D}	1.36 ± 0.18^{D}	1.43 ± 0.15^{D}
Instar II	10.32 ± 0.24^{bB}	10.31 ± 0.8^{bC}	11.83 ± 0.71^{aC}	10.82 ± 0.93^{C}
Instar III	28.39 ± 0.89^{bA}	31.30 ± 0.48^{aA}	31.95 ± 0.52^{aA}	30.55 ± 1.74^{A}
Post feeding stage	28.29 ± 0.62^{bA}	28.79 ± 1.06^{abB}	29.50 ± 0.53^{aB}	28.86 ± 0.85^{B}
Between stage F-value = 4975.52**; (P-value < 0.001) Between group F-value = 19.291**; (P-value < 0.001) Interaction between stage and group F-value = 6.621**; (P-value < 0.001)				

** Significant at 0.01 level

Means having different small letter as superscript differ significantly within a row

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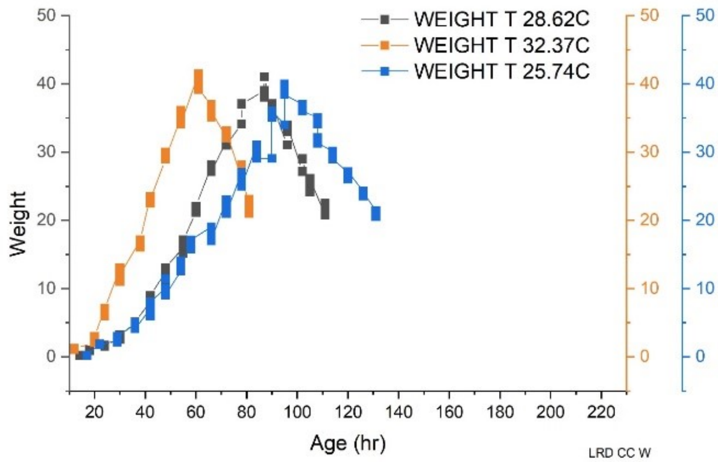

Fig. 4.71. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani* from hatching upto pupation under laboratory conditions

Developmental duration

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were 16 ± 2.06, 12 ± 2.67, 28 ± 4.04, 29 ± 2.63, 29 ±4.06, 118 ± 6.81 respectively. The total duration (hrs) for the completion of the life cycle was 232 ± 33.18.

In group II, the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 13 ± 2.18, 10 ± 1.18, 19±2.58, 27 ± 4.08, 21 ± 3.81, 89 ± 6.82 respectively. The total duration (hrs) for the completion of the life cycle was 179 ± 28.92.

In group III, the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 7 ± 3.04, 9 ± 2.17, 14 ± 3.11, 19 ± 3.77, 15 ± 4.46, 76 ± 7.91 respectively. The total duration (hrs) for the completion of the life cycle was 140 ± 22.18 (Table 4.100).

Table 4.100. Duration (hrs) of life cycle of *C. chani* under laboratory conditions

Group	Duration of different life cycle stages(hrs)						Total time taken from egg stage till emergence
	Incubation period of eggs	I instar	II instar	III instar	Post feeding Stage	Pupation stage	
I	16 ± 2.06	12 ± 2.67	28 ± 4.04	29 ± 2.63	29 ±4.06	118 ± 6.81	232 ± 33.18
II	13 ± 2.18	10 ± 1.18	19±2.58	27 ± 4.08	21 ± 3.81	89 ± 6.82	179 ± 28.92
III	7 ± 3.04	9 ± 2.17	14 ± 3.11	19 ± 3.77	15 ± 4.46	76 ± 7.91	140 ± 22.18

The laboratory rearing data was compared with the outdoor rearing data for validation. In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *C. chani* was 232 ± 33.18 (Group-I), 179 ± 28.92 (Group-II) and 140 ± 22.18 (Group-III) respectively. This was significantly shorter than the outdoor rearing results obtained for winter (320.33± 24.03), monsoon (246±20.08) and summer (192.33±8.15) respectively.

Hemipyrellia ligurriens

Length

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F=342.90; P = < 0.001). The length of instar I (2.41 ± 0.05) and instar II (4.53 ± 0.01) was significantly higher in Group I compared to other groups, while the length Instar III (8.22 ± 0.02) was significantly higher in Group II compared to other groups. The length of post feeding stage (7.98 ± 0.01) was significantly higher in Group III compared to Group I (7.75 ± 0.02) and Group II (7.95 ± 0.02) (Table.4.101) (Fig. 4.72 and Fig.4.74).

Table 4.101. Length (mm) of larval instars of *H. ligurriens* under laboratory conditions

Stage	Group			
	I.	II.	III.	Overall stage
Instar I	2.41 ± 0.05^{aD}	1.81 ± 0.01^{bD}	1.76 ± 0.02^{bD}	1.99 ± 0.32^{D}
Instar II	4.53 ± 0.01^{aC}	4.33 ± 0.02^{bC}	3.77 ± 0.05^{cC}	4.21 ± 0.34^{C}
Instar III	7.98 ± 0.09^{bA}	8.22 ± 0.02^{aA}	6.73 ± 0.04^{cB}	7.64 ± 0.70^{B}
Post feeding stage	7.75 ± 0.02^{bB}	7.95 ± 0.02^{aB}	7.98 ± 0.01^{aA}	7.90 ± 0.11^{A}
Between stage F-value = 48772.16**; (P-value < 0.001) Between group F-value = 874.59**; (P-value < 0.001) Interaction between stage and group F-value = 342.90**; (P-value < 0.001)				

** Significant at 0.01 level

Means having different small letters as superscripts differ significantly within a row

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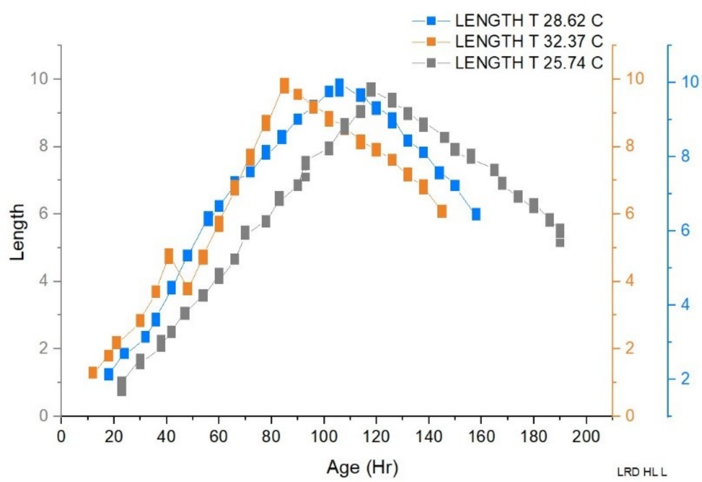


Fig. 4.72. Developmental rate (Length (mm) Vs. Age (hrs) of *H. ligurriens* from hatching upto pupation under laboratory conditions

Weight

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F=7.86; P=< 0.001). The weight of instar II (7.59 ± 0.54) and instar III (25.67 ± 0.53) was significantly higher in Group I compared to other groups (Table.4.102) (Fig. 4.73 and Fig.4.75).

Table 4.102. Weight (mg) of larval instars of *H. ligurriens* under laboratory conditions

Stage	Group			Overall stage
	I.	II.	III.	
Instar I	0.95 ± 0.16^{D}	0.79 ± 0.04^{D}	0.83 ± 0.02^{D}	0.86 ± 0.11^{D}
Instar II	7.59 ± 0.54^{aC}	5.24 ± 0.13^{bC}	5.63 ± 0.13^{bC}	6.16 ± 1.13^{C}
Instar III	25.67 ± 0.53^{aB}	24.15 ± 0.37^{bB}	24.75 ± 0.46^{bB}	24.86 ± 0.77^{B}
Post feeding stage	26.68 ± 0.41^{A}	26.71 ± 0.67^{A}	27.31 ± 0.61^{A}	26.9 ± 0.59^{A}
Between stage F-value = 9404.27**; (P-value < 0.001) Between group F-value = 18.39**; (P-value < 0.001) Interaction between stage and group F-value = 7.86**; (P-value < 0.001)				

** Significant at 0.01 level

Means having different small letters as superscripts differ significantly within a row

Means having different capital letters as superscripts differ significantly within a column

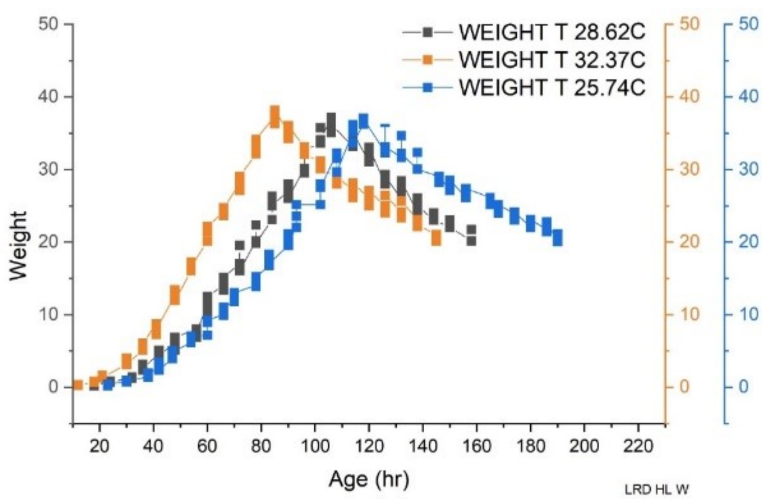


Fig. 4.73. Developmental rate (Weight (mg) Vs. Age (hrs) of *H. ligurriens* from hatching upto pupation under laboratory conditions

Developmental duration

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were 22 ± 3.15, 15 ± 2.33, 18 ± 2.59, 40 ± 4.77, 64 ± 5.16, 131 ± 9.03 respectively. The total duration (hrs) for the completion of the life cycle was 290 ± 29.17.

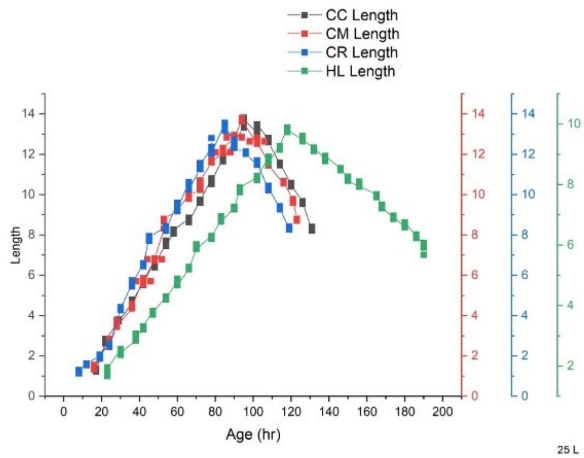
In group II, the developmental duration (hrs) of incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 17 ± 4.02, 14 ± 3.04, 20 ± 3.02, 20 ± 3.02, 46 ± 5.23, 44 ± 4.20, 117 ± 11.68 respectively. The total duration (hrs) for the completion of the life cycle was 258 ± 16.38.

In group III, the developmental duration (hrs) of incubation of eggs, Ist instar, IInd instar, IIIrd instar, post feeding stage and pupation were, 11 ± 2.84, 9 ± 2.17, 11 ± 2.85, 37 ± 4.99, 55 ± 3.12, 96 ± 8.16, respectively. The total duration (hrs) for the completion of the life cycle was 219 ± 21.11 (Table 4.103).

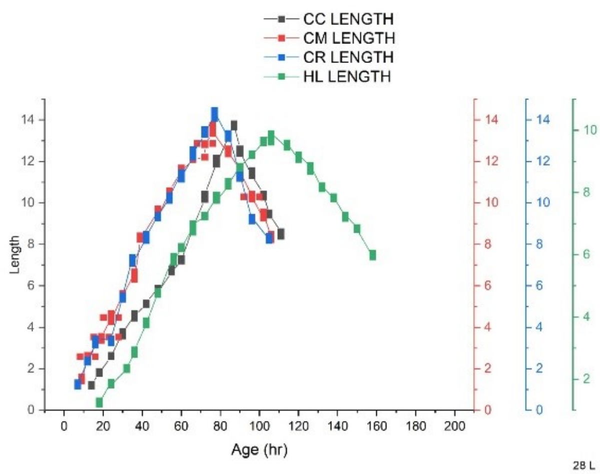
Table 4.103. Duration (hrs) of life cycle of *H. ligurriens* under laboratory conditions

Group	Duration of different life cycle stages(hrs)						Total time taken from egg stage till emergence
	Incubation period of eggs	I instar	II instar	III instar	Post feeding Stage	Pupation stage	
I	22 ± 3.15	15 ± 2.33	18 ± 2.59	40 ± 4.77	64 ± 5.16	131 ± 9.03	290 ± 29.17
II	17 ± 4.02	14 ± 3.04	20 ± 3.02	46 ± 5.23	44 ± 4.20	117 ± 11.68	258 ± 16.38
III	11 ± 2.84	9 ± 2.17	11 ± 2.85	37 ± 4.99	55 ± 3.12	96 ± 8.16	219 ± 21.11

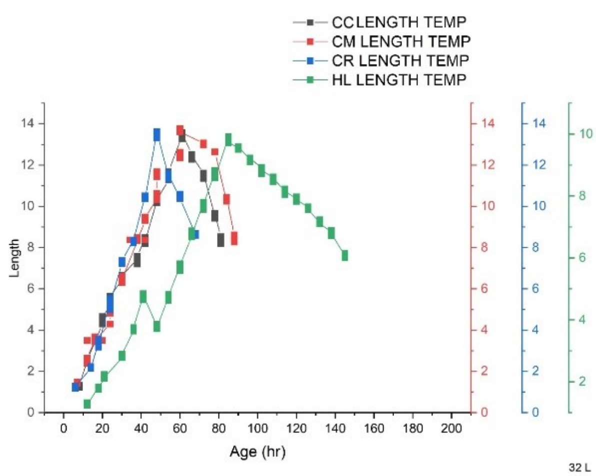
The laboratory rearing data was compared with the outdoor rearing data for validation. In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *H .ligurriens* was 290 ± 29.17 (Group-I), 258 ± 16.38 (Group-II) and 219 ± 21.11 (Group-III) respectively (Table 4.103).This was significantly shorter than the outdoor rearing results obtained for winter (457.33 ± 54.31), monsoon (430.33 ± 33.5) and summer (300.00 ± 23.07) respectively.



Group I

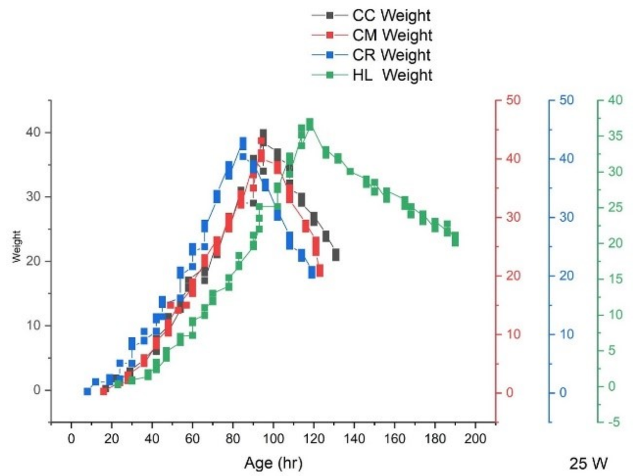


Group II

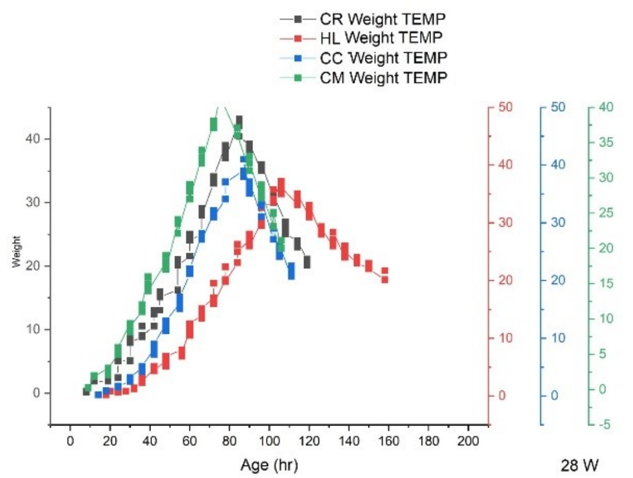


Group III

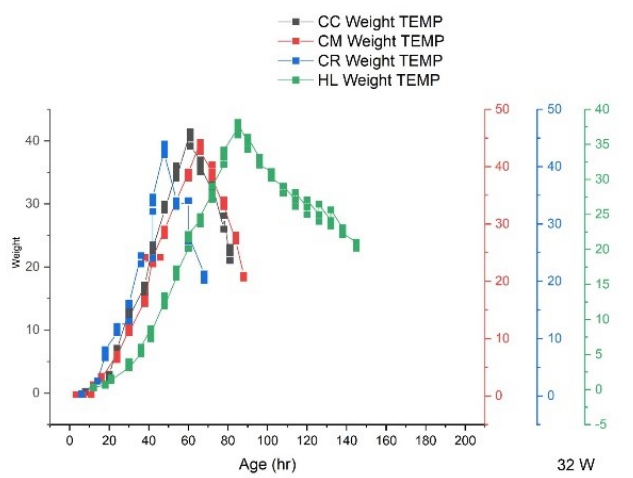
Fig. 4.74. Developmental rate (Length (mm) Vs. Age (hrs) of *C. chani, C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation under laboratory conditions



Group I



Group II



Group III

Fig. 4.75. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani, C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation under laboratory conditions

4.5. Relation between life cycle of flies and time since death assessment

4.5.1. Estimation of Post Mortem Interval (PMI)

The fitted equation (best fit model) for predicting duration in hours in terms of length of larvae *C. chani, C. megacephala, C. rufifacies* and *H. ligurriens* are given in table 4.104. This equation was useful for estimating the PMI.

In the case of *C. chani*, coefficient of determination (R^2) for the predicted equation is almost equal to 0.94 which indicates that about 94 percent of variability in length can be explained by duration and R2 for the predicted equation in the case of *C. megacephala, C. rufifacies* and *H. ligurriens* is above 0.894, 0.771, and 0.855 respectively. Pattern of variation of the observed and predicted values of the three different seasons for four different species are given in Fig. 4.76 - 4.79.

Table 4.104. Regression equation model for the estimation of PMI

Species	Season	Model Fitted	R^2
Chrysomya chani	Winter	$\ln(length) = 2.834 - 42.998/t$	0.940
	Monsoon	$\ln(length) = 2.723 - 38.353/t$	0.935
	Summer	$\ln(length) = 4.429 - 19.850/t$	0.945
Chrysomya megacephala	Winter	$\ln(length) = 2.862 - 42.245/t$	0.933
	Monsoon	$\ln(length) = 2.633 - 21.249/t$	0.911
	Summer	$\ln(length) = 2.621 - 17.648/t$	0.894
Chrysomya rufifacies	Winter	$\ln(length) = 2.535 - 23.123/t$	0.835
	Monsoon	$\ln(length) = 2.576 - 18.595/t$	0.868
	Summer	$\ln(length) = 4.162 - 15.321/t$	0.771
Hemipyrellia ligurriens	Winter	$\ln(length) = 2.370 - 43.547/t$	0.855
	Monsoon	$\ln(length) = 2.494 - 43.829/t$	0.902
	Summer	$\ln(length) = 2.284 - 28.378/t$	0.869

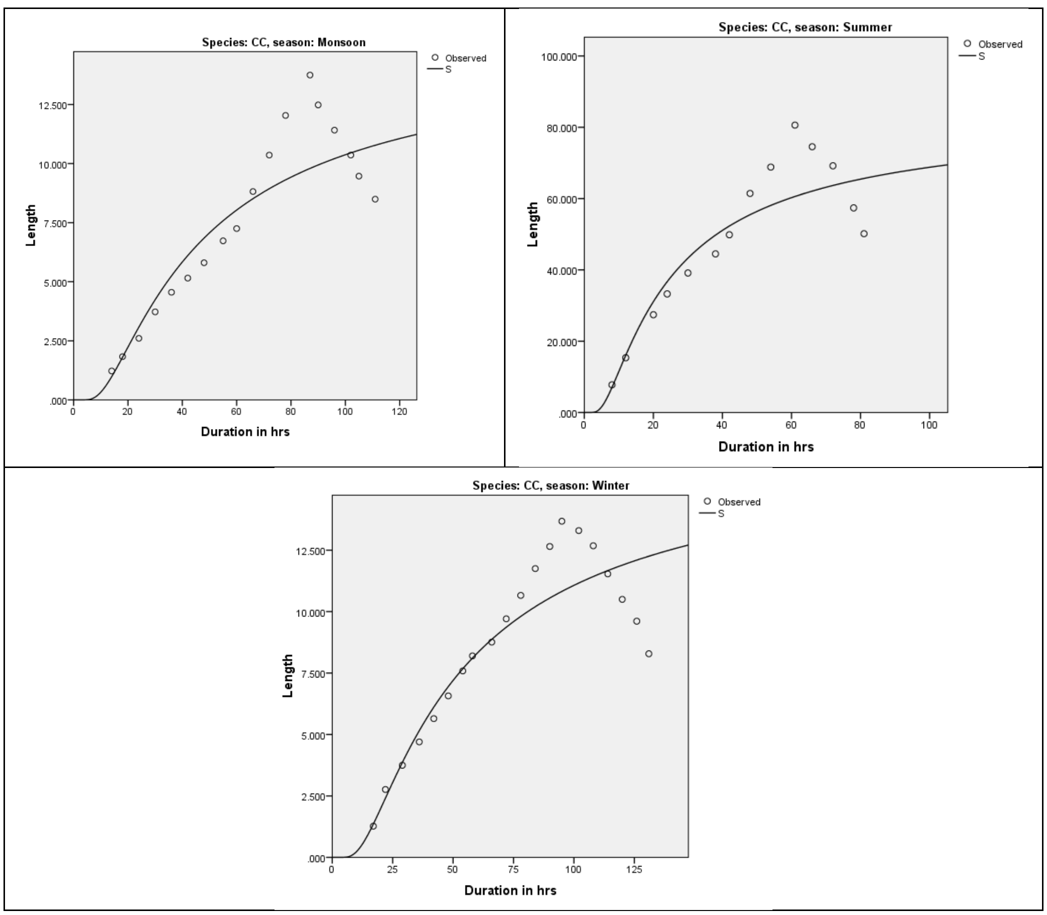


Fig. 4.76. Growth curves of *C. chani* for the estimation of the PMI

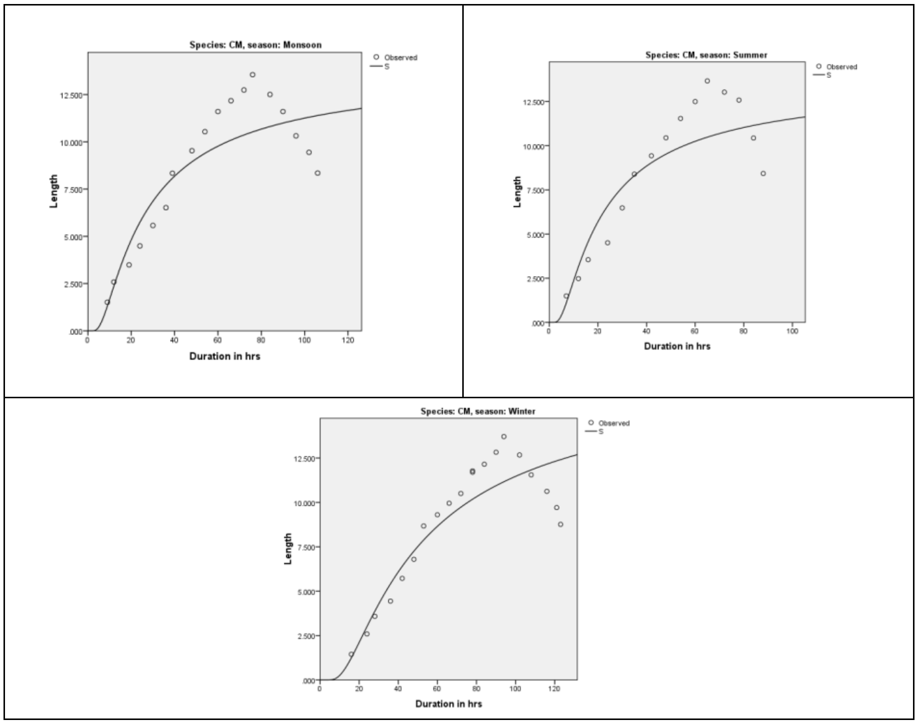


Fig. 4.77. Growth curves of *C. megacephala* for the estimation of the PMI

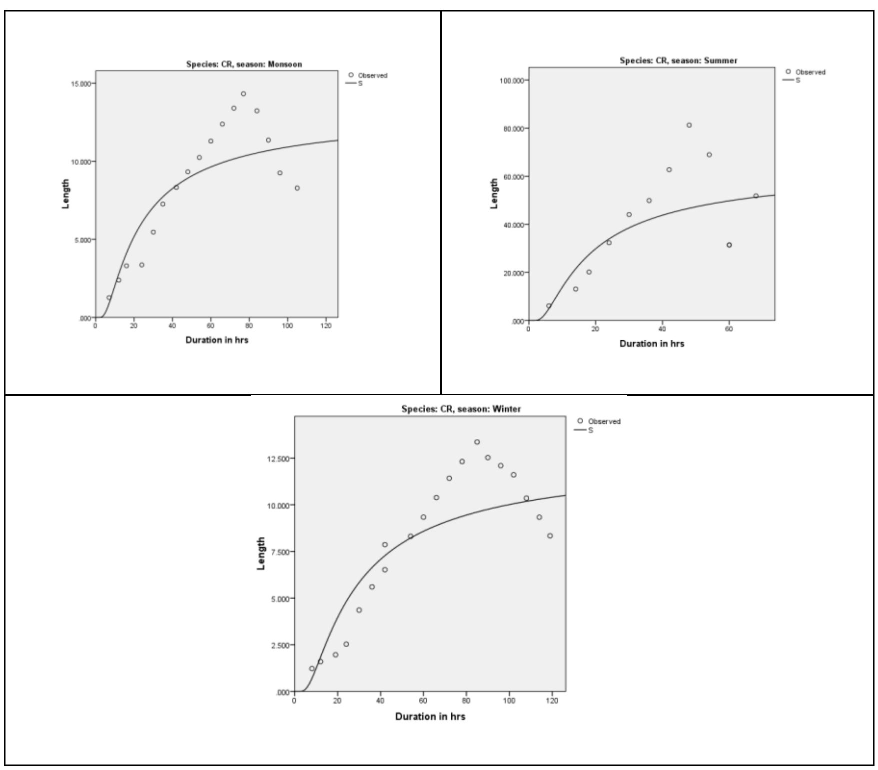


Fig. 4.78. Growth curves of *C. rufifacies* for the estimation of the PMI

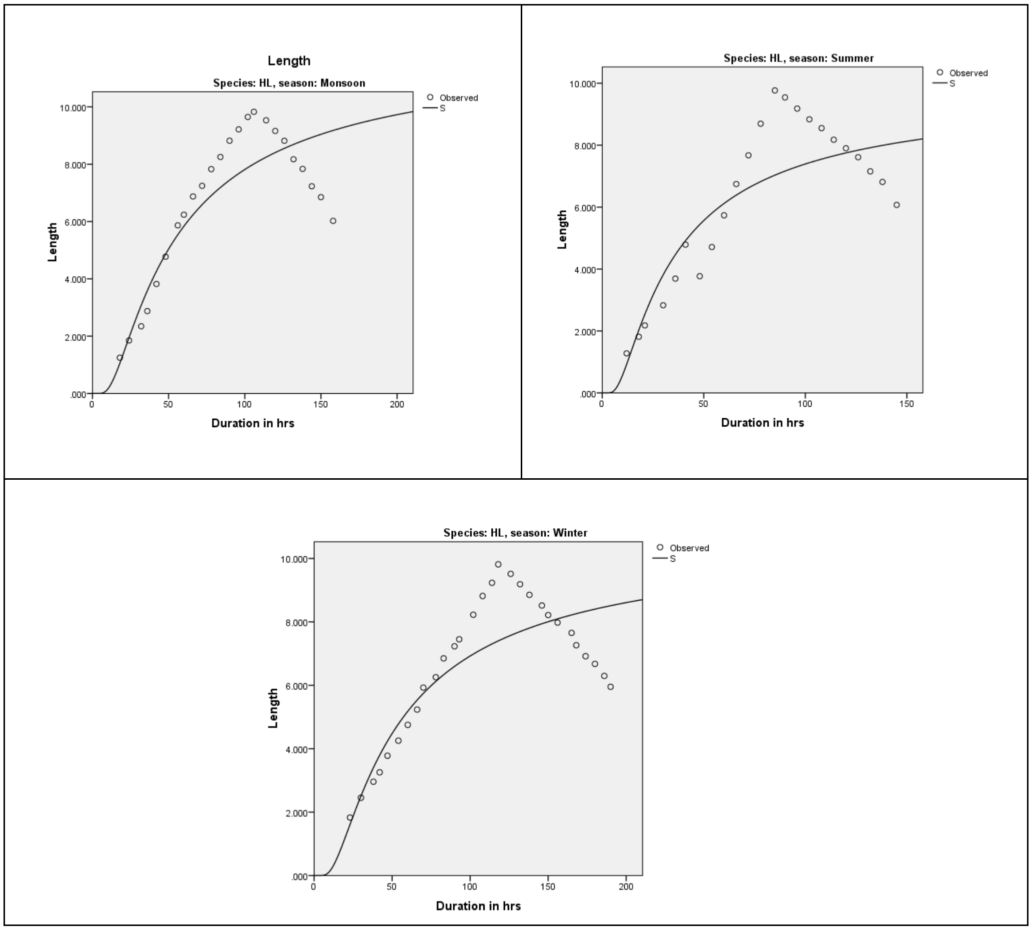


Fig. 4.79. Growth curves of *H. ligurriens* for the estimation of the PMI