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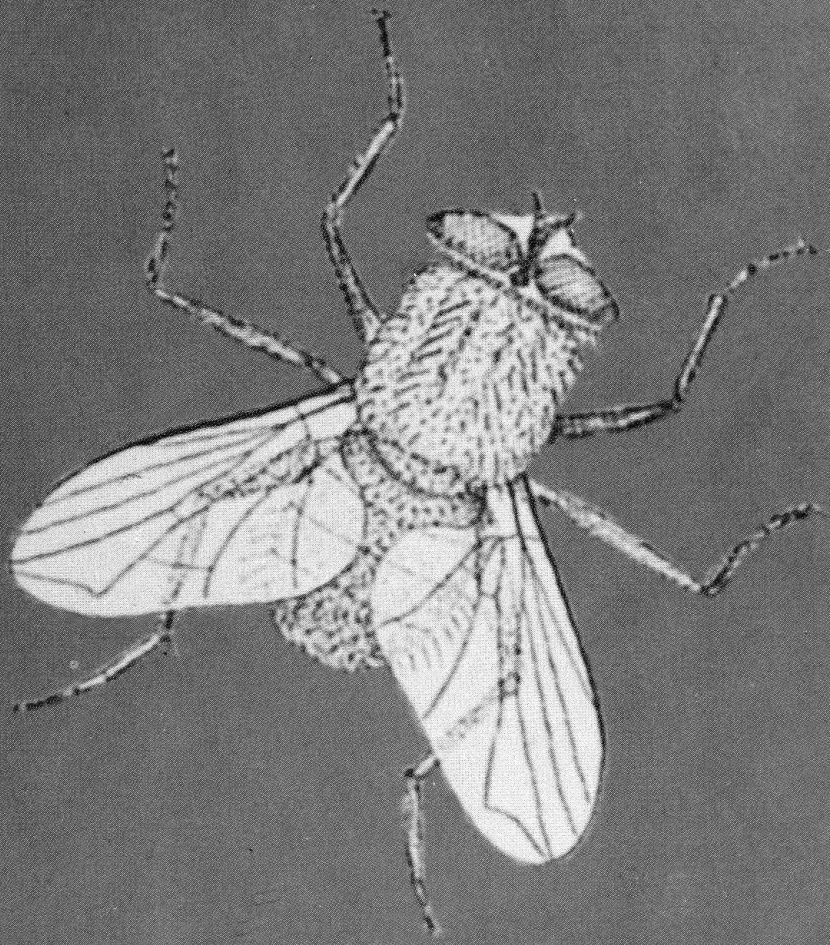

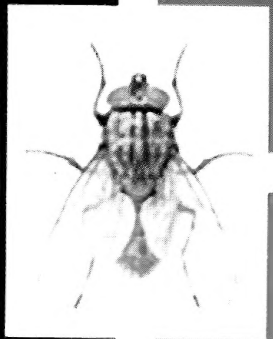

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Diversity of the ground inhabiting ant fauna at Department of Atomic Energy campus, Kalpakkam (Tamil Nadu)

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Abstract

Ant sampling was carried out in different locations of the Department of Atomic Energy (DAE) Campus at Kalpakkam during dry season (March – June 2008). Pit-fall traps and hand-picking methods were used to collect ants from 20 different sampling sites. A total of 31 species, 15 genera, and 5 subfamilies of ants were collected. The Myrmicinae were the most common, with 7 genera and 16 species, followed by the Formicinae (4 genera and 8 species), the Ponerinae (2 genera and 2 species), the Pseudomyrmecinae (1 genus and 4 species) and the Dolichoderinae was represented by only 1 species. Interestingly 86.6% of the genera, 83.8% of the species, and 92.4% of the individuals collected belonged to three subfamilies (Myrmicinae, Ponerinae, and Formicinae). The five most species-rich genera were *Monomorium*, *Camponotus*, *Tetraponera*, *Crematogaster* and *Tetramorium*. The taxonomic structure of the myrmecofauna sampled, resembles that of Western and Eastern Ghats and other tropical regions in two ways: Firstly, many rare species and a few abundant species: Secondly, the dominance of subfamilies such as Myrmicinae, Ponerinae and Formicinae. The species accumulation curve indicated that the likelihood of getting more number of species in DAE campus and this finding was supported by rarefaction curve

Keywords: *Ant diversity, Ground-inhabiting ants, Pit-fall trap, DAE Campus, Kalpakkam.*

Introduction

The use of indicator taxa, i.e. taxa that are theoretically representative of other taxa at a given site, has become important in studies of biodiversity in light of the need for rapid, reliable and cost-effective assessments that can be used in conservation and monitoring programs (Oliver and Beattie, 1993 and Kerr *et al.*, 2000). Determining the level of diversity of these groups should permit predictions about the other taxa to be present (Pearson and Carroll 1998, Lawton *et al.*,1998, Lindenmayer, 1999 and Kerr *et al.*, 2000).Traditionally, majority of studies used vascular plants and vertebrates as indicator taxa (Agosti and Alonso, 2000). However, recently the importance and appropriateness of using invertebrate groups

have been recognized (Pearson, 1994, Oliver and Beattie, 1996a and 1996b). Ants in particular are an excellent choice for use as an indicator taxon (Longino and Colwell, 1997 and Agosti and Alonso, 2000) due to their high local diversity, numerical and biomass dominance in almost every terrestrial habitat. Moreover, their important functions in ecosystems, organization in communities that are sensible to variations in the environment, relatively good base of taxonomic knowledge, and ease of sampling (Carroll and Janzen, 1973, Holldobler and Wilson, 1990, Bestelmeyer *et al.,* 2000, Brown, 2000 and Schultz and McGlynn 2000) are also responsible for their choice as indicator species. Ground-inhabiting ants are particularly promising group as they represent a large portion of the myrmecofauna. The ant fauna of India remains relatively unexplored (Rastogi *et al.,* 1997). Barring a few isolated studies, very little information is available on ants in India, especially bio-ecology and their usefulness as bioindicators of environmental health. Site-specific reports are essential because biodiversity profile varies regionally. Studies on ant faunal diversity in Tamil Nadu still remains rudimentary. Hence, an attempt was made to study the diversity pattern of ground inhabiting ant fauna of DAE Campus at Kalpakkam, Tamil Nadu. This exercise assumes greater significance considering the fact that DAE campus is going to be a nuclear complex soon. Thus, it in imperative to take stock of present biodiversity status for future impact assessment studies.

Materials and Methods

Study area

The DAE campus at Kalpakkam encompasses seashore and a vast plain area of the Bay of Bengal. The coastal system forms the complex natural site where intense interactions occur among land, sea and atmosphere.The unique interaction throws biological consortia peculiar to this system. It spreads through the biologically diverse and productive habitat for native flora and fauna and aesthetically blended with introduced vegetation. All the study sites were located inside the DAE campus. Totally 20 representative sampling sites comprising of different landscapes viz., undisturbed scrub jungle, near water bodies, riparian woods, sandy area, casurina monoculture, area with meagre native vegetation and building area (Fig.1) were selected for the study.

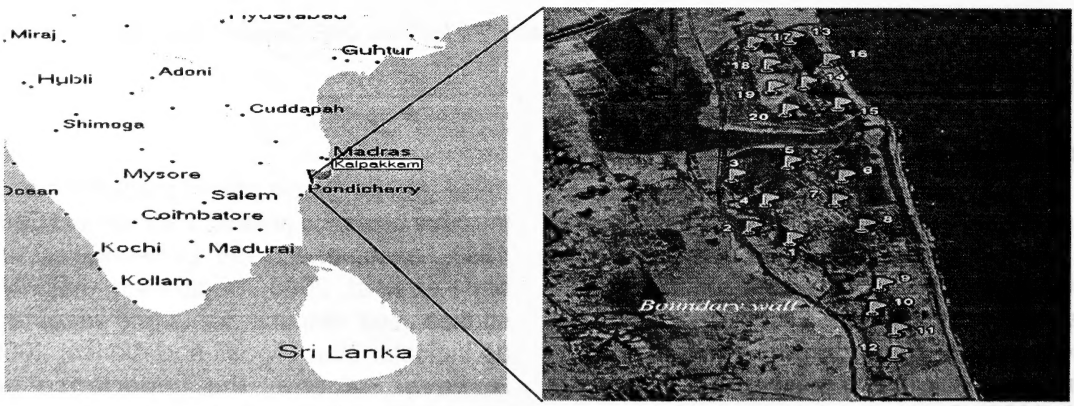

Fig.1: Map showing study area and sampling locations

Methodology

Ant sampling was carried out in different locations of the DAE Campus during dry season (March – June 2008). Pit-fall traps and hand-picking methods were used to collect ants in different sampling sites. Pit-fall trapping method permits foraging workers to be captured and provides information on the species present in the sampling area. The trap consisted of a one-liter plastic jar with an opening of 7cm in diameter and was placed at ground level. Six pit-fall traps were installed in a more or less straight transect line with each trap approximately 10mtrs apart. Each jar carried 25 ml of 0.05% methyl parathion. The traps were set up between 15.00 and 17.00 hrs and were collected on the next day evening. Ants trapped in the jars were preserved in labelled containers of 70% alcohol. In addition to trapping method described above, an intensive all-out-search to physically collect representative of as many species of ants as possible was made in each sampling unit. In hand-picking collection, two observers walked randomly around each transects (site viz) and to the extent possible, the effort involved in this process was kept same. Ants associated with leaf litter were also collected qualitatively to cover overall species spectrum, quantitative collection method was not preformed because leaf litter was not available at many locations in sandy area of the campus. No attempt was made to estimate abundance by these methods. Data collected through pit-fall was taken to quantify abundance. Collected ant species samples were identified primarily based on Bolton (1995) and Fauna of British India, Bingham (1903). Some specimens were sent to specialist to confirm their identity.

Results

Taxonomic structure of the fauna

A total of 31 species, 15 genera, and 5 subfamilies of ants were collected. The Myrmicinae were the most common, with 7 genera and 16 species, followed by the Formicinae (4 genera and 8 species), the Ponerinae (2 genera and 2 species), the Pseudomyrmicinae (1 genus, 4 species) and the Dolichoderinae was represented by only one species. Interestingly 86.6% of the genera, 83.8% of the species, and 92.4% of the individuals collected belonged to three subfamilies (Myrmicinae, Ponerinae, Formicinae) (Table-1).

Table-1: Total number and percentage of species, genera, and individuals collected per subfamily.

Subfamily	Genera		Species		Individuals	
	No.	%	No.	%	No.	%
Formicinae	4	26.67	8	25.81	214.00	17.88
Myrmicinae	7	46.67	16	51.61	658.00	54.97
Ponerinae	2	13.33	2	6.45	234.00	19.55
Pseudomyrmecinae	1	6.67	4	12.90	15.00	1.25
Dolichoderinae	1	6.67	1	3.23	76.00	6.35
Total (5)	15	100	31	100	1197	100

The five most species-rich genera were *Monomorium* (5 sp.), *Camponotus* (4 sp.), *Tetraponera* (4 sp.), *Crematogaster* (3 sp.) and *Tetramorium* (3 sp.). Out of 15 genera recorded these five genera collectively contribute 70.28% of total species encountered (Table-2). Twenty one species could be identified to the species level: *Diacamma rugosum, Camponotus variegates, Solenopsis invicta, Crematogaster subnuda, Tapinoma melanocephalum, Myrmicaria brunnea, Camponotus sericeus, Pachycondyla sulcata, Plagiolepis longipes, Monomorium scabriceps, Monomorium floricola, Paratrechina longicornis, Oecophylla smaragdina, Monomorium destructor, Camponotus compressus, Monomorium latinode, Pheidole latinoda, Tetraponera rufonigra, Meranoplus bicolor, Tetraponera nigra, Tetramorium walshi.*

Patterns in species richness

The number of ant species found in each

Table–2: Species richness of genera.

Subfamily	Genera	Species	
		No.	%
Formicinae	*Camponotous*	4	12.90
	Oecophylla	1	3.23
	Paratrechina	2	6.45
	Plagiolepis	1	3.23
Myrmicinae	*Crematogaster*	3	9.68
	Meranoplus	1	3.23
	Monomorium	5	16.13
	Myrmicaria	1	3.23
	Pheidole	2	6.45
	Solenopsis	1	3.23
	Tetramorium	3	9.68
Ponerinae	*Diacamma*	1	3.23
	Pachycondyla	1	3.23
Dolichoderinae	*Tapinoma*	1	3.23
Pseudomyrmecinae	*Tetraponera*	4	12.90
Total		31	100

sampling unit varied from six to ten in most samples, with an average of eight (Fig.2). In the first sampling unit itself, 13 species were encountered. To know the accumulation pattern and area vs. species relationship, species accumulation curve was plotted. The graph (Fig. 3), indicated increase in record of new species with the increase in sampling attempts. More than 60% of the species were recorded at 8th sampling effort and even at 9th sampling attempt the graph showed increasing trend which clearly indicated the possibility of getting more species.

Michaelis-Menten type model describes well about the accumulation of species records as the number of sampling attempt increases. This model has clearly demonstrated that, with increase in sampling attempts the likelihood of adding new species is most likely. Fig-4. depicts the rarefaction curve using MMMean and Coleman curve estimators of species richness. Michaelis-Menten model and Coleman curve were used for sampling data after randomizing them 50 times using the procedure of Colwell (1997). This indicated that the sampling area was rich enough to fetch 44 speices and as the average for all sites was 34 species.

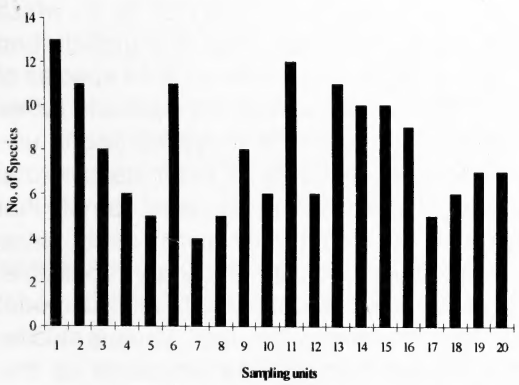

Fig.2: Distribution of number of species encountered in each sampling unit.

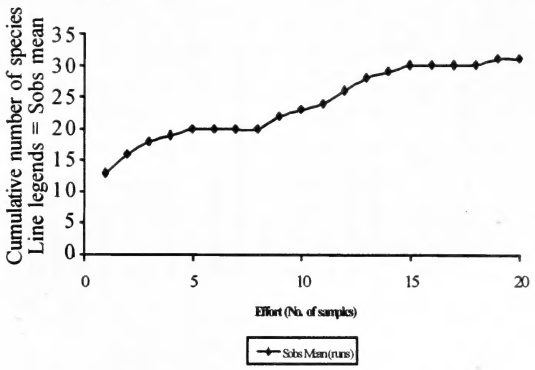

Fig.3: Species accumulation curve.

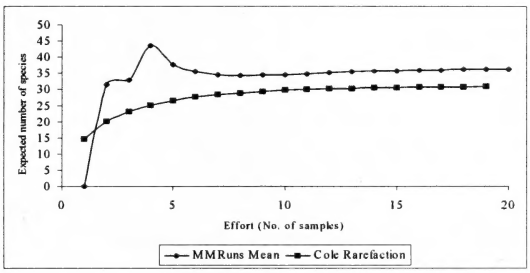

Fig.4: Rarefaction curves of performance of Michaelis-Menten richness estimators (MM Mean) and Coleman curve as a function of randomized sample accumulation.

Pattern in species abundance profile

The number of individuals trapped in pit-fall ranged from 24 to 142 with an average of 60 (Fig.5). Abundance was high at sampling sites 1, 11 and 14 because certain common species Viz., *Diacamma rugosum, Camponotus variegates, Myrmicaria brunnea, Pheidole* spp. dominated those sites. When the relative abundance of species was plotted against the rank, the plot often lead to approximately straight line. The more horizontal the line, the more equitable the distribution. In the present case rank order abundance plot demonstrated that a small number of very abundant species and a large number of rare species were captured (Fig.6).

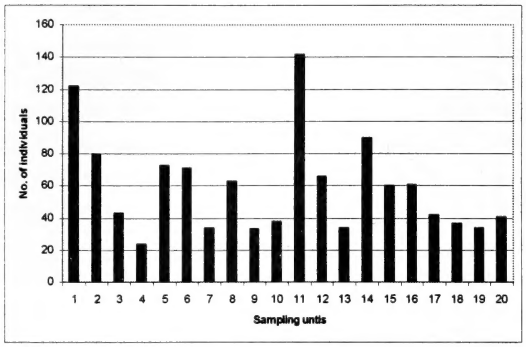

Fig.5: Abundance profile of ants collected at different sampling units at DAE campus.

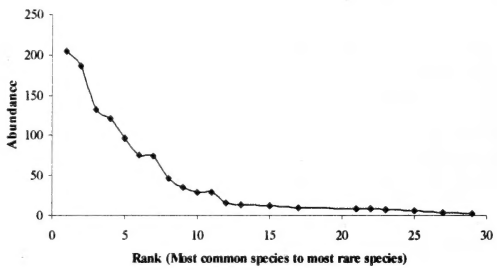

Fig.6: Rank order abundance plot of ant fauna at Kalpakkam.

Discussion

The results indicate that the diversity of the ground-inhabiting ant fauna of DAE campus was relatively high (31 species and 15 genera), as compared to that of other regions of Tamil Nadu with a similar sampling effort and methodology (Vinodhini *et al.,* 2003, Rajagopal *et al.,* 2005, Kaleeswaran, 2006 and Ramesh, 2007). Where as comparatively high diversity was reported from western Ghats and localities of Bangalore (Gadhakar *et al.,* 1993, Rastogi *et al.,* 1997, Sunil Kumar *et al.,* 1997, Anu and Sabu 2007, and Varghese, 2008). This difference in diversity could be due to inadequate studies in Tamil Nadu. Moreover, the differences in richness could possibly result from interactions existing between the ant fauna of the surrounding vegetation and associated fauna present at that specific geographical location. A more complete and comparative study of the biodiversity of the ant fauna of the state may throw more light on this aspect.

The taxonomic structure of the myrmecofauna sampled, resembles that of Western and Eastern Ghats and other tropical regions in two ways. Firstly, many rare species and a few abundant species were collected (Malsch, 2000). Secondly, the subfamilies such as Myrmicinae, Ponerinae, and Formicinae were dominant. The Myrmicinae alone accounted for nearly 50% of the genera, species, and individuals sampled (Gadagkar *et al.,*1993, Rastogi *et al.,* 1997, Anu and Sabu, 2007, Ramesh, 2007 and Ward, 2000). However, the relative importance of the Ponerinae and Formicinae subfamilies in the ants collected, differed with that of ants collected in both the Atlantic forest and the Amazonian forest. In these two regions, the Ponerinae subfamily was significantly predominant (Majer and Delabie, 1994, Delabie *et al.,* 2000, Vasconcelos and Delabie, 2000 and Tavares, 2002).

The species accumulation curve showed increasing trend even after 50% of sampling efforts, this clearly indicates that the likelihood of getting more species were bright. This was supported by rarefaction curve (Fig. 4), which clearly indicated that, sites like undisturbed scrub jungle might provide up to 44 species of ant. Common richness indices provide rather abstract figures, thus it is appropriate to use extrapolation methods to estimate the total number of species from empirical sample that make up the community under study since complete inventories are practically impossible. Hence, Michaelis-Menten mathematical model and Coleman curve were used. Various studies have shown that estimators such as the MMMean and Coleman rarefaction are more

reliable when compared to other estimators (Colwell and Coddington, 1994 and Sanjayan *et al.*, 2002).

Overall abundance pattern in different sites varied considerably due to their habitat heterogeneity and species composition. This was evident in certain sampling sites 1, 11 and 14 were common species viz., *Diacamma rugosum, Camponotus variegates, Myrmicaria brunnea, Pheidole* spp. dominated. As observed by many workers (Malsch, 2000 and Ramesh, 2007) species abundance pattern indicated a relatively small proportion of abundant species against large number of rare species.

Acknowledgements

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Diversity and Abundance of ants along an elevational gradient in Jammu-Kashmir Himalaya - I

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Abstract

Ant diversity was studied at an altitude of 1000mtrs and 2000mtrs above mean sea level along an elevational gradient in Jammu-Kashmir Himalaya. Ants were collected with the help of pitfall traps, winkler's and hand collection along a transect of 250mtrs at each site. Species richness was estimated with the help of Colwell's EstimatorS. Subfamily Myrmicinae has been found to be 66%, followed by Formicinae 26.81%, Ponerinae 4.84% and Dolichoderinae 2.35%. The data generated reflects that with decrease in temperature and humidity, composition of species changes as in case of Myrmicinae, the generalist species are replaced by more high altitude specialists like *Myrmica* and *Aphaenogaster.* In case of Formicinae, the interpretation resembles Myrmicinae as cold specialist *Formica* increases in abundance. But interestingly, the overall abundance increases from 1000mtrs to 2000mtrs with number of species almost same at both the elevations.

Keywords: *Ants, diversity, species richness, species abundance, elevational gradient, estimation indices, Jammu-Kashmir Himalaya.*

Introduction

Since the origin of Biogeography, many important studies have been carried on diversity of Insects along elevational gradients. But among insects, ants have been used more frequently by various workers in recent times.

Himalaya is listed as one of the biodiversity hotspots, harbours a number of endemic species since its origin in Paleogene period about 70 million years ago (Bharti, 2008). Within the Himalayan range, the area of Jammu-Kashmir is biogeographically most complex and diverse.

Since the recognition of elevational gradients by Linnaeus, these continued to serve as a heuristic tool and natural experimental site for generations of scientists; Van Humboldt (1849), Darwin (1839, 1859), Wallace (1876, 1878) and Whittaker (1960) to mention a few. Wheeler (1917), Weber (1943) and Gregg (1963) observed ants at high elevations above 2000 meters in mountains of North America, Sudan and Colorado respectively. According to Hutchinson (1959), Preston (1962a and 1962 b), Connell and Orians (1964), MacArthur (1965, 1969 and1972), Brown and Lomolino (1998) and Sanders (2002) there are two general predictions of how species richness and elevation are related; either species richness decreases monotonically with increasing elevation or richness peaks at mid elevations due to

increase in productivity. Rahbek (1995), while studying the elevational gradients of species richness emphasized on the importance to discriminate between patterns reflecting recent diversification and those reflecting long term accumulation of species.

During extensive studies on elevational gradients in Madagascar, Fisher (1996a and 1996b, 1997, 1998, 1999, 2002 and 2004) concluded that species richness is peaked at mid-elevation and emphasized that it could be the result of the mixing of two distinct, lower and montane forest ant assemblages. Samson *et al.* (1997) surveyed ant communities along an elevational gradient in the Philippines extending from lowland dipterocarp forest (250m) elevation to mossy forest (1750m) and found that very few ants occur at high elevations in the tropics. From Sabah, Borneo, Bruhl *et al.* (1998) studied stratification of ants in a primary rain forest. They observed dominance of Myrmicinae (39.9%) followed by Formicinae (31.5%), Ponerinae (11.5%) and Dolichoderinae (10.2%). Later, Bruhl *et al.* (1999) monitored altitudinal distribution of leaf litter ants along a transect in primary rain forest on Mount Kinabalu. The number of ant species decreased exponentially without evidence of a peak in species richness at mid-elevation.

Gunsalam (1999), Yamane and Hashimoto (1999), Noon-anant (2003) and Watanasit (2003), found that a combination of various ant sampling methods yield better results in the evaluation of ant species. The role of scale and species richness in defining the hierarchical theory of species diversity was discussed by Whittaker *et al.* (2001). Lomolino (2001), Sanders *et al.* (2003) discussed the patterns of ant species richness along elevational gradients in an arid ecosystem and role of area, geometry and Rapoport's rule in species richness. While, Xu *et al.* (2001) observed ant communities and their species diversity with altitudinal zonation on west and east slope of Gaoligongshan Mountain in China. Watt *et al.* (2002) worked on the effect of diversity and abundance of ants in relation to forest disturbances in Cameroon and supported the view that deforestation can reduce arthropod species richness.

Araujo and Fernandes (2003) monitered the distribution of ants along altitudinal gradients from 800m to 1500m, while Robinson *et al.* (2003) studied wood ant (*Formica lugubris*) population in Upper Dearne Woodlands, to investigate relationship between ant activity and factors such as light level, slope and vegetation. Schonberg *et al.* (2004) analysed arboreal ant species richness in primary forest, secondary forest and pasture habitat of a tropical Montane Landscape.

More recently, Gunawardene *et al.* (2008), Kumar and Mishra (2008), Malsch *et al.* (2008) and Sabu *et al.* (2008) monitored ant species richness along elevational gradient, in lowland forests and in agroecosystems. In one of the significant contributions, Nogues-Bravo *et al.* (2008) assessed scale effects and human impact on the elevational species richness gradients. From Himalaya, Bharti (2008) analysed altitudinal diversity of ants and found that about 45% of Himalayan ant fauna is endemic to this region. The present study is the first contribution dealing with diversity and abundance of ants from Himalaya.

Materials and Methods

The sampling sites for the study were spaced by an altitude of 1000 meters, since a shift in an altitude of 1000 meters in Himalayan region has pronounced effect on temperature, precipitation, humidity, decomposition, vegetation etc. (Mani, 1962). For this study, the sampling was carried using standard protocols

for ant collection along an elevational gradient following Fisher (2004). At each elevation, 50 pitfall traps and 50 leaf litter samples (winkler's) were used in parallel lines, 10 meters apart along 250 meter transect. The site for each transect was chosen in the interior of forest with the intent of sampling representative microhabitats found at each elevation.

Leaf litter samples were sifted in a 1 m × 1 m quadrant, every 5 meter along the transect using a litter sifter (Bestelmeyer *et al.*, 2000) through a wire sieve with square holes of 1 cm × 1 cm. Ants and other invertebrates were extracted from the sifted litter during a 48-hour period in mini-winkler sacks (Fisher, 1999, 2004). The litter samples were shaken with the help of machete to agitate the invertebrates, hence increasing the potential for further collection from the litter.

The pitfall traps consisted of test tubes with an 18mm internal diameter and 150mm long, partly filled to a depth of about 50 mm with soapy water and 5% ethylene glycol solution, inserted into PVC sleeves and buried with the rim flush with the soil surface, provided with a lid to prevent rainfall from flooding the traps. Material was collected after 48 hours and stored in 70% ethanol. In addition to above mentioned methods, ants were also collected by hand picking method. Ants were then separated from other invertebrates, pin-mounted and identified to species level.

Data analysis

Data was analysed by Incidence-based coverage estimator (ICE), species observed (Mao Tau). Chao 1, Chao 2 and bootstrap mean. Species richness and Alpha diversity was estimated by using Shannon wiener, and Simpson's D diversity indices. The program EstimateS (Colwell, 2006) was used to calculate these standard estimators.

Results and Discussion

A total of 1,446 ants belonging to 19 species were collected. Ponerinae and Dolichoderinae are represented by single genera each, while Myrmicinae and Formicinae by 5 genera each. More than half of the species belong to subfamily Myrmicinae (66%), followed by Formicinae (26.81%), Ponerinae (4.84%) and Dolichoderinae (2.35%). Hand collection yielded maximum number of specimens (45.27%) followed by Winkler's (28.81%) and Pitfall Trap (25.92%).

At 1000mtrs (Table -1, Graph-1, Pi chart-I & III) subfamily Myrmicinae was found to be maximum (49.96%). Genus *Crematogaster* represents 47.56% of the total catch and majority of the specimens were collected by hand picking method followed by winkler's and pitfall. Subfamily Formicinae represents 34.40% with genus *Camponotus* forming the bulk with 37.56%, again hand picking method was found to be most effective followed by winkler's and pitfall. Subfamily Dolichoderinae and Ponerinae are represented by single genus. But in case of Ponerinae maximum catch was found to be in winkler's collection and in terms of number of specimens, Ponerinae out numbered Dolichoderinae. This fact could be attributed to the humidity present in leaf litter.

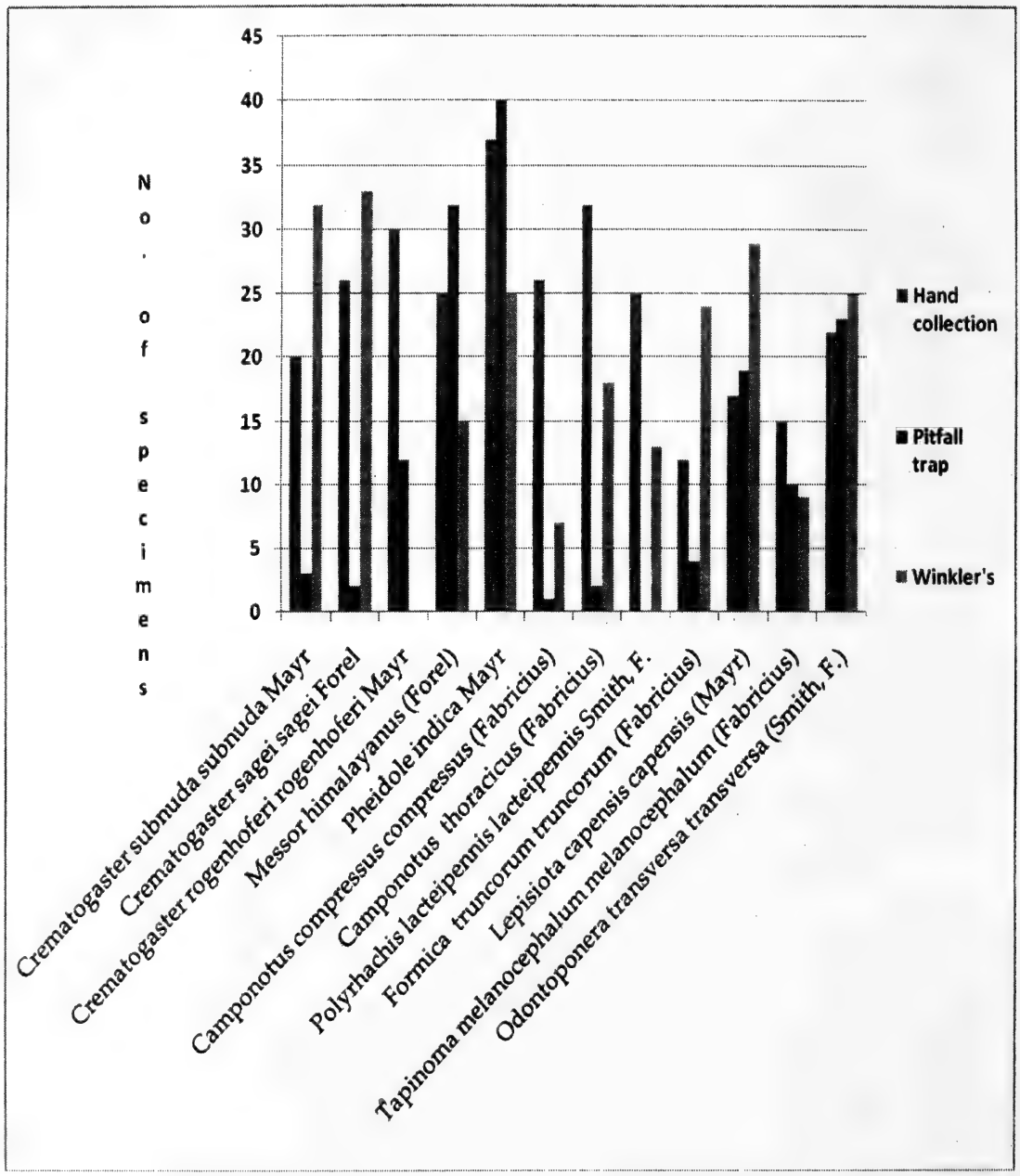
At 2000mtrs (Table-2, Graph-2, Pi chart-II & IV) subfamily Myrmicinae represents 79.64%, genus *Myrmica* as the dominant one with 88.10%. Subfamily Formicinae (20.36%) is mainly represented by *Formica* (72.32%). Two species of *Camponotus,* one each of *Formica* and *Lepisiota* have been found at both the altitudes. At 1000 mtrs, the average temperature was 22°C and relative humidity 52%. The total catch in terms of number of specimens was 665 (Table-1), while with temperature 13.7°C and relative humidity 45%,

the total catch has been found to be 781 at 2000 mtrs.

Species richness by different indices have been depicted in table-5 and species abundance and effectiveness of sampling methods by Sobs (species observed) Mao Tau (Graph-5) while Alpha diversity indices have been depicted in Table-6. The data generated reflects that with decrease in temperature and humidity, composition of species changes ;as in case of Myrmicinae the generalist species are replaced by more high altitude specialists like *Myrmica* and *Aphaenogaster.* In case of Formicinae the interpretation resembles Myrmicinae as cold specialist *Formica* increases in abundance. But interestingly, the overall abundance increases from 1000mtrs to 2000mtrs with number of species almost same at both the elevations. At this point of time, it is difficult to conclude that with more increase in altitude, the number of species and abundance would increase, but Bharti (2008) has observed that with increase in altitude in Himalaya, genera like *Myrmica, Lasius, Aphaenogaster* and *Temnothorax* gradually dominate the ant fauna and are represented by maximum number of endemic species, with Myrmicinae most speciose subfamily followed by Formicinae.

Table-1: (Showing data at 1000 mtrs)

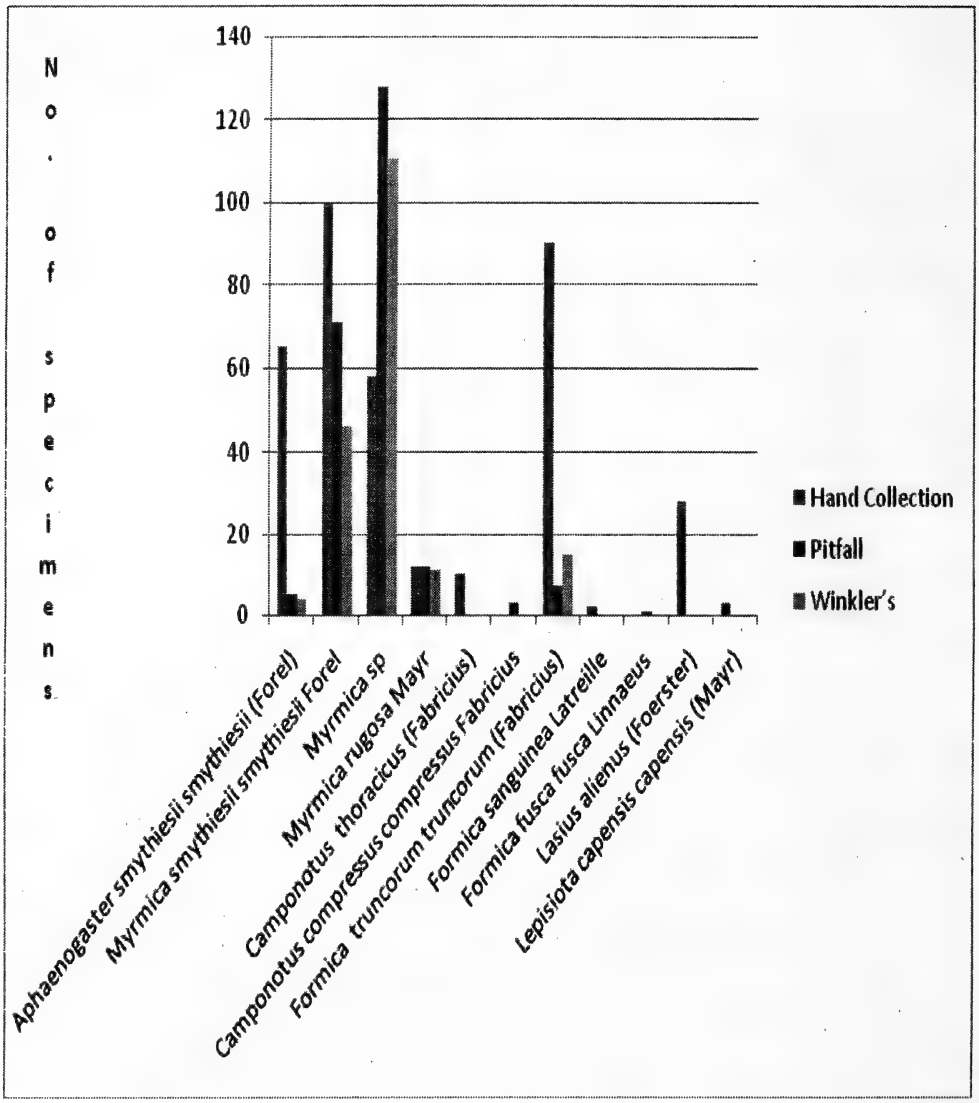
Subfamily	Species	Hand collection	Pitfall trap	Winkler's	Total	Total %age	%age within subfamily
Myrmicinae	*Crematogaster subnuda subnuda* Mayr	20	3	32	55	8.27%	16.75%
	Crematogaster sagei sagei Forel	26	2	33	61	9.17%	18.37%
	Crematogaster rogenhoferi rogenhoferi Mayr	30	12		42	6.36%	12.65%
	Messor himalayanus (Forel)	25	32	15	72	10.82%	21.69%
	Pheidole indica Mayr	37	40	25	102	15.34%	30.72%
Total		**138**	**89**	**105**	**332**	**49.96%**	**100.00%**
Formicinae	*Camponotus compressus compressus* (Fabricius)	26	1	7	34	5.11%	14.85%
	Camponotus thoracicus (Fabricius) [*Camponotus dichrous* Andre]	32	2	18	52	7.82%	22.71%
	Polyrhachis lacteipennis lacteipennis Smith, F.	25		13	38	5.71%	16.59%
	Formica truncorum truncorum (Fabricius) [*Formica truncicola* Nylander]	12	4	24	40	6.06%	17.47%
	Lepisiota capensis capensis (Mayr)	17	19	29	65	9.70%	28.38%
Total		112	26	91	229	34.40%	100%
Dolichoderinae	*Tapinoma melanocephalum melanocephalum (Fabricius)*	15	10	9	34	5.11%	
Ponerinae	*Odontoponera transversa transversa* (Smith, F.)	22	23	25	70	10.53%	
Grand Total		287	148	230	665	100%	



Graph-1: (Showing the no. of specimens per species at 1000mtrs)

Table-2: (Showing data at 2000 mtrs)

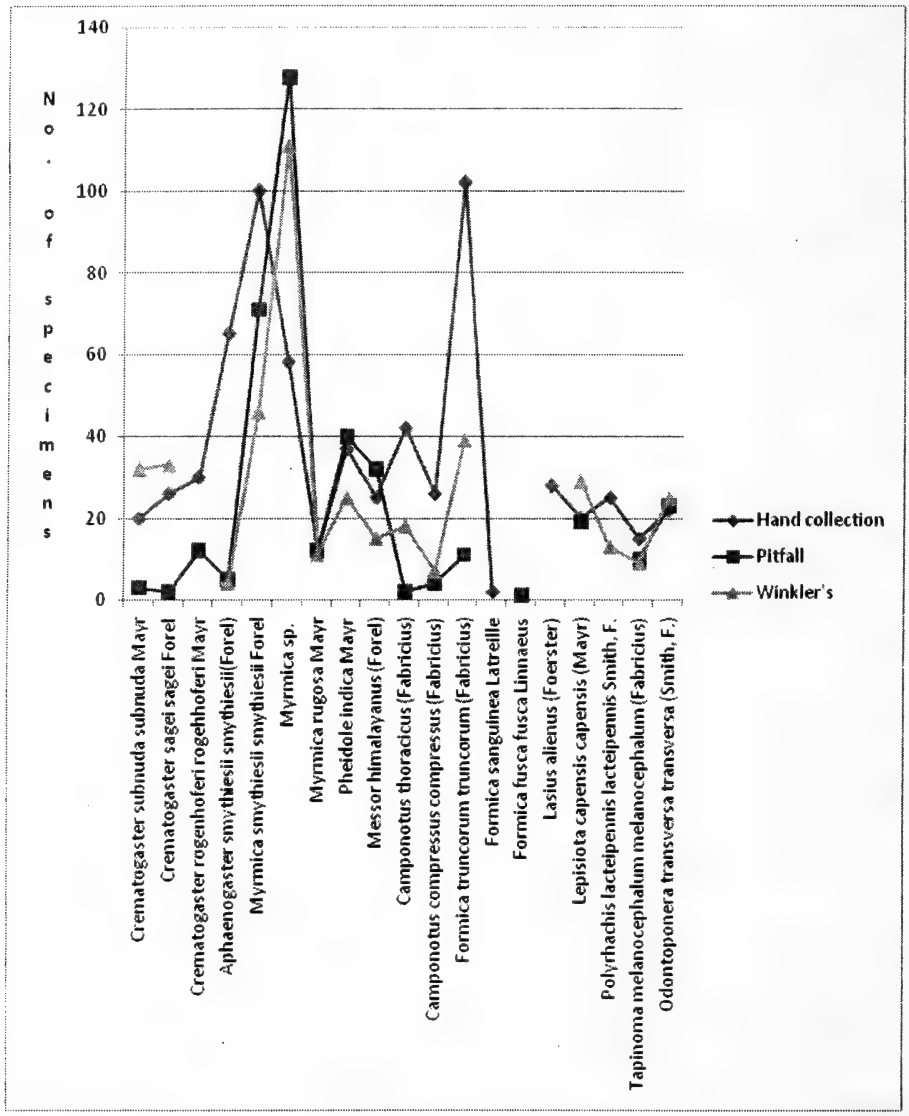
Subfamily	Species	Hand collection	Pitfall trap	Winkler's	Total	Total %age	%age within subfamily
Myrmicinae	*Aphaenogaster smythiesii smythiesii* (Forel)	65	5	4	74	9.48%	11.90%
	Myrmica smythiesii smythiesii Forel	100	71	46	217	27.78%	34.89%
	Myrmica sp.	57	128	111	296	37.90%	47.58%
	Myrmica rugosa Mayr	12	12	11	35	4.48%	5.63%
Total		**234**	**216**	**172**	**622**	**79.64%**	**100.00%**
Formicinae	*Camponotus thoracicus* (Fabricius) [*Camponotus dichrous* Andre]	10			10	1.28%	6.29%
	Camponotus compressus compressus Fabricius		3		3	0.38%	1.89%
	Formica truncorum truncorum (Fabricius) [*Formica truncicola* Nylander]	90	7	15	112	14.39%	70.43%
	Formica sanguinea Latreille	2			2	0.26%	1.26%
	Formica fusca fusca Linnaeus		1		1	0.13%	0.63%
	Lasius alienus (Foerster)	28			28	3.54%	17.61%
	Lepisiota capensis capensis (Mayr)	3			3	0.38%	1.89%
Total		133	11	15	159	20.36%	100%
Grand Total		367	227	187	781	100%	



Graph-2: (Showing the no. of specimens per species collected from 2000mtrs)

Table-3: (Showing combined data at both elevations)

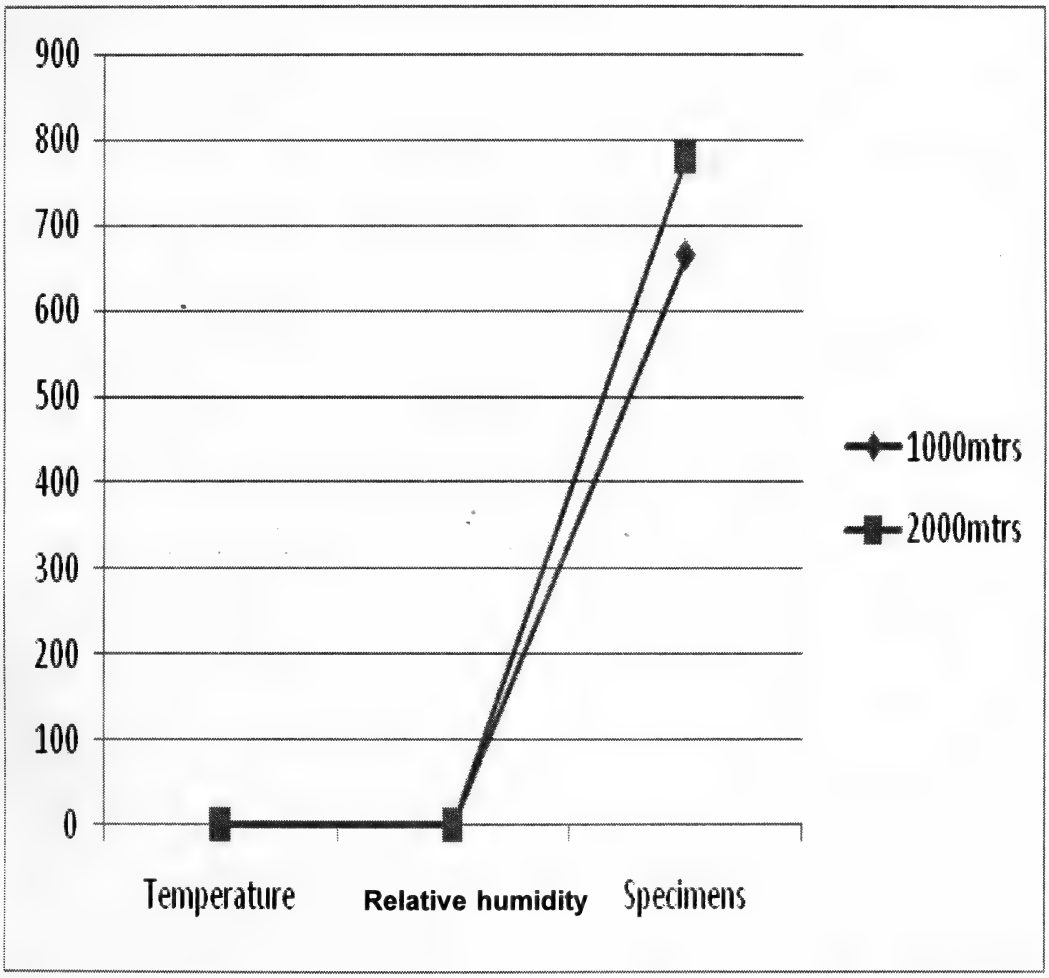
Subfamily	Genus	Species	Hand collection	Pitfall trap	Winkler's	Total	Total %age
Myrmicinae	*Crematogaster*	*Crematogaster subnuda subnuda* Mayr	20	3	32	55	3.80%
		Crematogaster sagei sagei Forel	26	2	33	61	4.22%
(5 Genera, 9 species)		*Crematogaster rogenhoferi rogenhoferi* Mayr	30	12		42	2.90%
	Aphaenogaster	*Aphaenogaster smythiesii smythiesii* (Forel)	65	5	4	74	5.11%
	Myrmica	*Myrmica smythiesii smythiesii* Forel	100	71	46	217	15.00%
		Myrmica sp.	57	128	111	296	20.52%
		Myrmica rugosa Mayr	12	12	11	35	2.42%
	Pheidole	*Pheidole indica* Mayr	37	40	25	102	7.05%
	Messor	*Messor himalayanus* (Forel)	25	32	15	72	4.98%
Total			**372**	**305**	**277**	**954**	**66.00%**
Formicinae	*Camponotus*	*Camponotus thoracicus* (Fabricius) [*Camponotus dichrous* Andre]	42	2	18	62	4.28%
		Camponotus compressus compressus (Fabricius)	26	4	7	37	2.56%
	Formica	*Formica truncorum truncorum* (Fabricius) [*Formica truncicola* Nylander]	102	11	39	152	10.50%
(5 Genera,8 species)		*Formica sanguinea* Latreille	2			2	0.14%
		Formica fusca fusca Linnaeus		1		1	0.07%
	Lasius	*Lasius alienus* (Foerster)	28			28	1.94%
	Lepisiota	*Lepisiota capensis capensis* (Mayr)	20	19	29	68	4.70%
	Polyrhachis	*Polyrhachis lacteipennis lacteipennis* Smith, F.	25		13	38	2.63%
Total			**245**	**373**	**106**	**388**	**26.81%**
Dolichoderinae	*Tapinoma*	*Tapinoma melanocephalum melanocephalum* (Fabricius)	15	10	9	34	2.35%
Ponerinae	*Odontoponera*	*Odontoponera transversa transversa* (Smith, F.)	22	23	25	70	4.84%
Grand Total			**654**	**375**	**417**	**1446**	**100%**



Graph-3: (Showing Abundance and effectiveness of collection methods at both the elevationas combined)

Table-4: (Showing relative humidity and average temperature at both elevations)

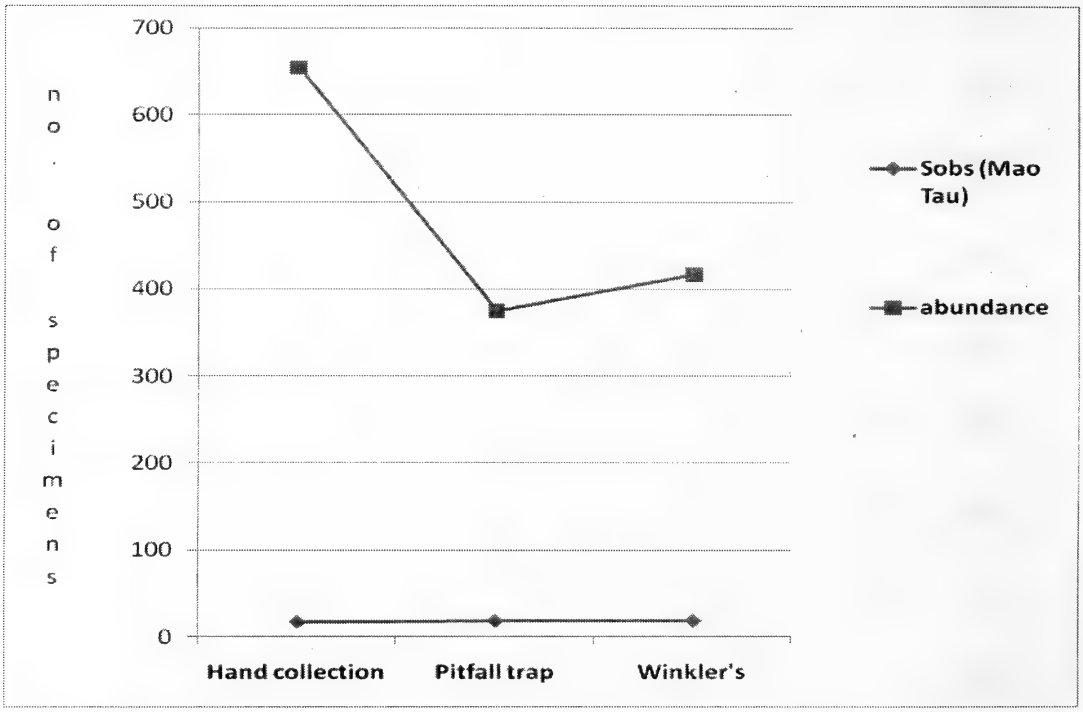
Altitude	1000 mtrs	2000 mtrs
Temperature	22°C	13.7°C
Relative Humidity	52%	45%
Specimens	665	781



Graph-4: (Showing correlation of temperature and humidity with species abundance)

Table-5: (Showing the species richness by different indices)

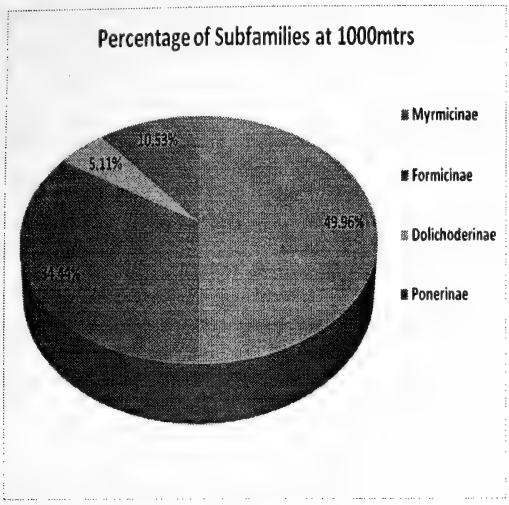
Samples	Individuals (computed)	Sobs (Mao Tau)	Sobs Mean (runs)	ACE Mean	ICE Mean	Chao 1 Mean	Chao 2 Mean	Jack 1 Mean	Jack 2 Mean	Bootstrap Mean
Hand collection	406.67	17.67	19	19.6	133	19	133	19	0	19
Pitfall trap	813.33	19	19	19	19.56	19	19	19.5	19.5	19.25
Winkler's	1220	19	19	19	19	19	19	19	18.33	19.15



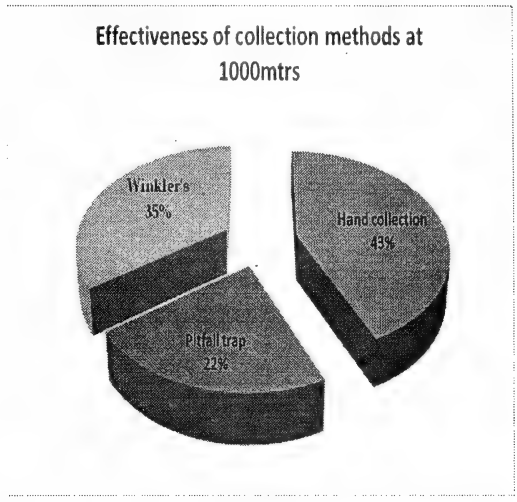
Graph -5: (Showing the species abundance and effectiveness of sampling method by Sobs (Mao Tao))

Table-6: (Showing Alpha diversity indices)

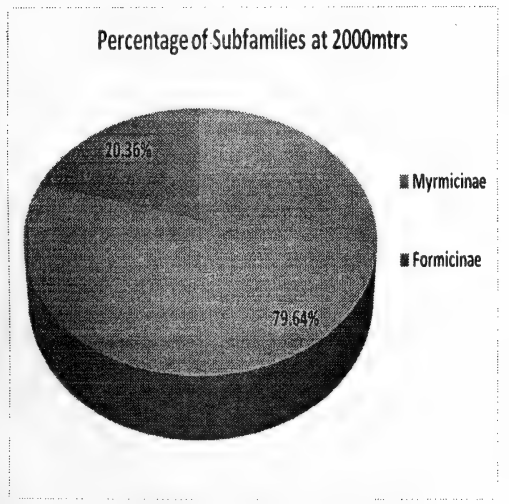
Samples	Winkler's	Alpha SD (analytical)	Shannon Mean	Simpson Mean
Hand collection	5.26	0.7	2.77	15.75
Pitfall trap	3.45	0.34	2.79	14.25
Winkler's	3.19	0.3	2.71	12.25



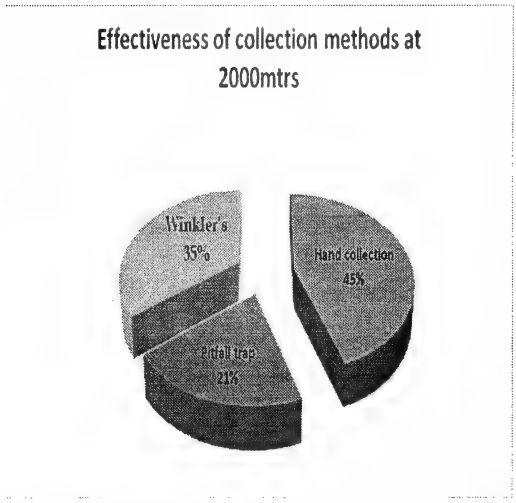
Pi chart-I



Pi chart-III



Pi chart-II



Pi chart-IV

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Influence of *Varroa jacobsoni* Oudemans Parasatization on the Protein profile and RNA content of *Apis mellifera* L. worker brood

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Abstract

Protein profile and RNA content of *Varroa jacobsoni* Oudemans infested *Apis mellifera* L. worker brood was studied and compared with non- infested brood. It was observed that total protein concentration in whole body extract was higher in non-infested pupa. The number of protein fractions obtained on SDS-PAGE were however more in the pupa infested with mite. The concentration of RNA was higher in healthy pupa as compared to infested one suggesting reduced transcription of genes encoding peptides and proteins.

Keywords: *Apis mellifera, Varroa jacobsoni, Protein profile, Worker brood.*

Introduction

The ectoparasitic mite *Varroa jacobsoni* Oudemans is today regarded as the most serious malady of honey bee colonies. *V. jacobsoni* was first detected by Dutch acarologist Jacobson on the Eastern honey bee, *Apis cerana* (Oudemans ,1904). *A. cerana* has been recognized as the mite's native host. Delfinado (1963) collected specimens of *V. jacobsoni* from *A. mellifera* brood in Hong Kong in 1962. This was the first report of the utilization of *A.mellifera* as an alternative host by *V.jacobsoni*. *Varroa* feeds on the haemolymph of adult bee, larvae and pupae. Haemolymph is probably lost at a variable rate in each bee, depending upon the time of feeding by parent mites and their progeny in relation to the bees development.

Cacho *et al.* (1996) studied the effect of *Varroa* parasitization on the glycoprotein expression of *A. mellifera* spermatozoa. They (Cacho *et al.*, 1996) compared the lectin binding patterns of the spermatozoa of non- parasitized and parasitized bees and observed that presence of *Varroa* altered the expression of glycoprotein on the spermatozoa.Yang and Cox-Foster (2005) reported that infestation by *Varroa* led to reduction in the transcription of genes encoding antimicrobial peptides and immunity- related enzymes causing immunosuppression in the infested bees. The present investigation were undertaken to study influence of parasitization on the protein profile of the infested worker pupa and to compare the RNA content of the infested and healthy pupae

in order to understand the pathophysiological changes exhibited by the infested bees.

Materials and Methods

Samples of *A.mellifera* worker brood were drawn from the colonies maintained by Department of Zoology, Panjab University Chandigarh. A random sample of 10 infested and 10 non- infested worker pupae (brown eye stage) was taken for each test after brushing off the bees from the comb.Each pupa was taken in 1ml of PBS and electrically homogenized. Estimation of total protein in the infested and non-infested sample was done following Lowry's standard procedure (Lowry *et al.*,1951). The protein types and protein fractions were determined by standard SDS-PAGE technique (Laemmli,1970). For the estimation of RNA the procedure of Schneider (1945) was utilized.

Results

Protein concentration was found to be higher (0.260 $\pm$ 0.0030mg/ml) in whole body extract of healthy pupa as compared to 0.176 $\pm$ 0.002mg/ml in the pupa infested with mite (Results are mean + SD. Values are significantly different from control at $p<0.0001$). A total of ten bands corresponding to different protein fractions were observed in worker brood not infested with mites. The molecular weights of these proteins ranged between 38.0 to 97.6kDa while the distance traveled was in the range of 0.8 and 5.6 cm. In case of infested sample on the other hand, twelve bands were observed (Table1).

Table-1: Protein types in non- infested and infested worker pupa of *A. mellifera* as observed by SDS-PAGE .

S.No.	Standards		*Apis mellifera* late pupa Non-infested		*Apis mellifera* late pupa Infested	
	Molecular weights (kDa)	Distance travelled (Cm)	Molecular weights (kDa)	Distance travelled (Cm)	Molecular weights (kDa)	Distance travelled (Cm)
1.	97.4	0.8	97.6	0.7	97.6	0.7
2.	66.0	2.5	90.2	1.1	90.2	1.1
3.	43.0	4.5	88.1	1.3	88.1	1.3
4.	29.0	5.6	79.4	1.5	79.4	1.5
5.	-	-	70.7	1.8	70.7	1.8
6.	-	-	67.5	2.2	67.5	2.2
7.	-	-	-	-	65.2	2.7
8.	-	-	59.3	2.8	59.3	2.8
9.	-	-	53.6	3.3	53.6	3.3
10.	-	-	48.1	3.8	48.1	3.8
11.	-	-	-	-	40.7	4.6
12.	-	-	38.0	4.8	38.0	4.8

Comparison of electropherogram of infested and non-infested sample revealed that the protein types with molecular weights of 65.2 and 40.7kDa were absent in case of non-infested brood of *A. mellifera*. The proteins fraction of 97.6, 90.2, 88.1, 79.4, 70.7,67.5, 59.3, 48.1 and 38.0kDa were common in both cases.

The RNA concentration in whole body extract of non-infested late worker pupa (brown eye) was found to be 0.017 ± 0.002mg/ml as compared to 0.008 ± 0.003mg/ml in late worker pupa infested with mite (Result are mean ± SD .Values are significantly different from control at $p<0.05$) .

Discussion

Physiological interference due to mite infestation was reported by Ball (1997) who observed depletion in host hemolymph as a consequence of feeding by the mite.The reduction in total proteins (estimated by Lowry's method) of the infested pupal extract observed during the present study could be a consequence of hemolymph depletion .Howevèr the protein types established by SDS-PAGE were two more in the infested than in the non-infested brood. The additional proteins are perhaps contributed by mite feeding or could be produced by the host in response to the presence of the parasite. The later however seems unlikely because the RNA content of the parasitized pupa was observed to fall suggesting reduced transcription of genes encoding for polypeptides. Bees parasitized by *V.jacobsoni* have been reported to the immunosupressed due to reduction in the synthesis of immunity related enzymes as reported by Yang and Cox-Foster (2005).

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Diversity of Odonata in District Poonch and Sudhnoti of Kashmir Valley – Pakistan, with a new record for the country

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Abstract

Detailed surveys were carried out from two districts viz. Poonch and Sudhnoti of Kashmir Valley during summer seasons of 2007 and 2008 to make an updated record of inhabiting Odonata. Ten localities were selected on the basis of variables keeping in view the habitat requirements of Odonata. The present study provides a record of 16 Anisopterous species spreading to 9 genera and 29 Zygopterous species spreading to 14 genera. Among these *Lestes patricia* is a new record for the country. The distribution, synonymy, richness and abundance of the species are discussed in this paper. The Kashmir Valley is rich in insect biodiversity, the odonate fauna of this valley needs to be further explored.

Keywords: *Odonata, Poonch, Sudhnoti, Kashmir Valley.*

Introduction

The Kashmir valley is the liberated part of State of Jammu and Kashmir. It lies between longitude 73° – 75° and latitude of 33° – 36° and is spread over an area of 13,297 Km^2. The topography is mostly hilly and mountainous alongwith valleys and plains. Climate is highland subtropical. Districts Poonch & Sudhnoti are mostly mountainous and lie at the foot hills of Himalayas. District Poonch has an area of 855 Km^2, however Sudhnoti is spread over 569 Km^2 (IPAK, 2008).

Odonates are important predator of serious pests in terrestrial and aquatic ecosystem. They consume noxious flies, mosquitoes, aphids, jassids, bollworms (Fraser, 1933) and black flies (Subramanian, 2005). They are good indicator of the condition of aquatic and terrestrial ecosystems (Brown

1991). Odonata themselves may also be significant prey items of birds, fishes and some other invertebrates such as spiders and predatory coleopterans (Kapoor, 1985).

Previously, Laidlaw (1915) and Fraser (1933-34) reported odonata from subcontinent, Kanth (1985), Khaliq (1990), Khaliq *et al.* (1994), Ali (1995), Yousuf *et al.* (2000) studied Odonata of different districts of Kashmir valley. Khaliq and Siddique (1995), Khaliq *et al.* (1990) and Khaliq *et al.* (1995) studied the odonates of Poonch district of Kashmir Valley. However, during the year 1995, Poonch was divided into two individual parts i.e Poonch and Sudhnoti. No significant work has been done on the odonate fauna of the area after partition. It is important to know the existing fauna of both the districts individually. The area has greater biodiversity and is rich in water resources. In view to this, it was planned to extensively explore the Odonata of Poonch and Sudhnoti to make an updated and authentic record.

Materials and Methods

Five sites (Fig.1.) were selected each from the district Poonch and Sudhnoti of Kashmir Valley. The sites were selected on the basis of variables which may be important according to Clark and Samway (1996) in influencing the distribution of adult Odonata. Among Poonch [Datot (L1), Hajira (L2), Rawalakot (L3), Banjosa (L4), Abbaspur (L5)] and Sudhnoti [Tattapani (L6), Palandri (L7), Azad Pattan (L8), Goraha (L9) and Tararkhal (L10)] were surveyed during the summer season of two consecutive years (2006 & 2007).

Methods of sampling were based on Wahizatul-Afzan *et. al.* (2006) with minor additions. The collected specimens were brought to Odonata section at National Insect Museum, Islamabad – Pakistan. The preservation methodology was based on Borror & White (1970). All the collected specimens were identified by running them through taxonomic keys. The taxonomic literature by Fraser (1933 - 1934), Khaliq (1990) and Subramanian (2005) were followed. Voucher specimens have been deposited in National Insect Museum , NARC – Islamabad.

Results

The study provides a record of 45 species of Odonata including, 16 Anisopterous species identified under 9 genera and 3 families (Table 1) and 29 Zygopterous species identified under 14 genera and 8 families (Table 2). Among Anisoptera, *(Trithemis pallidinervis)* is first time recorded from Poonch district. However in Zygoptera (*Lestes patricia and L. viridulus*) are first time reported from both the districts. Among these, *Lestes patricia* (Zygoptera) is a new record for country's Zygopterous fauna. Richness of species (Fig. 2) was observed, which shows that 45 species of Odonata were recorded from district Poonch. However from Sudhnoti, 37 species were collected. Abundance of species (Fig. 3) was also taken into consideration, showing *Orthetrum triangulare triangulare* (Anisoptera), *Agriocnemis pygmaea* (Zygoptera) as one of the most common, abundant and widely distributed species of the area, recorded from seven and nine localities respectively. Amongst Anisoptera (*Anax nigrolineatus)* and Zygoptera (*Ceriagrion cerinorubellum*) appear to be less common or even rare and were recorded from single locality only. Due to lot of topographic diversity and aquatic habitats, further surveys can unhide more species of Odonata from these areas.

Table 1: Valid names, Synonyms and distributional details for the collected Anisopterous species.

Family	Species	Synonyms	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Libellulidae Rambur, 1842	*Orthetrum triangulare triangulare* Selys, 1878	*Libellula triangularis* Selys, 1878 *Libellula delesserti* Selys, 1878 *Libellula melanica* Selys, 1883 *Pseudothemis nigrifrons* Matsumura, 1898 *Orthetrum ganeshii* Mehrotra, 1961 *Orthetrum chandrabali* Mehrotra, 1961	-	+	+	-	+	+	+	-	+	+
	O. pruinosum neglectum Burmeister, 1839	*Libellula pruinosa* Burmeister, 1839 *Libellula neglecta* Rambur, 1842 *Libellula petalura* Brauer, 1865 *Libella clelia* Selys, 1878 *Orthetrum schneideri* Forster, 1903	-	+	+	+	+	-	+	-	-	-
	O. sabina Drury, 1770	*Libellula sabina* Drury, 1770 *Libellula gibba* Fabricius, 1798 *Libellula leptura* Burmeister, 1839 *Libellula ampullacea* Schneider, 1845 *Lepthemis divisa* Selys, 1878 *Orthetrum nigrescens* Bartenev, 1929 *Orthetrum viduatum* Lieftinck, 1942	+	+	-	-	-	-	+	-	-	-
	O. glaucum Brauer, 1865	*Libellula glaucum* Brauer, 1865 *Orthetrum gangi* Sahni, 1965	-	+	+	-	+	+	-	-	-	-
	Sympetrum meridionale Selys, 1841	*Libellula basilaris* Palisot de Beauvois, 1805 *Tramea burmeisteri* Kirby, 1889	-	+	+	-	+	-	-	-	-	-
	Tramea basilaris Palisot de Beauvois, 1805	*Acisoma ascalaphoides* Rambur, 1842 *Acisoma inflata* Selys, 1882 *Acisoma variegatum* Kirby, 1898	-	+	+	+	+	-	+	-	-	-
	Palpopleura sexmaculata sexmaculata Fabricius, 1787	*Libellula sexmaculata* Fabricius, 1787	-	+	+	-	-	-	-	-	-	-
	Trithemis festiva Rambur, 1842	*Libellula festiva* Rambur, 1842 *Libellula infernalis* Brauer, 1865 *Trithemis proserpina* Selys, 1878	-	+	-	-	-	+	-	-	-	-
	**T. pallidinervis* Kirby, 1889	*Sympetrum pallidinervis* Kirby, 1889 Trithemis dryas Selys, 1891	-	+	-	-	-	-	+	-	-	-
	Crocothemis servilia Drury, 1773	*Libellula servilia* Drury, 1773 *Libellula ferruginea* Fabricius, 1793 *Libellula soror* Rambur, 1842 *Crocothemis* reticulata Kirby, 1886	+	-	-	+	-	-	+	+	-	-
	C. erythraea Brulle, 1832	*Libellula erythraea* Brullé, 1832 *Libellula rubra* de Villers, 1789 (nec Müller, 1764) *Libellula ferruginea* Vander Linden, 1825 (nec Fabricius, 1775) *Libellula coccinea* Charpentier, 1840 *Libellula inquinata* Rambur, 1842 *Crocothemis chaldaeora* Morton, 1920	+	-	-	+	-	-	+	-	-	-
	Pantala flavescens Fabricius, 1798	*Libellula flavescens* Fabricius, 1798 *Libellula viridula* Palisot de Beauvois, 1805 *Libellula analis* Burmeister, 1839 *Libellula terminalis* Burmeister, 1839 *Sympetrum tandicola* Singh, 1955	+	-	+	+	-	+	-	-	-	-
Gomphidae Rambur, 1842	*Mesogomphus lineatus* Viswanathan & Varadaraj, 1985	*Gomphus lineatus* Selys, 1850 *Onychogomphus lineatus* Selys, 1854 *Lindenia lineata* Kirby, 1890 *Mesogomphus lineatus* Fraser, 1924	-	-	-	-	+	-	-	-	-	-

Table-1: Continued

Family	Species	Synonyms	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Aeshmidae Rambur, 1842	*Anax immaculifrons* Rambur, 1842		-	-	+	-	-	-	-	+	-	-
	A. nigrolineatus Fraser, 1935	*Anax bacchus* Martin 1908 *Anax guttatus* 1921 *Anax fumosus* 1923 *Anax nigrolineatus* 1935	-	-	+	-	-	-	-	+	-	-
	A. parthenope Selys, 1839	*Aeschna parthenope* Selys, 1839 *Anax julius* Brauer, 1865 *Anax bacchus* Hagen, 1867 *Anax major* Gotz, 1923 *Anax parisinus* Rambur, 1842 *Anax geyri* Buchholz, 1955 *Anax jordansi* Buchholz, 1955	-	-	+	-	-	-	-	+	-	-

*New Record for the district

Table-2: Vaild names, Synonyms and distributional details for the collected Zygopterous species.

Family	Species	Synonyms	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Lestidae Calvert, 1901	*Lestes thoracicus* Laidlaw, 1920	**L. viridulus* Rambur, 1842	-	-	+	+	+	-	+	-	-	-
	L. umbrinus Selys, 1891	*Orolestes motis* Baijal and Agarwal, 1955	-	+	-	-	-	-	+	-	-	-
	**L. patricia*		-	-	-	+	+	-	-	-	-	+
	**L. viridulus* Rambur, 1842	*Lestes viridulus* Kirby, 1890	-	-	-	-	-	+	-	+	-	-
Synlestidae Tillyard,	*Megalestes major* Selys, 1962		-	+	+	-	-	-	+	+	-	-
Chlorocyphidae Cowley, 1937	*Rhinocypha unimaculata* Selys, 1853	*Paracypha unimaculata*, Fraser 1949 *Libellago unimaculata* Walker, 1853	-	+	+	+	-	-	-	+	-	-
	R. quadrimaculata Selys, 1853	*Aristocypha quadrimaculta* Laidlaw, 1950 *Libellago quadrimaculata* Walker, 1853	+	+	-	-	-	+	-	-	-	+
	R. trifasciata Selys, 1853	*Libellago trifasciata* Walker, 1853 *Aristocypha trifasciata* Laidlaw, 1950	+	-	-	+	-	-	-	-	+	+
	R. hilaryae Fraser, 1927		-	+	-	+	+	-	-	-	-	-
	R. immaculata Selys, 1871		-	+	+	-	-	-	-	+	-	-
Coenagrionidae Kirby, 1890	*Pseudagrion rubriceps* Selys, 1876	*Archibasis ceylonica* Kirby, 1891 *Pseudagrion flavifrons* Needham and Gyger, 1939	-	+	+	-	+	+	+	-	-	+
	P. laidlawi Fraser, 1922		-	+	-	-	-	-	-	+	+	-

Table-2: Continued

Family	Species	Synonyms	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
	P. decorum Rambur, 1842	*Agrion decorum* Rambur,1842	-	+	+	-	-	-	-	+	-	-
	P. hypermelas Selys, 1876		+	+	+	+	-	-	-	+	-	+
	P. spencei Fraser, 1922		-	-	-	+	-	-	-	-	+	+
	Ceriagrion cerinorubellum Brauer, 1865	*Agrion cerinorubellum* Brauer, 1865 *Pyrrhosona cerinorubellum* Brauer, 1865	-	-	+	-	-	-	-	-	-	-
	C. coromandelianum Fabricius, 1798	*Agrion coromandelianum* Fabricius, 1798 *Agrion cerinum* Rambur, 1842	+	+	+	-	+	+	+	+	+	-
	Aciagrion hisopa Selys, 1876	*Pseudagrion hisopa* Selys, 1876 *Aciagrion aciculare* Lieftink, 1929	+	-	-	-	+	-	-	-	-	+
	Ischnura forcipata Morton, 1907	*Ischnura musa* Bartenev, 1913 *Ischnura gangetica* Laidlaw, 1913 *Agriocnemis nainitalensis* Sahni, 1965 *Coenagrion needhami* Navas, 1933	+	+	+	-	+	+	+	+	-	-
	I. elegans Vander Linden, 1820	*Agrion elegans* Vander Linden, 1820 *Ischnura lamellata* Kolbe, 1885	-	-	+	-	+	+	-	-	+	-
	I. aurora Brauer, 1865	*Agrion aurora* Brauer, 1865 *Agrion delicatum* Hagen, 1876 *Ischnura delicata* Hagen, 1876 *Micronympha aurora* Kirby, 1890 *Nanosura aurora* kennedy, 1920 *Ishnura bhimtalensis* Sahni, 1965	+	+	+	-	+	+	+	-	-	+
	I. senegalensis Rambur, 1842	*Agrion senegalensis* Rmbur, 1842 *Enallagma brevispina* Selys, 1876	-	+	-	-	+	-	-	-	+	+
	Agriocnemis pygmaea Rambur, 1842	*Agrion pygmaeum* Rambur, 1842 *Agriocnemis australis* Selys, 1877 *Agriocnemis velaris* Selys, 1882 *Agrion kagiensis* Matsumura, 1911 *Agriocnemis hyacinthus* Tillyard, 1913	+	-	+	+	+	+	+	+	+	+
	Rhodischnura nursei Morton, 1907	*Ischnura nursei* Morton, 1907	+	+	-	-	-	-	-	+	-	-
	Calicnems eximia Selys, 1863	*Calicnemis atkinsoni* Selys, 1886	-	-	+	+	-	-	-	-	-	-
Platycnemidae Tillyard, 1917	*Copera marginipes* Rambur, 1842	*Platycnemis marginipes* Rambur, 1842 *Platycnemis lacteola* Selys, 1863 *Psilocnemis marginipes* Selys, 1863 *Psilocnemis striatipes* Selys, 1863 *Copera acutimargo* Krug, 1898 *Disparoneura bhatnagri* Sahni, 1965	-	+	+	-	-	-	-	-	-	-
Protoneuridae Tillyard, 1917	*Elattoneura nigerrima* Laidlaw, 1935	*Disparoneura nigerrima* Laidlaw, 1917	-	-	+	-	-	-	-	+	-	+

Table-2: Continued

Family	Species	Synonyms	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Calopterygidae Selys, 1850	*Neurobasis chinensis* Linnaeus, 1758	*Libellula chinensis* Linnaeus, 1758 *Agrion nobilitata* Fabricius, 1776 *Agrion chinensis* Guerin, 1829 *Calopteryx disparilis* Rambur,1842 *Calopteryx chinensis* Rambur, 1842 *Calopteryx sinensis* Walker, 1853 *Neurobasis c. chinensis* Fraser, 1934	-	+	+	-	+	-	+	-	-	-
Euphaeidae Selys, 1853	*Baydera indica* Selys, 1853	*Epallage indica* Selys,1853	+	+	-	-	-	+	+	-	-	-

*** New record for districts**
° New record for the country

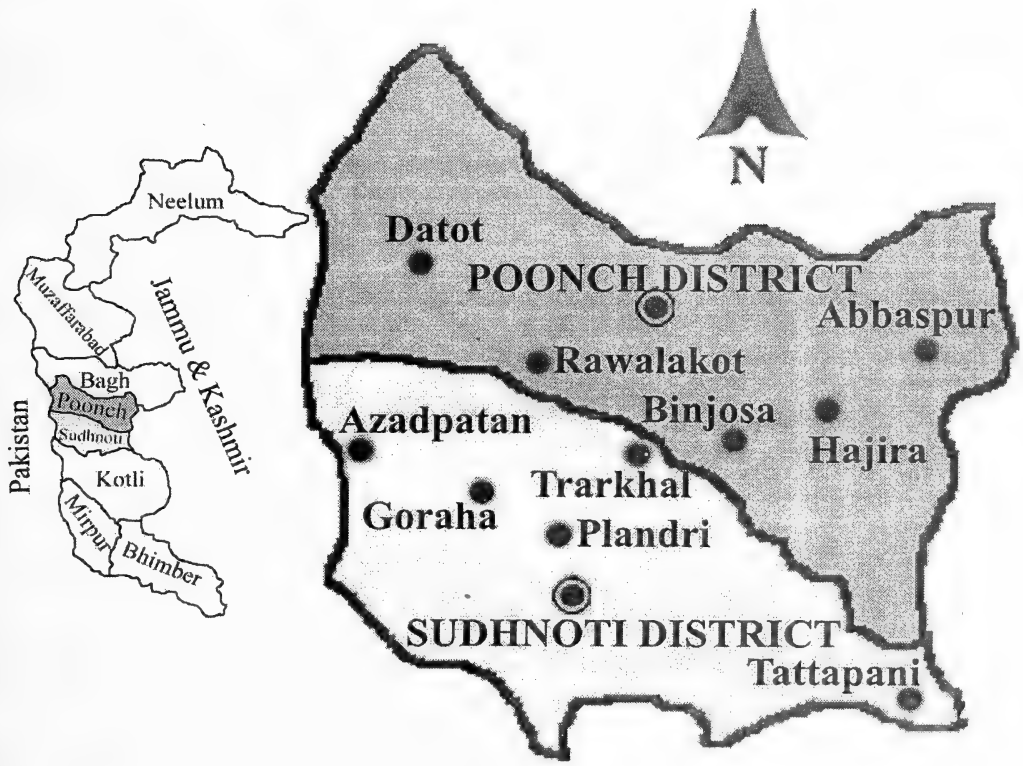

Fig.1: Map of District Poonch and Sudhnoti, Kashmir Valley.

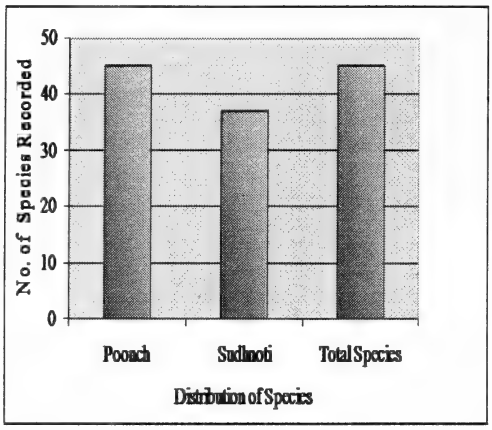

Fig.2: Richness of Species Observed in Poonch & Sudhnoti Districts of Kashmir Valley.

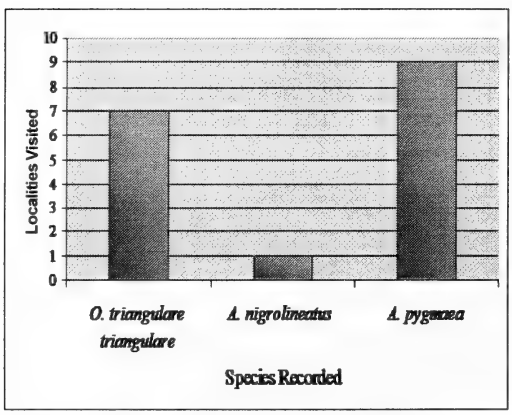

Fig.3: Abundance of Species observed in Poonch & Sudhnoti Districts of Kashmir Valley.

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Seasonal Patterns of Ants (Hymenoptera: Formicidae) in Punjab Shivalik

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Abstract

Seasonal patterns of Ants were analysed in five seasons in Punjab Shivalik range of North-West Himalaya. Various collection methods like Pitfall traps, Winkler's, Fish bait and Hand picking were used. 40 species belonging to 8 subfamilies have been observed for seasonal patterns and subfamily Myrmicinae followed by Formicinae were found to be dominant. Temperature and Relative humidity have been correlated with seasonal patterns.

Keywords: *Seasonal patterns, Ants, Shivalik, Disturbed ecosystem, Anthropogenic activity, North-West Himalaya.*

Introduction

Various studies have been carried on community composition on ants, their habitats, foraging behaviour and other ecological aspects. However, studies dealing specifically with seasonal patterns of ants are comparatively few. To start with, Davidson (1977) studied foraging ecology and community organisation in desert seed-eating ants. Levings (1983) studied the seasonal, annual and site variations in the ground ant communities of a tropical forest. Zorilla *et al.* (1986), while studying structural characteristics of an ant community during succession observed that ant communities in the pastures present a sequence of successional variation. Andersen (1986) worked on diversity, seasonality in ant community organisation of ants at woodland site in South-eastern Australia.

Fellers (1989) observed daily seasonal activity in woodland ants. Johnson (1992) monitored seasonal structure of ant communities. Belshaw and Bolton (1993) studied the effect of forest disturbance on leaf litter ant fauna and concluded that most primary forest leaf litter ant species continue to survive in parts of the agricultural landscape which has largely replaced their original habitat. Byrne (1994) observed the correlation between availability of nests and soil type. Fellowes (1996) discussed community composition of Hong Kong ants with respect to spatial and seasonal patterns. Smith *et al.* (1997) studied variation in structure and function of ant communities during stress and disturbance. Rico-Gray *et al.* (1998) observed richness and seasonal variation of ant-plant association mediated by plant derived resources in Mexico. Whitford (1999) studied seasonal and diurnal activity patterns in ant communities in vegetation transition region of New Mexico.

Retana and Cerda (2000) observed the patterns of diversity and composition of Mediterranean ground ant communities. Vanderwoude *et al.* (2000) observed long term ant communities responses to selective harvesting of timber from spotted forest in Southeast Queensland.

Clough (2004) worked on the factors influencing ant assemblages and ant community composition in a subtropical sub-urban environment and concluded that ant communities in sub-urban environments respond to disturbance in a similar manner to ant communities in tropical forest and rainforests. Touyama and Kameyama (2004) worked on foraging behavior with relation to temperature. Coelho and Ribeiro (2006) expressed the response of ant species assemblage to contrasting types of forests in Brazil. Recently, Basu (2008) analysed seasonal and spatial patterns in ground foraging ants and observed that all ant species showed marked seasonality. Suwabe *et al.* (2008) assessed difference in seasonal activity pattern between non-native and native ants in subtropical forest of Okinawa Island, Japan. With this state of affairs, the present study was aimed to generate knowledge about seasonal patterns of ant species in Punjab Shivalik.

The area chosen for study is a disturbed ecosystem, subject to anthropogenic activities. The Punjab Shivalik extends between river Ravi in north and river Ghaggar in south; (between latitude 30°34' 10.82" and 32°33' 02.95"N; longitude 74°50' 30.30" and 76° 57.26"E). The Punjab Shivalik is about 280 km long with variable width of 5 km to 12 km. The Shivalik experiences koeppen's cwg category climate (Mittal *et al.*, 2000) based on annual and monthly means of temperature and rainfall. This is characterized by humid, tropical and dry winter, extreme seasonal temperatures, long dry-short wet season and potential evapotranspiration exceeding precipitation, which varies from 800 to 1200 mm annually.

The Punjab Shivalik falls in the sub-moist to humid and less hot region. The temperature in the area varies from about 2°C in winters to a maximum of about 42°C in summers, and the annual rainfall varies between 400 to 600 mm (Tiwana and Jerath, 2006). Champion and Seth (1968) categorised the forests of Punjab Shivalik into following types: 1. Northern dry mixed deciduous forests, 2. Chir-Pine Forests, 3. Dry deciduous scrub forests, 4. Khair and *Dalbergia* sissu forests, 5. Dry Bambo brakes, 6. Subtropical *Euphorbia* scrub. On the basis of texture, climate, topography and denudational process, the soil of Punjab Shivalik is divided into following types: 1. Grey-Brown Podgolic and Fores soil, 2. Kandi soil.

Materials and Methods

For collection of ants different sites (Talwara, Hajipur, Chohal, Ropar, Pathankot, Jugial) falling in Punjab Shivalik were visited. The selected sites were visited frequently/ repeatedly so as to cover different seasons of the year and five seasons are recognised in the state of Punjab (Mavi and Tiwana, 1993);

Summer season	:	Mid April to end of June
Rainy season	:	Early July to September
Autumn season	:	September to end of November
Winter season	:	Early December to end of February
Spring season	:	March to Mid April

For collection of ants following methods were used: Pitfall traps were placed, made up of test tubes (with an 18mm internal diameter and 150mm long) partly filled to a depth of about 50mm with soapy water and 5% ethylene glycol solution. Leaf litter samples were sifted in a

1 m × 1 m quadrant, every 5 meter along the transect using a litter sifter through a wire sieve with square holes of 1 cm × 1 cm and placed in mini Winkler's sac (Fisher, 2004). Ants were then extracted after 48 hours. Ants were also collected by hand picking method i.e. searching logs, stumps, dead and live branches, twigs, low vegetation, termite mounds and under stones.

To increase the effectiveness of this study sampling sites were chosen interior into forest. Temperature and Relative humidity of the above mentioned areas were recorded during different seasons of the year. Collected specimens were preserved in 70% alcohol to prevent degradation. The collected specimens were mounted on triangles, as per standard procedure in ant taxonomy. Dry specimens bearing all relevant data are kept in wooden boxes. For identification, Bolton (1994) and Bingham (1903) were followed and the identified material was compared with reference collection housed in the laboratory.

Table-1: (List of species collected during Summer Season)

Subfamilies	Genus	Name of Species
Myrmicinae	*Pheidole*	*Pheidole latinoda angustior* Forel *Pheidole indica* Mayr *Pheidole spathifera aspatha* Forel
	Meranoplus	*Meranoplus bicolor* (Guerin-Meneville)
	Myrmicaria	*Myrmicaria brunnea brunnea* Saunders
	Tetramorium	*Tetramorium walshi* (Forel)
	Monomorium	*Monomorium criniceps* (Mayr) *Monomorium glabrum* (Andre) *Monomorium destructor* (Jerdon) *Monomorium pharaonis* (Linnaeus) *Monomorium indicum indicum* Forel
	Messor	*Messor instabilis* (Smith, F.)
	Crematogaster	*Crematogaster subnuda subnuda* Mayr
Ponerinae	*Pachycondyla*	*Pachycondyla luteipes luteipes* (Mayr) *Pachycondyla tesseronoda* (Emery) *Pachycondyla bispinosa* Smith, F. *Pachycondyla nigrita nigrita* (Emery) *Pachycondyla rufipes rufipes* (Jerdon)
	Harpegnathos	*Harpegnathos venator venator* (Smith, F.)
	Leptogenys	*Leptogenys diminuta laeviceps* (Smith, F.)
	Odontoponera	*Odontoponera transversa transversa* (Smith, F.)

Table-1: Continued

Subfamilies	Genus	Name of Species
Cerapachyinae	*Cerapachys*	*Cerapachys longitarsus* (Mayr)
Formicinae	*Oecophylla*	*Oecophylla smaragdina smaragdina* (Fabricius)
	Lepisiota	*Lepisiota frauenfeldi integra* (Forel) *Lepisiota opaca pulchella* (Forel)
	Cataglyphis	*Cataglyphis setipes* (Forel)
	Camponotus	*Camponotus parius* Emery *Camponotus compressus compressus* (Fabricius) *Camponotus rufoglaucus rufoglaucus* (Jerdon) *Camponotus sericeus sericeus* (Fabricius)
	Polyrhachis	*Polyrhachis lacteipennis lacteipennis* Smith, F.
	Paratrechina	*Paratrechina longicornis longicornis* (Latreille)
Dolichoderinae	*Bothriomyrmex*	*Bothriomyrmex wroughtonii wroughtonii* Forel
	Tapinoma	*Tapinoma melanocephalum melanocephalum* (Fabricius)
	Chronoxenus	*Chronoxenus myops* (Forel)
Dorylinae	*Dorylus*	*Dorylus orientalis orientalis* Westwood *Dorylus labiatus* Schuckard
Aenictinae	*Aenictus*	*Aenictus pachycerus pachycerus* (Smith, F.)
Pseudomyrmecinae	*Tetraponera*	*Tetraponera allaborans* (Walker) *Tetraponera rufonigra* (Jerdon)

Table-2: (List of species collected during Rainy Season)

Subfamilies	Genus	Name of Species
Myrmicinae	*Pheidole*	*Pheidole latinoda angustior* Forel *Pheidole indica* Mayr *Pheidole spathifera aspatha* Forel
		Meranoplus bicolor (Guerin-Meneville)
	Myrmicaria	*Myrmicaria brunnea brunnea* Saunders
	Tetramorium	*Tetramorium walshi* (Forel)
	Monomorium	*Monomorium criniceps* (Mayr) *Monomorium glabrum* (Andre) *Monomorium destructor* (Jerdon) *Monomorium pharaonis* (Linnaeus) *Monomorium indicum indicum* Forel

Table-2: Continued

Subfamilies	Genus	Name of Species
	Messor	*Messor instabilis* (Smith, F.)
	Crematogaster	*Crematogaster subnuda subnuda* Mayr
Ponerinae	*Pachycondyla*	*Pachycondyla luteipes luteipes* (Mayr) *Pachycondyla tesseronoda* (Emery) *Pachycondyla bispinosa* Smith, F. *Pachycondyla nigrita nigrita* (Emery) *Pachycondyla rufipes rufipes* (Jerdon)
	Harpegnathos	*Harpegnathos venator venator* (Smith, F.)
	Leptogenys	*Leptogenys diminuta laeviceps* (Smith, F.)
	Odontoponera	*Odontoponera transversa transversa* (Smith, F.)
Cerapachyinae	*Cerapachys*	*Cerapachys longitarsus* (Mayr)
Formicinae	*Oecophylla*	*Oecophylla smaragdina smaragdina* (Fabricius)
	Lepisiota	*Lepisiota frauenfeldi integra* (Forel) *Lepisiota opaca pulchella* (Forel)
	Cataglyphis	*Cataglyphis setipes* (Forel)
	Camponotus	*Camponotus parius* Emery *Camponotus compressus compressus* (Fabricius) *Camponotus rufoglaucus rufoglaucus* (Jerdon) *Camponotus sericeus sericeus* (Fabricius)
	Polyrhachis	*Polyrhachis lacteipennis lacteipennis* Smith, F.
	Paratrechina	*Paratrechina longicornis longicornis* (Latreille)
Dolichoderinae	*Bothriomyrmex*	*Bothriomyrmex wroughtonii wroughtonii* Forel
Dorylinae	*Tapinoma*	*Tapinoma melanocephalum melanocephalum* (Fabricius)
	Dorylus	*Dorylus orientalis orientalis* Westwood *Dorylus labiatus* Schuckard
Aenictinae	*Aenictus*	*Aenictus pachycerus pachycerus* (Smith, F.)
Pseudomyrmecinae	*Tetraponera*	*Tetraponera allaborans* (Walker) *Tetraponera rufonigra* (Jerdon)

Table-3: (List of species collected during Autumn Season)

Subfamilies	Genus	Name of Species
Myrmicinae	*Pheidole*	*Pheidole latinoda angustior* Forel *Pheidole indica Mayr* *Pheidole spathifera aspatha* Forel
	Meranoplus	*Meranoplus bicolor* (Guerin-Meneville)
	Myrmicaria	*Myrmicaria brunnea brunnea* Saunders
	Tetramorium	*Tetramorium walshi* (Forel)
	Monomorium	*Monomorium criniceps* (Mayr) *Monomorium glabrum* (Andre) *Monomorium destructor* (Jerdon) *Monomorium pharaonis* (Linnaeus) *Monomorium indicum indicum* Forel
	Messor	*Messor instabilis* (Smith, F.)
	Crematogaster	*Crematogaster subnuda subnuda* Mayr
Ponerinae	*Pachycondyla*	*Pachycondyla luteipes luteipes* (Mayr) *Pachycondyla tesseronoda* (Emery) *Pachycondyla bispinosa* Smith, F. *Pachycondyla nigrita nigrita* (Emery) *Pachycondyla rufipes rufipes* (Jerdon)
	Leptogenys	*Leptogenys diminuta laeviceps* (Smith, F.)
	Odontoponera	*Odontoponera transversa transversa* (Smith, F.)
Formicinae	*Oecophylla*	*Oecophylla smaragdina smaragdina* (Fabricius)
	Lepisiota	*Lepisiota frauenfeldi integra* (Forel) *Lepisiota opaca pulchella* (Forel)
	Cataglyphis	*Cataglyphis setipes* (Forel)
	Camponotus	*Camponotus parius* Emery *Camponotus compressus compressus* (Fabricius) *Camponotus rufoglaucus rufoglaucus* (Jerdon) *Camponotus sericeus sericeus* (Fabricius)
	Polyrhachis	*Polyrhachis lacteipennis lacteipennis* Smith, F.
Dolichoderinae	*Bothriomyrmex*	*Bothriomyrmex wroughtonii wroughtonii* Forel

Table-3: Continued

Subfamilies	Genus	Name of Species
Tapinoma	*Tapinoma*	*Tapinoma melanocephalum melanocephalum* (Fabricius)
Dorylinae	*Dorylus*	*Dorylus orientalis orientalis* Westwood *Dorylus labiatus* Schuckard
Aenictinae	*Aenictus*	*Aenictus pachycerus pachycerus* (Smith, F.)
Pseudomyrmecinae	*Tetraponera*	*Tetraponera allaborans* (Walker) *Tetraponera rufonigra* (Jerdon)

Table-4: (List of species collected during Winter season)

Subfamilies	Genus	Name of Species
Myrmicinae	*Monomorium*	*Monomorium destructor* (Jerdon)
Formicinae	*Camponotus*	*Camponotus compressus compressus* (Fabricius)
	Paratrechina	*Paratrechina longicornis longicornis* (Latreille)
	Lepisiota	*Lepisiota frauenfeldi integra* (Forel)
Dolichoderinae	*Tapinoma*	*Tapinoma melanocephalum melanocephalum* (Fabricius)

Table-5: (List of species collected during Spring Season)

Subfamilies	Genus	Name of Species
Myrmicinae	*Pheidole*	*Pheidole latinoda angustior* Forel *Pheidole indica* Mayr *Pheidole spathifera aspatha* Forel
	Meranoplus	*Meranoplus bicolor* (Guerin-Meneville)
	Myrmicaria	*Myrmicaria brunnea brunnea* Saunders
	Tetramorium	*Tetramorium walshi* (Forel)
	Monomorium	*Monomorium glabrum* (Andre) *Monomorium destructor* (Jerdon) *Monomorium pharaonis* (Linnaeus) *Monomorium indicum indicum* Forel
	Messor	*Messor instabilis* (Smith, F.)
	Crematogaster	*Crematogaster subnuda subnuda* Mayr

Table-5: Continued

Subfamilies	Genus	Name of Species
Ponerinae	*Pachycondyla*	*Pachycondyla luteipes luteipes* (Mayr) *Pachycondyla tesseronoda* (Emery) *Pachycondyla bispinosa* Smith, F. *Pachycondyla nigrita nigrita* (Emery) *Pachycondyla rufipes rufipes* (Jerdon)
	Leptogenys	*Leptogenys diminuta laeviceps* (Smith, F.)
	Odontoponera	*Odontoponera transversa transversa* (Smith, F.)
Formicinae	*Oecophylla*	*Oecophylla smaragdina smaragdina* (Fabricius)
	Lepisiota	*Lepisiota frauenfeldi integra* (Forel) *Lepisiota opaca pulchella* (Forel)
	Cataglyphis	*Cataglyphis setipes* (Forel)
	Camponotus	*Camponotus parius* Emery *Camponotus compressus compressus* (Fabricius) *Camponotus rufoglaucus rufoglaucus* (Jerdon) *Camponotus sericeus sericeus* (Fabricius)
	Polyrhachis	*Polyrhachis lacteipennis lacteipennis* Smith, F.
	Paratrechina	*Paratrechina longicornis longicornis* (Latreille)
Dolichoderinae	*Bothriomyrmex*	*Bothriomyrmex wroughtonii wroughtonii* Forel
	Tapinoma	*Tapinoma melanocephalum melanocephalum* (Fabricius)

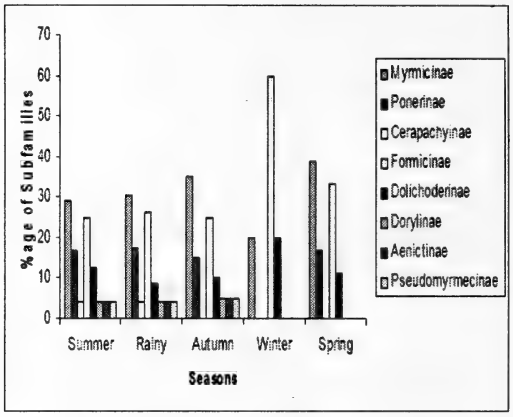
Table: 6 (Showing number of species collected w.r.t. temperature in different seasons of the year [2007-2008])

Seasons	Number of Species Collected	Temperature °C		Average temperature °C
		Maximum	Minimum	
Summer	40	36.54	20.81	28.67
Rainy	39	32.89	23.16	28.02
Autumn	36	31.6	16.4	24.0
Winter	5	19.6	2.26	10.93
Spring	31	20.63	19.08	19.85

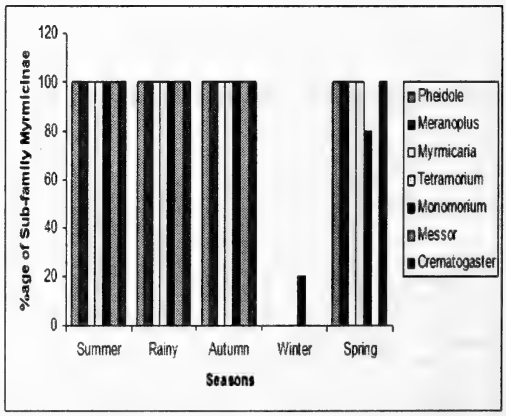
Results and Discussion

During the present study a total of 40 species have been recognised from Punjab Shivalik representing 8 subfamilies namely Myrmicinae, Ponerinae, Cerapachyinae, Formicinae, Dolichoderinae, Dorylinae, Aenictinae and Pseudomyrmecinae. Representatives of subfamilies Myrmicinae, Formicinae and Dolichoderinae were found throughout the year. These subfamilies were able to withstand extreme temperature fluctuation ranging from 2.26°C to 36.54°C (Table-6). All the 8 subfamilies were reported during summer season with Myrmicinae representing 29.16% of the total catch followed by Formicinae (25%) (Table-1, Graph-1). Dorylinae, Aenictinae and Psuedomyrmecinae were scanty. Rainy season was also dominated by subfamilies Myrmicinae and Formicinae. In autumn season, seven subfamilies were recorded, but no representative of subfamily Cerapachyinae was recorded. Extreme temperatures of winter were braved by subfamily Myrmicinae, Formicinae and Dolichoderinae. Within subfamily Myrmicinae genus *Monomorium* and species *Monomorium destructor* was the only representative that was found throughout the year. In subfamily Ponerinae, genus *Harpegnathos* was found only during summer and rainy season and no representative of Ponerinae was found in winter season. Similarly, subfamily Cerapachyinae was found only in summer and rainy season. Genus *Lepisiota, Camponotus* and *Paratrechina* of subfamily Formicinae were found throughout the year. Genus *Tapinoma* of subfamily Dolichoderinae was found in all the seasons of the year, whereas genus *Chronoxenus* was collected only during summer season. In subfamily Dorylinae, genus *Dorylus*, the only representative of the subfamily reported during this study was found missing in winter and spring season. Similarly, genus *Aenictus* (single representative) of subfamily Aenictinae was found in summer, rainy and autumn seasons. Genus *Tetraponera* representing subfamily Pseudomyrmecinae from Punjab Shivalik was found only during summer, rainy and autumn seasons.

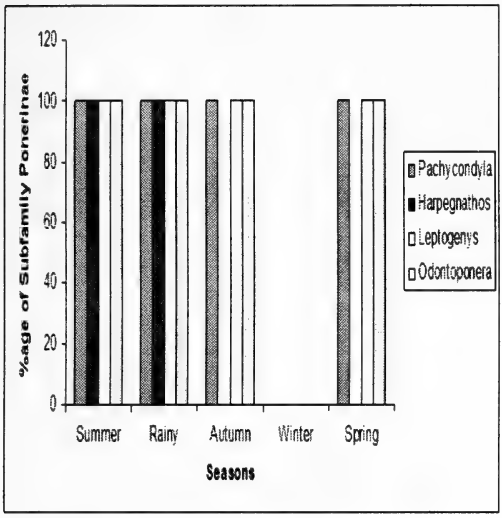
So, it can be concluded that species richness was maximum during summer season (36.54°C - 20.81°C), as a total of 40 species representing 24 genera and 8 subfamilies were collected during this season, whereas in winter season (19.6°C - 2.26°C) only 5 species belonging to subfamily Myrmicinae, Formicinae and Dolichoderinae were reported.



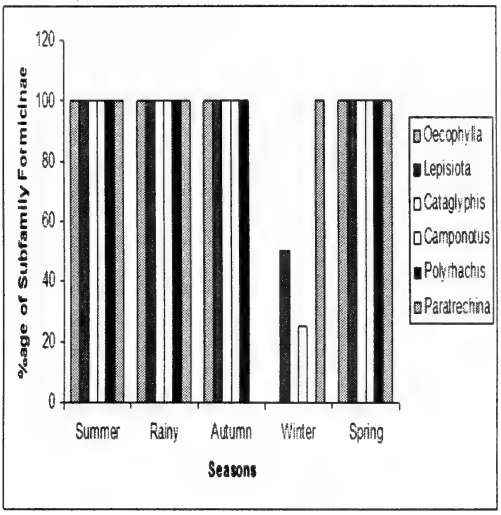
Graph-1: {Percentage representation of Subfamilies in different seasons of the year (2007-08)}



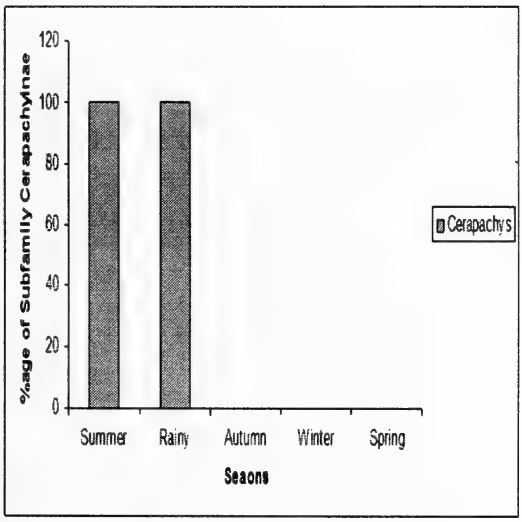
Graph-2: {Percentage representation of Subfamily Myrmicinae in different seasons of the year (2007-2008)}



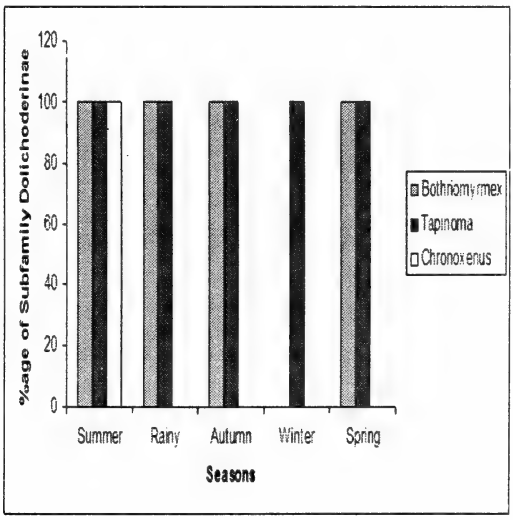
Graph-3: {Percentage representation of Subfamily Ponerinae in different seasons of the year (2007-2008)}



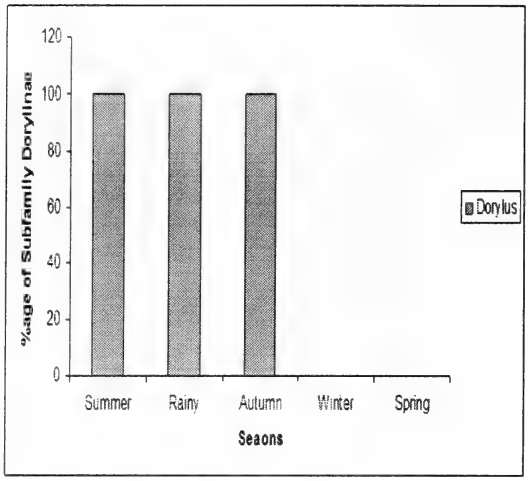
Graph-5: {Percentage representation of Subfamily Formicinae in different seasons of the year (2007-2008)}



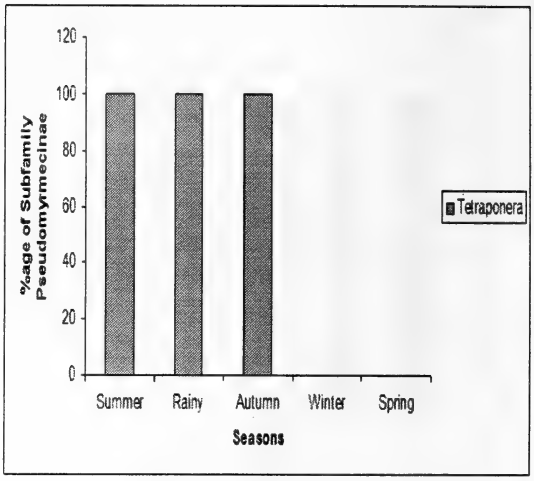
Graph-4: {Percentage representation of Subfamily Cerapachyinae in different season of the year (2007-2008)}



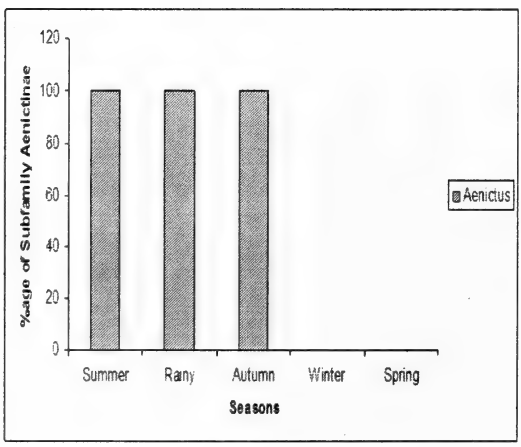
Graph-6: {Percentage representation of Subfamily Dolichoderinae in different season of the year (2007-2008)}



Graph-7: {Percentage representation of Subfamily Dorylinae in different seasons of the year (2007-2008)}



Graph-9: {Percentage representation of Subfamily Pseudomyrmecinae in different seasons of the year (2007-2008)}



Graph-8: {Percentage representation of Subfamily Aenictinae in different seasons of the year (2007-2008)}

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Occurrence of Odonata in Northern areas of Pakistan with seven new records

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Abstract

The study was undertaken to explore the Odonata (Dragonflies & Damselflies) of Northern Areas of Pakistan. The area has an assemblage of high mountains with unlimited water resources in the form of rivers, streams, springs and melted snow. New records of Odonata need to be explored from the area. The surveys were carried out during the months of April – August for four consecutive years (2004 – 2008). Help was also taken from the collection housed at National Insect Museum, Islamabad. Valid names alongwith their synonyms, distribution, habitat description and abundance for all the collected species are discussed in this paper. As total, 21 genera spreading to 37 species of Odonata, comprising of seven new records for the area including one new record for the country have been presented. A checklist for the area has also been included.

Keywords: *Odonata, Dragonflies, Damselflies, Northern Areas, Pakistan.*

Introduction

The Northern areas of Pakistan have an area of 72,496 sq. kms. Physiographically, it includes a set and series of high ranges of mountains (i.e Himalayas, Karakorum and the Hindukush) which are separated by the intervening valleys (Survey of Pakistan, 1997). Odonata of Northern areas are not well investigated in the past. In this regard, a need for comprehensive taxonomic work on Odonata of the area was felt and the present study was undertaken to record the un-explored Odonate fauna of Northern areas of Pakistan.

Odonates are economically important insects. They are predaceous both as larvae and adult. The larvae are aquatic and are found in all types of water bodies ranging from soaks and seepages to lakes, streams, rivers, temporary pools and water-filled holes of trees (Trueman and Rowe, 2001). Larvae are known to consume tadpoles, fish fry, and mosquito

larvae (Boyd, 2005). Adults normally feed on small insects, including beetles, moths (Silsby, 2001), mosquitoes (Pedigo, 2002) and black flies (Subramanian, 2005). In Pakistan, they are known to feed on jassids, thrips (Ali, 1983), stem borers, leaf folders and leaf hoppers (Najam, 1984). They are highly sensitive to habitat disturbances, thus play a vital role as bio-indicators (Clausnitzer, 2003).

According to Trueman and Rowe (2001) there are 6500 named species of Odonata so far described all over the world. In past, Jehangir (1997) studied the Odonata of Gilgit and Baltistan and recorded 21 dragonfly and 7 damselfly species. Hussain (2006), reported 9 dragonfly species and a single damselfly species from districts Gilgit and Astor. In contrast to above, there is a lot of potential to explore un-seen Odonate fauna of the area.

Materials and Methods

All the districts of Northern areas of Pakistan were surveyed during four consecutive years (2004 – 2008). As a total 46 different sites under 7 districts (Fig.1.) have been visited for collecting the adults of Odonata.

Northern areas (Gilgit and Baltistan):

Gilgit Territory:

District: Diamer (Goru, Chillas, Darail, Goner Farm).

District: Astor (Rama, Boomroy, Youghum, Gorikot, Pakora, Moorghulum).

District: Hunzanagar (Hunza, Aliabad, Borath Lake, Sost).

District: Gilgit (Juglote, Danyore, Sultanabad, Chinar Bagh, Gilgit, Kashroat, Sonikot, Chinar Bagh, Aliabad).

District: Ghizzer (Gackhuch Bala, Gackuch Zireen, Saling).

Baltistan Territory:

District: Skardu (Sat Para, Kharmang, Shangrilla, Shigar, Skardu, Gol, Mehdiabad, Hussainabad, Oolding, Newranga, Sundus, Ashkoli).

District: Ghanche (Khaplu, Bara, Balghar, Yougo, Surmo, Kharko, Hushe, Chumik).

Collection was done during the months of April to August (2004 – 2008). Methods of sampling were based on Wahizatul-Afzan *et. al.* (2006) with minor additions. When catching over water, dip nets were used. However for collection on wing or while siting over any dry surface or vegetation, aerial netting was done. The collected specimens were killed in glass bottles containing cotton swab moistened with ethyl acetate. The killed specimens were then shifted to paper envelopes for bringing them to laboratory. The preservation methodology was based on Orr (2003). Adults were softened by giving them a water bath in hot water, after softening they were properly set by using setting boards. The collection was then identified under microscopes {Labomet CZM4 (4X)} following the taxonomic keys of Fraser, (1933 – 34) and Subramanian (2005). The identified specimens have been deposited in National Insect Museum, NARC – Islamabad.

Results

The surveys revealed a collection of 37 species of Odonata including 28 Anisopterous species spreading to 15 genera and 09 Zygopterous species spreading to 6 genera (Table 1). As a whole seven species viz. *Sympetrum fonscolombei, S. commixtum, S. meridionale, Orthetrum taeniolatum, O. glaucum, Mortonagrion gautama and Libellago greeni* are recorded for the first time from this area. Amongst these *Mortonagrion gautama* is new to the country record. Abundance of species was also observed, which showed that

Crocothemis servilia (Anisoptera) and *Ischnura forcipata* (Zygoptera) are the most common and abundant species of the area, recorded from 16 and 23 different localities respectively. However amongst Anisoptera (*Aeschna juncea, Ophiogomphus reductus, Diplacodes lefbvrei, Orthetrum taeniolatum and Palpopleura sexmaculata sexmaculata*) and Zygoptera (*Libellago greeni and Mortonagrion guatama*) appeared to be less common or rare and were recorded from single locality only.

Discussion

The Northern areas of Pakistan are bestowed upon with variable habitats having lot of water streams and springs. Further collection surveys can unhide the existing but un-explored species of the area.

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Fig.1: Map - Northern areas of Pakistan showing all the surveyed localities.

Table 1: Collected species of dragonflies along with their synonyms, distribution and habitat description.

S.No.	Scientific names	Synonyms	Distribution in Northern areas	Habitat description
	Aeshnidae Rambur, 1842			
01.	*Aeshna juncea* Linnaues 1758	*Libellula juncea*, Linnaeus, 1758 *Aeshna Americana* Bartenev, 1929	Gilgit {Astor (Rama, Boomroy)}.	Collected from standing water spots with lot of grassy vegetation
02.	*Anax immaculifrons* Rambur, 1842		Baltistan {Ghanche (Balghar, Kharko, Yougo, Surmo), Skardu (Shigar, Hussainabad, New Ranga, Olding, Sundus)}.	Collection was made from poorly vegetated banks of slow running water streams, some specimens were also collected while sitting on small rocks within water and from small bushes near streams.
03.	*Anax nigrolineatus* Fraser, 1935	*Anax bacchus* Martin 1908 *Anax guttatus* 1921 *Anax fumosus* 1923 *Anax nigrolineatus* 1935	Gilgit {Diamer (Goru), Astor (Rama)}, Baltistan {Skardu (Gackuch Bala)}.	Found flying along running water, field areas and from the marshy spots.
04.	*Anax parthenope* Selys, 1839	*Aeschna parthenope* Selys, 1839 *Anax julius* Brauer, 1865 *Anax bacchus* Hagen, 1867 *Anax major* Gotz, 1923 *Anax parisinus* Rambur, 1842 *Anax geyri* Buchholz, 1955 *Anax jordansi* Buchholz, 1955	Gilgit {Gilgit (Juglote, Darel, Chinarbagh, Juglote, Kashroat)}, Baltistan {Skardu (New Ranga), Shegar	It is a strong flier and was collected from about 1000 ft. altitude. The spots were having both streams and springs water.
	Cordulegasteridae Calvert, 1893			
05.	*Cordulegaster brevistigma* Selys, 1854	*Anax bacchus* 1908 *Anax guttatus* 1921 *Anax fumosus* 1923 *Anax nigrolieatus* 1935	Baltistan {Ghanche (Balghar)}, Gilgit {Gilgit (Chinar Bagh), Hunzanagar (Sost)}.	Found among tall and high vegetation beside water sources.
	Gomphidae Rambur, 1842			
06.	*Ophiogomphus reductus* Calvert, 1898	*Ophiogomphus forficula* Okumura, 1937	Baltistan {Skardu (Shangrilla)}.	A single male and a female was collected while mating at the edges of stagnant weedy water spot near Shangrilla Lake.
	Libellulidae Rambur, 1842			
07.	*Acisoma panorpoides panorpoides* Rambur, 1842	*Acisoma ascalaphoides* Rambur, 1842 *Acisoma inflata* Selys, 1882 *Acisoma variegatum* Kirby, 1898	Gilgit {Diamer (Chillas)}.	Specimens were collected as they were sitting on small rocks within and around water spots.
08.	*Crocothemis erythraea* Brulle, 1832	*Libellula erythraea* Brullé, 1832 *Libellula rubra* de Villers, 1789 (nec Müller, 1764) *Libellula ferruginea* Vander Linden, 1825 (nec Fabricius, 1775) *Libellula coccinea* Charpentier, 1840 *Libellula inquinata* Rambur, 1842 *Crocothemis chaldaeora* Morton, 1920	Baltistan {Ghanche (Balghar, Kharko, Surmo), Skardu (Shigar, Olding, Shangrilla)}; Gilgit (Hunza).	The species was found among the grasses and bushes present aside standing water lake and along slow moving water streams in diferent areas

Table-1: Continued

S.No.	Scientific names	Synonyms	Distribution in Northern areas	Habitat description
09.	*Crocothemis servilia* Drury, 1773	*Libellula servilia* Drury, 1773 *Libellula ferruginea* Fabricius, 1793 *Libellula soror* Rambur, 1842 *Crocothemis* reticulata Kirby, 1886	Baltistan {Ghanche (Balghar, Kharko, Yougo, Khaplu), Skardu (New Ranga, Shigar, Hussainabad, New Ranga, Sundus, Bara, Shangrilla, Skardu, Oolding)}, Gilgit {Gilgit (Danyore, Juglote), Diamer (Chillas)}.	Found flying over fast running water streams, sitting on submerged grasses, swampy places along banks of streams.
10.	*Diplacodes lefebvrei* Rambur, 1842	*Libellula lefebvrei* Rambur, 1842 *Libellula parvula* Rambur, 1842 *Libellula flavistyla* Rambur, 1842 *Libellula tetra* Rambur, 1842 *Libellula concinna* Rambur, 1842 *Libellula morio* Schneider, 1845 *Diplacodes unimacula* Förster, 1906 *Diplacodes limbata* Fraser, 1949	Baltistan {Skardu (Oolding)}.	Caught from the margins of weedy ponds.
11.	*Libellula quadrimaculata* Linnaeus, 1758	*Libellula quadripunctata* Fabricius, 1781 *Libellula maculata* Harris, 1782 *Libellula ternaria* Say, 1839 (part) *Libellula quadrimaculata asahinai* Schmidt, 1957 *Libellula relicta* Belyshev, 1973	Baltistan {Ghanche (Kharko, Yougo), Skardu (Shigar, Hussainabad, Sundus, Bara)}, Gilgit {Astor (Rama,Boomroy)}.	It is mountaneous species and was recorded from standing water ponds. Some of the specimens were collected while they were perching on the long grassy vegetation.
12.	*Orthetrum anceps* Schneider, 1845	*Libellula anceps* Schneider, 1845 *Libellula ramburii* Selys, 1848	Baltistan {Ghanche (Balghar, Kharko, Yougo, Khaplu, Surmo), Skardu (Shegar, Hussainabad, New Ranga, Sundus)},Gilgit {Diamer (Chillas), Gilgit (Chinar Bagh, Juglote)}.	Collection was done from fresh water streams and grassy vegetation around spring water ways. Specimens were also found sitting on dead bushes and rock stones.
13.	*Orthetrum brunneum brunneum* Fonscolombe, 1837	*Libellula brunnea* Fonscolombe, 1837	Baltistan {Ghanche (Balghar, Khaplu), Skardu (Hussainabad, Sundus, Shangrilla, Skardu, Shigare, Ashkoli)}, Gilgit {Gilgit (Juglote, Chinarbagh, Danyore, Kashroat)}.	Collection was done from standing as well as from moving water of streams and springs.
14.	*Orthetrum cancellatum* Linnaeus, 1758	*Libellula cancellata* Linnaeus, 1758	Baltistan {Ghanche (Balghar, Yougo, Surmo), Skardu (Shigar, Sundus, New Ranga, Shangrilla, Olding)}.	Collected from miscellaneous spots i.e. water lakes, from weeds growing on the banks of very slow running water ways and from water standing in empty tree holes and other pits with water.
15.	*Orthetrum chrysostigma luzonicum* Burmeister, 1839	*Libellula chrysostigma*, Burmeister, 1839 *Libellula barbarum* Selys, 1849 *Orthetrum todii* Pinney, 1970	Gilgit {Gilgit (Juglote)}, Baltistan {Skardu (Shangrilla)}.	Collected from spring water spots with a lot of lush green vegetation growing around it.
16.	°*Orthetrum glaucum* Brauer, 1865	*Libellula glaucum* Brauer, 1865 *Orthetrum gangi* Sahni, 1965 *Orthetrum glaucum* Kirby, 1890 *Orthetrum nicevillei* Kirby, 1894	Baltistan {Ghanche (Balghar, Yougo), Skardu (Olding)}, Gilgit {Gilgit (Chinarbagh), Diamer (Chillas, Darail), Astor (Boomroy)}.	Found preying over minute insects hiding between grassy bushes around fresh water sources.
17.	*Orthetrum pruinosum neglectum* Burmeister, 1839	*Libellula pruinosa* Burmeister, 1839 *Libellula neglecta* Rambur, 1842 *Libellula petalura* Brauer, 1865 *Libella clelia* Selys, 1878 *Orthetrum schneideri* Forster, 1903	Baltistan {Skardu (Shegar), Mehdiabad}. Gilgit {Astor (Yougham)}.	Recorded from ponds and some other spots with standing water.

Table-1: Continued

S.No.	Scientific names	Synonyms	Distribution in Northern areas	Habitat description
18.	*Orthetrum sabina* Drury, 1770	*Libellula sabina* Drury, 1770 *Libellula gibba* Fabricius, 1798 *Libellula leptura* Burmeister, 1839 *Libellula ampullacea* Schneider, 1845 *Lepthemis divisa* Selys, 1878 *Orthetrum nigrescens* Bartenev, 1929 *Orthetrum viduatum* Lieftinck, 1942	Gilgit {Gilgit (Juglote, Danyore)}.	In both the collection spots there was standing water with muddy and swampy areas around it.
19.	°*Orthetrum taeniolatum* Schneider, 1845	*Libellula taeniolata* Schneider, 1845 *Orthetrum hyalinum* Kirby, 1886 *Orthetrum brevistylum* Kirby, 1896 *Orthetrum garhwalicum* Singh and Baijal, 1954	Baltistan {Skardu (Shangrilla)}.	Captured from standing as well as very fast moving water spots.
20.	*Orthetrum triangulare triangulare* Selys, 1878	*Libellula triangularis* Selys, 1878 *Libellula delesserti* Selys, 1878 *Libellula melanica* Selys, 1883 *Pseudothemis nigrifrons* Matsumura, 1898 *Orthetrum ganeshii* Mehrotra, 1961 *Orthetrum chandrabali* Mehrotra, 1961	Gilgit {Gilgit (Juglote, Kashroat), Diamer (Chillas, Darail)}, Baltistan {Ghanche (Hushe, Chumick)}.	Specimens were collected from different standing water spots. They were not recorded from any running water spot in the visited areas.
21.	*Palpopleura sexmaculata sexmaculata* Fabricius, 1787	*Libellula sexmaculata* Fabricius, 1787	Baltistan {Skardu (Oolding)}.	
22.	*Pantala flavescens* Fabricius, 1798	*Libellula flavescens* Fabricius, 1798 *Libellula viridula* Palisot de Beauvois, 1805 *Libellula analis* Burmeister, 1839 *Libellula terminalis* Burmeister, 1839 *Sympetrum tandicola* Singh, 1955	Gilgit {Gilgit (Kashroat, Sultan abad)}.	The specimens were collected from variable spots i.e standing water spots, moving water spots, long grasses and dry branches of some dwarf plantations.
23.	°*Sympetrum commixtum* Selys, 1884	*Diplax commixta* Selys, 1884	Gilgit {Astor (Moorgulum, Gorikot)}.	Found flying and feeding around standing water areas.
24.	°*Sympetrum fonscolombei* Selys, 1840	*Libellula flaveola*, Fonscolombe 1837	Gilgit {Astor (Yougham, Boomroy, Pakora)}.	This is mountainous species and mostly found around standing water sitting in bushes and grass.
25.	°*Sympetrum meridionale* Selys, 1841	*Libellula meridionalis* Selys, 1841 *Libellula hybrida* Rambur, 1842 *Diplax meridionalis* Braur, 1868 *Sympetrum meridionals* Meyer, 1874	Gilgit {Astor (Pakora, Gorikot)}.	Recorded from the dry branches of plants present along the margins of ponds and some small standing water points.
26.	*Traemea virginia* Rambur, 1842	*Libellula virginia* Rambur, 1842	Gilgit {Gilgit (Juglote), Ghizer (Saling)}.	Specimens were collected while they were perching in sunlight, earlier in the afternoon.
27.	*Trithemis aurora* Burmeister, 1839	*Libellula aurora* Burmeister, 1839 *Trithemis soror* Brauer, 1868 *Trithemis adelpha* Selys, 1878 *Trithemis fraterna* Albarda, 1881 *Trithemis congener* Kirby, 1890	Baltistan {Skardu (Skardu, Shegar)}.	The collection was done along the banks of streams. The specimens were busy in feeding, mating and hunting while flying near the muddy banks. The spot was also having grasses which were submerged in the stream water.
28.	*Trithemis festiva* Rambur, 1842	*Libellula festiva* Rambur, 1842 *Libellula infernalis* Brauer, 1865 *Trithemis proserpina* Selys, 1878	Gilgit {Gilgit (Danyore, Juglote)}.	Collected during mating, while sweeping the net blindly in the air with in a crop field.

Table: 2 Collected species of damselflies along with their synonyms, distribution and habitat description.

S.No.	Scientific names	Synonyms	Distribution in Northern areas	Habitat description
	Chlorocyphidae Cowley, 1937			
01.	°*Libellago greeni* Laidlaw 1924	*Micromerus greeni* Laidlaw, 1924	Gilgit {Gilgit (Danyore)}.	Recorded while mating within a grassy spot along moving water.
	Coenagrionidae Kirby, 1890			
02.	**Mortonagrion gautama,* Fraser 1923	*Indagrion gautama* Fraser, 1922	Gilgit {Gilgit (Danyor)}.	Found in stagnant water pond at Danyore. The pond was surrounded by thick as well as thin long and dwarf vegetation. The species was recorded when it was busy in preying over minute insects.
03.	*Ceriagrion coromandelianum* Fabricius, 1798	*Agrion coromandelianum* Fabricius, 1798 *Agrion cerinum* Rambur, 1842	Gilgit {Diamer (Darail, Chillas)}.	Collected while hovering stagnant water and from vegetation grown aside water streams.
04.	*Enallagma cyathigerum* Charpantier, 1840	*Agrion cyathigerum* Charpantier, 1840 *Agrion annexum* Stephens, 1835 (nec Charpentier, 1825) *Agrion pulchrum* Hagen, 1840 *Agrion charpentieri* Selys, 1840 *Agrion annexum* Hagen, 1861 *Enallagma robustum* Selys, 1875 *Enallagma continentale* Belyshev, 1956 *Enallagma nigrolineatum* Belyshev and Haritonov, 1975	Baltistan {Ghanche (Kharko, Yougo), Skardu (Shigar, Hussainabad, New Ranga, Olding, Sundus, Shangrilla)}, Gilgit {Hunzanagar (Hunza)}.	The species was found feeding among the grasses and bushes present aside standing water lake and along slow moving water ways.
05.	*Ischnura aurora* Brauer, 1865	*Agrion aurora* Brauer, 1865 *Agrion delicatum* Hagen, 1876 *Ischnura delicata* Hagen, 1876 *Micronympha aurora* Kirby, 1890 *Nanosura aurora* kennedy, 1920 *Ishnura bhimtalensis* Sahni, 1965	Gilgit {Diamer (Darail, Chillas)}.	Found flying among thin vegetation present a little distant to water streams. Also collected while sitting on swampy places. Sometimes found between the submerged vegetation along streams and springs.
06.	*Ischnura elegans* Vander Linden, 1820	*Agrion elegans* Vander Linden, 1820 *Ischnura lamellata* Kolbe, 1885	Baltistan {Skardu (Gol, Shigar, Husainabad, New Ranga, Olding, Sundus), Ghanche (Kharko)}, Gilgit {Diamer (Darel), Gilgit (Juglote, Danyore, Kashroat, Chinar Bagh), Ghizer (Saling)}.	Collection was done from grassy vegetation around water spots
07.	**Ischnura forcipata* Morton, 1907	*Ischnura musa* Bartenev, 1913 *Ischnura gangetica* Laidlaw, 1913 *Agriocnemis nainitalensis* Sahni, 1965 *Coenagrion needhami* Navas, 1933	Baltistan {Ghanche (Balghar, Kharko, Yougo, Khaplu, Surmo), Skardu (Gol, Satpara, Shigar, Hussainabad, New Ranga, Shangrilla), }, Gilgit { Diamer (Chillas (Goner farm, Darel, Goru), Gilgit (Chinar Bagh, Danyore, Sonikot, Juglote), Astor (Rama), Hunzanagar (Borath Lake), Ghizer (Gackuch Bala, Gackuch Zireen)}.	It is a common species of the region and thus collected from a variable number of ecological habitats including grasses growing among stagnant water and along running water, some times found among thick and dense vegetation present aside and a little away from water streams. Also found flying among small grasses present a little distant to water streams.
08.	*Ischnura senegalensis* Rambur, 1842	*Agrion senegalensis* Rmbur, 1842 *Enallagma brevispina* Selys, 1876	Baltistan {Ghanche (Balgar)}.	Caught from grassy and swampy spot.
	Synlestidae			
09.	*Megalestes major* Selys, 1962		Baltistan {Skardu (Oolding), Aliabad}.	Collection was done from spots with high grassy vegetation. Some specimens were collected along water side as well as some from nearby small mountains.

*** New record for Country**
° New record for Northern areas

Check list of Odonata of Northern areas of Paksistan

SUB ORDER ANISOPTERA
Family: Aeshnidae Rambur, 1842
Genus *Aeshna* Fabricius 1775
Aeshna juncea Linnaues 1758

Genus *Anax* Leach, 1815
Anax immaculifrons Rambur, 1842
Anax nigrolineatus Fraser, 1935
Anax parthenope Selys, 1839

Family: Cordulegasteridae Calvert, 1893
Genus *Cordulegaster* Leach, 1815
Cordulegaster brevistigma Selys, 1854

Family: Gomphidae Rambur, 1842
Genus *Ophiogomphus* Selys, 1854
Ophiogomphus reductus Calvert, 1898

Family: Libellulidae Rambur, 1842
Genus *Acisoma* Rambur, 1842
Acisoma panorpoides panorpoides Rambur, 1842

Genus *Crocothemis* Brauer, 1868
Crocothemis erythraea Brulle, 1832
Crocothemis servilia Drury, 1773

Genus *Diplacodes* Kirby, 1889
Diplacodes lefebvrei Rambur, 1842

Genus *Libellula* Linnaeus, 1758
Libellula quadrimaculata Linnaeus, 1758

Genus *Orthetrum* Newman, 1833
Orthetrum anceps Schneider, 1845
Orthetrum brunneum brunneum Fonscolombe, 1837
Orthetrum cancellatum Linnaeus, 1758
Orthetrum chrysostigma luzonicum Burmeister, 1839
Orthetrum glaucum Brauer, 1865
Orthetrum pruinosum neglectum Burmeister, 1839
Orthetrum sabina Drury, 1770
Orthetrum taeniolatum Schneider, 1845
Orthetrum triangulare triangulare Selys, 1878

Genus *Palpopleura* Rambur, 1842
Palpopleura sexmaculata sexmaculata Fabricius, 1787
Pantala flavescens Fabricius, 1798

Genus *Sympetrum* Newman, 1833
Sympetrum commixtum Selys, 1884
Sympetrum fonscolombei Selys, 1840
Sympetrum meridionale Selys, 1841

Genus *Traemea* Hagen, 1861
Traemea virginia Rambur, 1842

Genus *Trithemis* Brauer, 1868
Trithemis aurora Burmeister, 1839
Trithemis festiva Rambur, 1842

SUB ORDER ZYGOPTERA
Family: Chlorocyphidae Cowley, 1937
Genus *Libellago* Selys, 1840
Libellago greeni Laidlaw 1924

Family: Coenagrionidae Kirby, 1890
Genus *Mortonagrion* Fraser, 1920
Mortonagrion gautama Fraser 1923

Genus *Ceriagrion* Selys, 1876
Ceriagrion coromandelianum Fabricius, 1798

Genus *Enallagma* Charpentier, 1840
Enallagma cyathigerum Charpantier, 1840

Genus *Ischnura* Charpentier, 1840
Ischnura aurora Brauer, 1865
Ischnura elegans Vander Linden, 1820
Ischnura forcipata Morton, 1907
Ischnura senegalensis Rambur, 1842

Family: Synlestidae
Genus *Megalestes* Selys, 1862
Megalestes major Selys, 1962

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Influence of Foraging time, Flight activity patterns and Duration of a foraging trip of *Apis* species (order: Hymenoptera) on *Brassica campestris* var. Sarson

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Abstract

Foraging behaviour of *Apis cerana* and *Apis mellifera* was studied at two field stations- Pallimore and Hiranagar in district Kathua of Jammu region (J&K), in order to determine their potential for working hours in the fields of sarson. Single colonies of each species were placed in the fields till the end of flowering. Commencement of the foraging activity of *Apis cerana* (0624±0.91 and 0622±0.55 hrs) was significantly earlier (P<0.0001) than *A. mellifera* (0648±0.68 and 0645±0.98 hrs) at both the fields respectively. However in the evening, *A. cerana* mean timings: 1842±0.84 and 1844±1.07 hrs, ceased its foraging activity significantly later (P<0.001) than *A. mellifera* mean timings: 1813±1.06 and 1817±2.10 hrs respectively at both the fields. For flight activity patterns, *A. mellifera* reached its maxima (0800-1400 hrs) before *A. cerana* (1000-1200 hrs) at Pallimore, while at Hiranagar peak activity of *Apis cerana* lies between 1000-1300 hours and that of *A. mellifera* lies between 1000-1400 hours. Duration of foraging trip was significantly more (P<0.05) in *A. mellifera* (24.14 minutes) than *A. cerana* (22.97 minutes) at Pallimore, but no significant differences (P>0.05) were observed at Hiranagar for *A. cerana* (23.77 minutes) and *A. mellifera* (24.54 minutes).

Keywords: - *Apis mellifera, Apis cerana, Brassica campestris, Foraging.*

Introduction

Honeybees are the most efficient pollinators of cultivated crops because of their floral fidelity (Wells and Wells, 1983 & Waser, 1986), potential for long working hours (Sihag, 1990), presence of pollen baskets, maintainability of high population, micromanipulation of flowers and adaptability to different climatic conditions (Verma and Partap, 1993).

The use of bees for pollination purpose is increasing day by day in different parts of the world. It is considered that the services that bees render to agriculture in the pollination of fruits, vegetables, legume and other seed crops are worth many times the return which bee-keepers receive in the form of honey and

bee-wax.

Materials and Methods

Foraging behaviour of *Apis cerana* and *A. mellifera* species of honey bees were studied by placing single colony of each of these species in sarson crop, till the end of following.

Foraging time

Foraging time of *Apis cerana* and *A. mellifera* was assessed in terms of timing of commencement and cessation of fight activity and was observed by recording the time when the first bee started its flight in the morning and last bee ceased in the evening. This data was recorded for a period of seven days during the full bloom (Verma and Dulta, 1986; Verma and Partap, 1993 and Kumar, 1998).

Flight activity patterns

Flight activity was measured in terms of number of worker bees of *A. cerana* and *A. mellifera* leaving the hive per minute. These observations were recorded daily at regular hourly intervals from 0700 hrs. in the morning to 1800 hours in the evening. From the recorded data peak hours of foraging activity were calculated for both *A. cerana* and *A. mellifera* in terms of maximum number of foragers leaving the hive at particular hours (Kumar, 1998).

Temperature and relative humidity were also recorded at the time of taking bee counts in the crop fields. All these observations were taken for a period of 7 days in each field.

Duration of a foraging trip

Duration of a foraging trip was studied randomly by marking 20 worker bees each of *A. cerana* and *A. mellifera* with nail polish of different colours on their thoracic region. The interval between bees leaving and entering the hive was recorded with the help of a stop watch (Mattu, 1982). In total, 10 observations were made daily during different hours of the day. These observations were repeated regularly for 7 days during the blooming period, in each study locations. The bees were regularly marked depending upon their casualities.

Results and Discussion

Foraging time

Bees moving out for the collection of nectar and pollens for their colony are called as the foragers. They forage for specific time of the day in the field. The data regarding the commencement and cessation of foraging time of *Apis cerana* and *A. mellifera* is presented in the Table 1. The mean timing of commencement of the foraging activity of the Indian hive bee, *Apis cerana* was 0624±0.91 and 0622±0.55 hrs, respectively at Pallimore and Hiranagar fields which was significantly earlier ($P<0.001$) than the European hive bee, *A. mellifera* whose mean foraging activity starts at 0648±0.68 hrs at Pallimore and 0645+0.98 hrs at Hiranagar field. However in the evening, *A. cerana* mean timings: 1842±0.84 and 1844±1.7 hrs ceased its foraging activity significantly ($P<0.001$) later than *A. mellifera* mean timings: 1813±1.06 and 1817±2.10 hrs, respectively at both the fields. Thus average duration of foraging activity lasted for 12.17±0.22 and 12.21±0.15 hrs for *A. cerana* & 11.64±0.16 and 11.72±0.18 hrs for *A. mellifera* respectively at both fields.

These observations are in conformation with the earlier reports of Kapoor and Dhaliwal (1989) on cauliflower at Hissar, Verma and Partap (1993) at Nepal for *B. juncea*. Verma and Partap (1993) also studies the foraging timing of *A. cerana* on cauliflower, cabbage, radish and lettuce at Nepal and concluded that it starts its foraging activity early in the morning and ceases late in the evening.

These differences in the mean timings of the commencement and cessation of foraging

activity may be due to differential interactions between genotype of the two species and the environment (Kumar, 1998).

Table-1: Commencement and cessation of foraging time of *Apis cerana* and *A. mellifera*.

Pallimore				**Hiranagar**			
Initiation		Cessation		Initiation		Cessation	
**A.c.*	**A.m.*	*A.c.*	*A.m.*	*A.c.*	*A.m.*	*A.c.*	*A.m.*
0624	0648	1842	1813	0622	0645	1844	1817
Duration (in hours)							
A. cerana		*A. mellifera*		*A. cerana*		*A. mellifera*	
12.18		11.65		12.21		11.72	

****A.c.= Apis cerana; *A.m. = Apis mellifera.***

Flight activity patterns

Indian and European hive bees were monitored for their foraging activity patterns at regular hourly intervals from 0700- 1800 hrs at both the fields as shown in Tables 2 & 3 and figure 1 & 2. The flight activity patterns of *Apis cerana* and *A. mellifera* at Pallimore and Hiranagar stations, presented in the tabulated form revealed *A. mellifera* reached its maxima (0800-1400 hours) before *A. cerana* (1000-1200 hours) at Pallimore while, the peak period of two species coincides at Hiranagar.

Parameters	**Pallimore**		**Hiranagar**	
	A. cerana	***A. mellifera***	***A. cerana***	***A. mellifera***
Peak activity(hrs)	1000-1200	0800-1400	1000-1300	1000-1400
Temperature (°C)	20.63-24.20	15.51-25.34	19.57-24.46	19.57-
Relative humidity (%)	72.86-59.43	77.28-53.86	78.43-60.71	78.43-53.43

Mishra *et al.* (1988) observed peak foraging activity of *A. cerana* between 1300-1400 hrs on mustard bloom. Thakur *et al.* (1982) recorded peak foraging activity of *A. mellifera* and *A. cerana* at 1200 hrs and smaller peak activities between 1400 hrs and 1500 hrs on mustard bloom. Chand *et al.* (1994) during their studies reported maximum peak activity of *A. cerana indica* at 1500 hrs. and 1600 hrs. and that of *A. mellifera* at 1300 hrs. on *Brassica juncea*. Peak periods of *A. cerana* was recorded as 1200 hrs. -1400 hrs. on cauliflower in Solan, H.P., by Dhaliwal and Bhalla (1980). Maximum peak activity of Hymenopterans and Dipterans were recorded at 1400 hrs. by Priti and Sihag (1997) on cauliflower, Hissar.

These differences in foraging activity patterns of *A. cerana* and *A. mellifera* may be due to the difference in the genotype and environmental interactions (Kumar, 1998).

Duration of a Foraging Trip

Foraging data on sarson crop showed that *A. cerana* spent on an average 22.97±0.40 minutes and 23.77±0.44 minutes for a single foraging trip at Pallimore and Hirangar, whereas this duration was 24.14+0.38 minutes and 24.54.035 minutes for *A. mellifera* at both Pallimore and Hiranagar respectively.

Statistical analysis of the data revealed that duration of a foraging trip (interval between the number of bees leaving and entering the hive) was significantly ($P<0.05$) more in *A. mellifera* than *A. cerana* at Pallimore. But no significant ($P>0.05$) difference was observed between *A. cerana* and *A. mellifera* at Hiranagar shown in tabulated form as: -

Parameters	Pallimore			Hiranagar		
	A.cerana X±S.E	***A.mellifera*** X±S.E		***A.cerana*** X±S.E.	***A.mellifera*** X±S.E	
Duration of a foraging trip (minute)	22.97±0.40	24.14±0.38	S	23.77±0.44	24.54±0.35	NS

X±S.E = Mean±Standard error about the mean; S = Significant ($P < 0.001$; 0.05); NS = Non-significant ($P > 0.05$)

These differences may be due to small size of the flower of *Brassica campestris* where *A. mellifera* spent more time in collecting nectar and pollens as compared to *A. cerana*. These results of the present author are in agreement with earlier findings of Verma and Partap (1993) who reported that duration of foraging trips of *A. mellifera* (25.29±0.57) was more than *A. cerana* (23.24±0.22).

Verma and Partap (1993) also observed duration of foraging trips of *A. cerana* as 26.8 minutes on cauliflower, 23.8 minutes on cabbage, 22.1 minutes on radish and 15.6 minutes on lettuce in Kathmandu, Nepal.

Table-2: Flight activity patterns of *Apis cerana* and *Apis mellifera* on sarson bloom at different hours of the day, at Pallimore (No. of bees leaving the hive/minute)

Apis cerana	Time in											
	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
$\bar{X}$	30.71	33.86	32.14	38.71	24.71	26.57	20.57	19.29	7.43	6.43	5.29	2.86
±S.E.	0.97	0.96	1.42	0.64	0.86	0.84	0.43	0.56	0.37	0.48	0.92	0.51
%	12.35	13.33	12.93	15.57	9.94	10.69	8.27	0.97	7.76	2.99	2.59	1.15
T	14.21	15.51	18.5	20.63	22.54	24.2	24.7	25.34	25.17	25.11	22.17	21.14
RH.	74.71	77.28	71.57	72.86	64.57	59.43	59.57	53.86	52.86	55.71	74.71	70.28
Apis mellifera												
$\bar{X}$	34.57	45.43	39.14	43.14	40.00	44.43	41.00	30.00	21.29	7.57	4.86	1.57
±S.E.	1.32	0.84	0.51	0.94	0.31	0.99	0.69	0.79	0.42	0.42	0.51	0.20
%	9.79	12.86	11.09	12.22	11.33	12.59	11.61	8.50	6.03	2.14	1.38	0.44
T	14.21	15.51	18.5	20.63	22.54	24.2	24.7	25.34	25.17	25.11	22.17	21.14
RH.	74.71	77.28	71.57	72.86	64.57	59.43	59.57	53.86	52.86	55.71	74.71	70.28

% = percentage; T = Temperature; R.H. = Relative humidity;
Peak foraging activity: 1000-1200 hours in *A. cerana;* 0800-1400 hours in *A. mellifera*

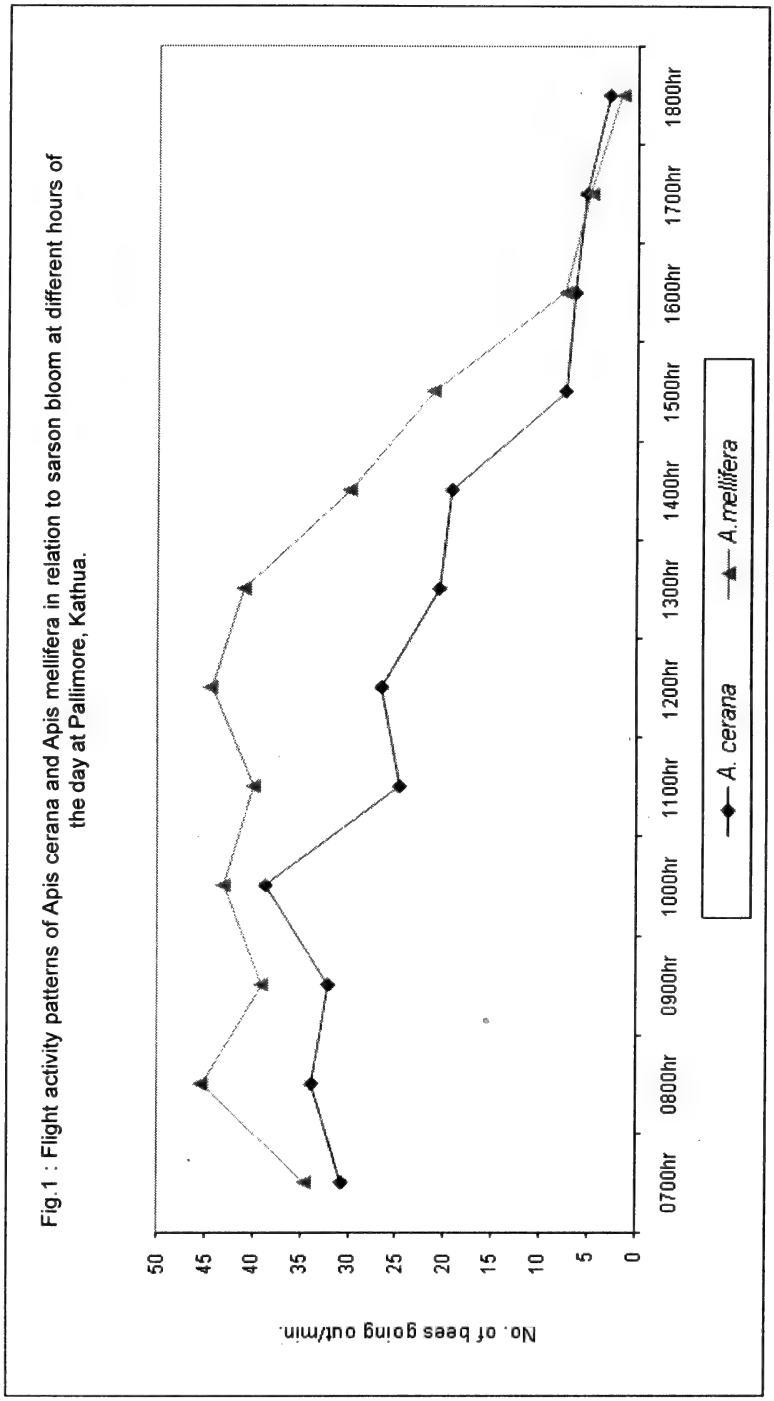

Fig.1 : Flight activity patterns of Apis cerana and Apis mellifera in relation to sarson bloom at different hours of the day at Pallimore, Kathua.

Table 3: Flight activity patterns of *Apis cerana* and *Apis mellifera* on sarson bloom at different hours of the day, at Hiranagar (No. of bees leaving the hive/minute)

Apis cerana	Time in											
	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
$\bar{X}$	13.57	17.29	21.86	33.29	30.14	40.43	32.14	22.71	17.57	15.00	5.71	3.29
±S.E.	0.57	0.97	0.86	1.23	0.80	1.13	1.35	1.08	1.25	0.82	0.56	0.29
%	5.36	6.83	8.64	13.16	11.91	15.98	12.70	8.98	6.94	5.93	2.26	1.30
T	14.36	15.63	18.36	19.57	21.68	23.4	24.46	25.06	25.17	24.64	22.16	21.00
R.H.	73.14	75.57	68.00	78.43	68.43	63.93	60.71	53.43	53.1	53.57	72.14	68.43
Apis. mellifera												
$\bar{X}$	17.43	38.29	40.43	51.00	41.43	53.14	42.86	38.86	25.43	20.71	4.71	1.29
±S.E.	0.65	0.68	1.32	0.93	0.97	2.16	0.74	0.59	1.46	0.61	0.36	0.18
%	4.64	10.19	10.76	13.58	11.03	14.15	11.41	10.35	6.77	5.51	1.25	0.34
T	14.36	15.63	18.36	19.57	21.68	23.4	24.46	25.06	25.17	24.64	22.16	21.00
R.H.	73.14	75.57	68.00	78.43	68.43	63.93	60.71	53.43	53.1	53.57	72.14	68.43

% = percentage; T = Temperature; R.H. = Relative humidity;

Peak foraging activity: 1000-1300 hours in *A. cerana;* 1000-1400 hours in *A. mellifera*

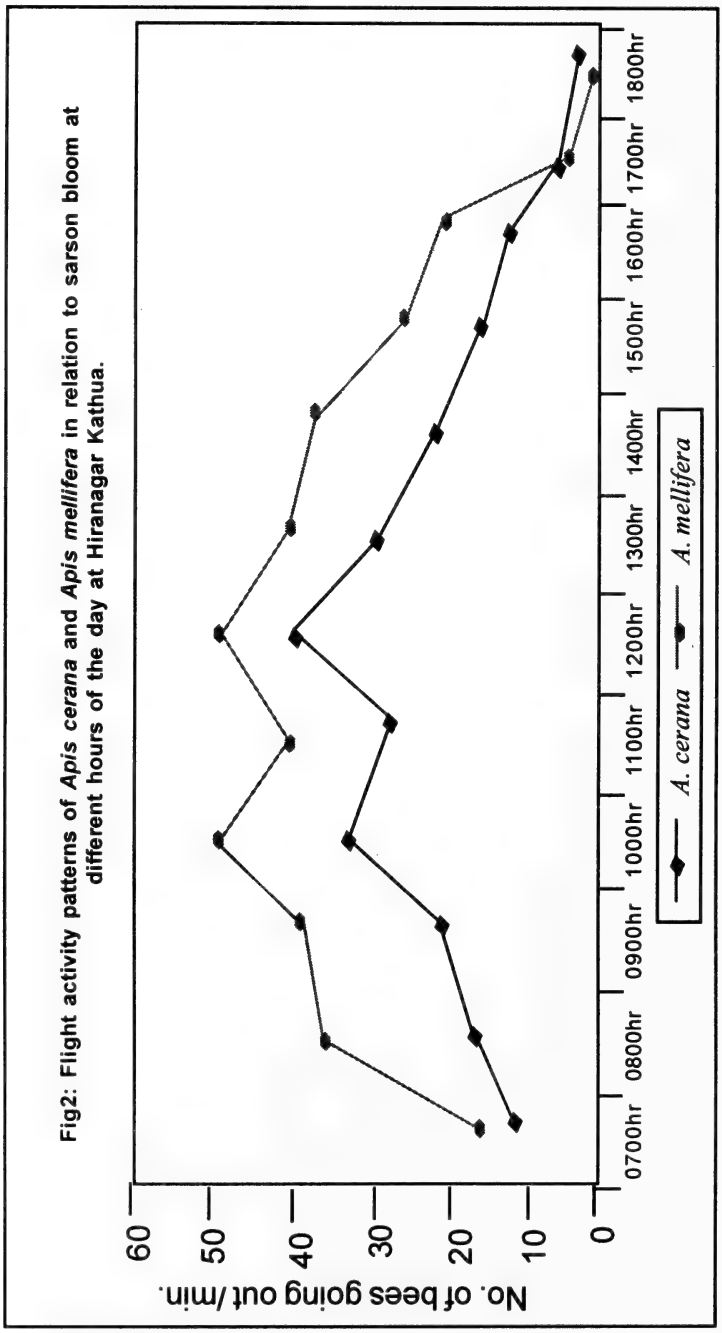

Fig2: Flight activity patterns of *Apis cerana* and *Apis mellifera* in relation to sarson bloom at different hours of the day at Hiranagar Kathua.

These variations in results may be because of different foraging efficiencies of these two species of honeybees in relation to morphology of different flowers.

Conclusion

It has been concluded that by placing both the colonies of bees (*A. cerana* and *A. mellifera*) in the fields of *B. campestris*, increases the peak period of pollination, hence enhances the yields of the crop.

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Some notes on medically important flies (Diptera: Calliphoridae) from India

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Abstract

Many cases of myiasis are reported every year from India, but in most of these cases the correct identification of fly maggots is lacking. Moreover, calliphorids and other families of Diptera like Sarcophagidae, Muscidae are vectors of number of diseases like cholera, poliomyelitis, typhoid fever, leprosy, tuberculosis etc. Keeping in view the medical importance of these flies, an attempt is made to enlist the calliphorid species from India.

Keywords: *Myiasis, Calliphoridae, India.*

Introduction

Myiasis is usually dealt with from the stand point of tissues and organs invaded, and classified under rhinal, aural, oral, ocular, cutaneous, subcutaneous, vaginal and gastro-intestinal myiasis. This method of dealing with the subject is not only unscientific but leads to endless confusion, as the same larva may be found in more than one organ and in wounds and cuts of all kinds. As to mention, the larvae of *Chrysomya bezziana*, the old world screw-worm fly may be found in all the above named cavities and in all forms of cutaneous and subcutaneous myiasis. The subject of myiasis should be best studied from the standpoint of the flies themselves, which may be classified as follows:

Obligatory (Specific myiasis producing flies): Include those species which lay their eggs or deposit their larvae in the living tissues.

Facultative (Semi-specific myiasis producing flies): Include species which normally lay their eggs or deposit their larvae in decomposing animal or vegetable matter, but occasionally place them in living tissue.

Accidental myiasis producing flies: Include those species which normally lay their eggs or larvae in stale or decomposing vegetable matter. Many human food stuffs are suitable breeding ground for these flies, and if these are not properly protected, washed or cooked, become infected and the larvae are accidentally ingested, and are able to live in the intestines.

Many cases of myiasis have also been reported from India (Bapat, 2000; Mahipal *et*

al., 2002; Sehgal *et al.*, 2002) but in most of these cases the correct identification of the fly maggots is lacking. Long lists of names of flies are given in the books of medicine, but no mention is made as to how the various larvae of Indian species could be identified. For this reason very little effort is made by medical practitioner to rear the larval forms into adults.

Apart from causing myiasis, the flies belonging to families Calliphoridae, Sarcophagidae and Muscidae are vectors/ transmitters of diseases like poliomyelitis, cholera, typhoid fever, bacillary dysentery, trachoma virus, enteric infections, leprosy, tuberculosis, etc.(Patton, 1922; Zumpt, 1965; Greenberg, 1971,1973).

Keeping in view the medical importance of these flies, an attempt is made to enlist the calliphorid species.

Results and Discussion

Morphology of the larvae

A calliphorid larva is generally identified and distinguished from other dipteran larvae on the basis of following characters:

A typical calliphorid larva has twelve segments; one cephalic, three thoracic and eight abdominal segments (Fig.1a).

The second segment bears an anterior spiracle on either side in second and third instar. It is a fan shaped multi-lobed respiratory structure which represents the sclerotized anterior end of the large tracheal branch (Keilin, 1944). The number of lobes is of systematic use as each species posses a limited range; for e.g. *Calliphora vomitoria* possesses 9-12 lobes in the third instar where as *Calliphora vicina* larvae possess 5-8 lobes(Fig.1b).

The first and second larval segments together contain the cephalopharyngeal skeleton. The following nine segments show few distinguishing features other than the arrangement of spines. The twelfth segment, however, shows several features of taxonomic interest, the most important being the posterior spiracle. In the first instar these are simple, kidney shaped structures, whereas in the second and third instars the spiracle consist of an outer heavily sclerotized ring, the peritreme, which surrounds the spiracular apertures. In the second instar the peritreme is incomplete at the ventral end and there are two apertures. In the third instar the peritreme is complete and a button is present at the ventral end, which represents the ecdysial scar of the second instar spiracle (Kurahashi, 1985). Three apertures are present in the third instar (Fig.1c).

In addition, there are present the four foci of a 'sun-ray' structure; these are presumed to strengthen the spiracle. Spiracle distance factor (SDF) is of utmost value in distinguishing larvae at species level. It is calculated by dividing the distance between the spiracles by the greatest diameter of one spiracle.

Last segment also possesses seven pairs of papillae on its posterior surface (numbered P1 to P7). The position of P2 in relation to P1 and P3 is of taxonomic value(Fig.1d).

Currently no identification key to immature stages of Indian calliphorids is available, so the above mentioned diagnostic characters will help the medical practitioner to distinguish the calliphorid larva from other dipteran larvae.

Notes on Indian Calliphorids

***Chrysomya megacephala* (Fabricius, 1794)**

Musca megacephala: Fab., 1794,Syst. Ent.,4:317

Chrysomya megacephala: Seguy, 1928, Encycl.Ent.,B II Dipt.,4:101

Chrysomya megacephala: James, 1977:542.

Type locality: Guinea

Distribution: Pantropical, widespread in Oriental region: India, Nepal, Thailand, Malaysia, Indonesia (Java,Maluku,Timor); widespread in Australian and Oceanian region.

Bionomics:

This fly is a common scavenger in India(Bharti & Singh,2003) and sometimes produces myiasis of man and domestic animals (Bakar *et al.*,1983). Adults are generally found on garbage and are attracted to decaying meat and human excrement. It could be found at an elevation of up to 2200m (Senior White *et al.*, 1940; Kurahashi&Thapa, 1994; Sukontason *et al.*, 2006).

***Chrysomya rufifacies* (Macquart,1843)**

Lucilia rufifacies: Macquart, 1843:303(146). Type locality: Nouvelle-Hollande [Australia]
Lucilia orientalis: Macquart, 1843:302(145). Type locality:Pondicherry,India
Lucilia pavonina: Schiner, 1868:305. Type locality: Kar Nicobar and Tellnschong
Somomyia barbata: Bigot, 1877:39. Type locality:India
Chrysomya cordieri: Seguy, 1925:303.Type locality: Sockaboemi, Java [Indonesia]
Chrysomya rufifacies: Senior-White, Aubertin&Smart, 1940:141
Chrysomya albiceps rufifacies: Kurahashi, 1971:3
Chrysomya rufifacies: James, 1977:542
Chrysomya rufifacies: Inder Singh, Kurahashi & Kano, 1979:11
Chrysomya rufifacies: Kurahashi & Thapa, 1994:224
Distribution: India, Nepal, Srilanka, South China, Thailand, Malaysia(Malaya,Borneo),Singapore,Indonesia (Java,Maluku), Philippines; Palaearctic region: China, Korea, Japan. Australian & Oceanian regions: Guam, Marshall Islands, Hawaii Islands, Indonesia, PNG, Vanuatu, New Caledonia, Fiji & Australia.

Bionomics:

Larvae of this fly are commonly known as 'hairy' maggots as the first and seventh abdominal segments bear several pairs of finger-like papillae. They are predacious in nature and attack other larvae of Calliphoridae, Sarcophagidae and Muscidae found in the same breeding place (Kurahashi et al., 1997; Bharti & Singh, 2003). This species is known to be involved in secondary myiasis.

***Chrysomya bezziana* Villeneuve,1914**

Chrysomya bezziana Villeneuve, 1914: 430. Type locality: Africa
Chrysomya bezziana: Kurahashi, 1971:3
Chrysomya bezziana: James, 1977: 541
Distribution: India; widely distributed in the oriental region, including New Guinea (Irian Jaya & PNG) and Bismarck Arch.

Bionomics:

This species is commonly known as "Old World screw-worm" fly. It is an obligate parasite (specific myiasis producing flies) and unlike Chrysomya megacephala and Chrysomya rufifacies never breeds in the dead bodies of animals. The larvae are commonly found in many diseased tissues and organs of the human body and particularly in the nose and accessory sinuses. These flies generally oviposit on fresh wounds and are attracted by smell of blood, but never deposit its egg or larvae on the unbroken skin. Eye infestation has been reported by number of scientists (Patton, 1922; Zumpt, 1965; Greenberg, 1971, 1973). C. bezziana is one of the most important producers of myiasis in man and domestic animals in the

old World tropical countries (Spradbery & Vanniasingham, 1980; Zahedi & Jeffery, 1982; Bakar et al., 1983; Vellayan et al., 1984).

***Calliphora pattoni* Aubertin,1931**

Calliphora pattoni: Aubertin, 1931. Ann. Mag. Nat. Hist., (10)8:615

Calliphora pattoni: Tumrasvin, Kurahashi and Kano, 1976, Bull. Tokyo Med. Dent. Univ., 23: 211-216. Type locality: India, Darjeeling

Distribution: India (West Bengal), Nepal, Thailand, Burma and Taiwan.

Bionomics:

This species is usually found in evergreen forests, alpine flowers and few flies are found on garbage piles around human dwellings. According to Senior-White et al., 1940 this species is larviparous. The fly is responsible for transmission of various bacteria for bacillary dysentery, typhoid fever and other salmonelloses, poliomyelitis etc. and for causing facultative myiasis.

***Calliphora vomitoria* (Linnaeus,1758)**

Musca vomitoria: Linnaeus, 1758:595. Type locality: Sweden

Calliphora vomitoria: James, 1964: 171

Distribution: Cosmopolitan, widespread in Palaearctic region: Canary Islands, Eurasia, Afghanistan, India, Nepal, Mongolia, China, Korea and Japan. Australasian and Oceanian regions: Hawaiian Islands, Australia, New Zealand. Widespread in Nearctic and Neotropical regions.

Bionomics:

Adults are commonly found in mountainous areas up to 3800m. Males are frequently found in evergreen forests, but females are abundant on human faeces, garbage piles, and decomposed materials around human dwellings. This fly is responsible for transmitting bacillary dysentery, typhoid fever, poliomyelitis etc.

***Lucilia sericata* (Meigen,1826)**

Musca sericata: Meigen, Sitz. Beschr. V, p.53, 1826

Lucilia basalis: Macquart, Mem. Soc. Royal Agric. Arts, Lille, p.305, 1842

Lucilia flavipennis Macquart (nec.kram.), Mem. Soc. Roy. Agric. Arts, Lille, P.296, 1842; id., Kipt. Exot. iii, p.139, 1842

Musca lagyra: Walker., List. Dipt. Brit. Mus. IV, p.885, 1849

Lucilia barberi: Townsend, Smiths. Misc. Li, p.121, 1908

*Lucilia girault*i: Townsend, smiths. Misc. Li. P.121, 1908

Type locality: Europe

Distribution: Cosmopolitan.

Bionomics:

There is a large amount of literature on this insect in connection with its habit of 'blowing' sheep (i.e. to lay eggs). Much investigatory works has also been done on its physio – chemical ecology. In temperate climates the fly is comparatively harmless, but in Africa and Australia it is one of the species most intimately connected with the blowing of wool, and is a serious pest (Senior White et al., 1940). Larvae are usually scavengers but frequently invade the injured human tissue. In most instances the damages produced by these larvae are traumatic, such as extension of the pre-existing wounds (Kurahashi, 1997).

***Lucilia illustris* (Meigen,1826)**

Musca illustris: Meigen, Sitz. Beschr. V, p.54, 1826

Musca parvula: Meigen , Sitz. Beschr. V, p.55, 1826

Musca equestris: Meigen, Sitz. Beschr. V, p.57, 1826

Lucilia lepida: Robineau –Desvoidy, Myodaires, p.453, 1830
Lucilia fraternal: Maquart, ibid.
Musca muralis: Walker, List Dipt. Brit. Museum
Lucilia Caesar: Hough (necl.) Zool. Bull. P.288, 1899;
Townsend (necl.), Ann. Ent. Soc. Amer. xxi, p.121, 1928.
Type locality: Europe
Distribution: India (Himalaya), Burma, China, North America.

Bionomics:
This species is responsible for causing enteric myiasis in man. Generally inhabits filthy places and therefore carries pathogenic organisms to human food such as polio virus. Sterile larvae of this species and of Lucilia sericata are used in maggot debridement therapy (MDT) for curing wounds.

Acknowledgements
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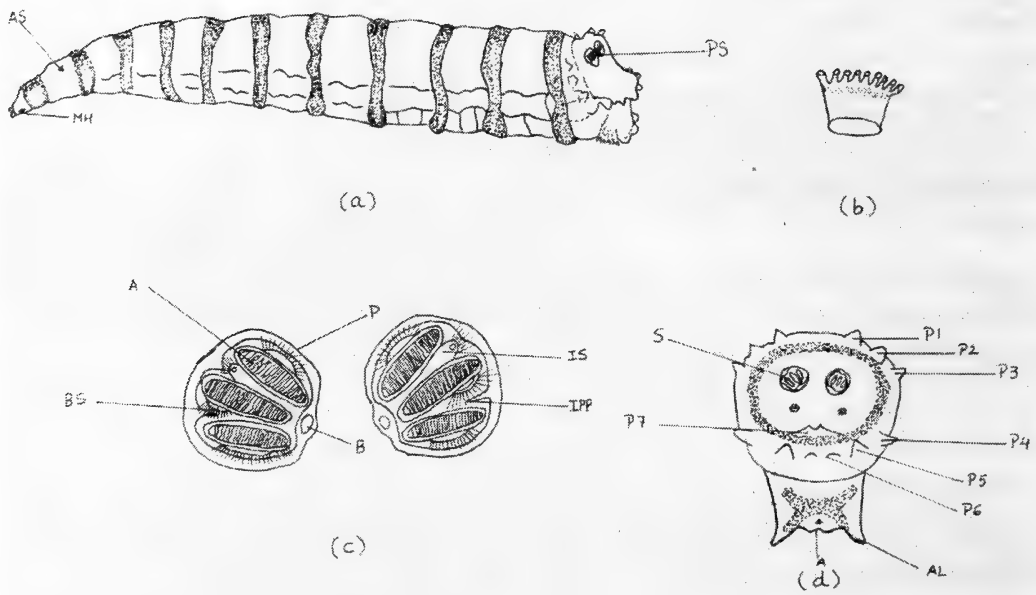

Fig: 1. Morphology of generalized third instar larva. a, entire larva (AS,Anterior spiracle; MH, mouth hooks; PS, posterior spiracle); b, anterior spiracle; c, posterior spiracles (A, aperture of slit; B, button; BS, blister structure; IPP, internal peritremal projection; IS, intermediate structure; P,peritreme); d, posterior view of 12thy segment of third instar larva (A, anus; AL, anal lobe; P1-p7, posterior papillae; S, posterior spiracle)

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Biochemical changes in the midgut during metamorphosis in *Apis cerana indica*

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Abstract

The digestive cells of midgut are responsible for the secretion of various enzymes and absorption of nutrients. During the process of metamorphosis midgut passes through, histolysis and histogenesis. As a result, destruction of larval tissue and construction of adult tissue occurs. The present work thus carried out is to know the changes that occurr in the biomolecules like DNA, RNA, Proteins, Carbohydrates etc., in relation with the remodeling of gut. Along with these biomolecules, various enzymes like amylase, invertase, protease and lipase are also estimated to know their status during metamorphosis of midgut in *Apis cerana indica*.

Keywords- *Apis cerana indica, Midgut, Metamorphosis, Biomolecules.*

Introduction

Apis cerana indica is one of the indigenous species of honeybees, used for apiculture in India. The alimentary canal of the bee can be divided into three distinct regions foregut, midgut and hindgut (Snodgrass, 1935 and 1956). The midgut is the main site for the digestive processes in most of the insects (Wigglesworth, 1972 and House, 1974), the midgut epithelium is composed of columnar cells which secretes the enzymes, carries out digestion and absorption (Wigglesworth, 1977). The ultrastructural and cytochemical aspect indicates that the midgut is remodeled during metamorphosis and shows the programmed cell death in the honey bee larvae and pupae (Gregorc and Bowen, 1996 and 1997, Cruz-landim and Cavalcante 2002 and 2003, Neves *et al.,* 2003 and Barsagade and Kelwadkar, 2008). The peptides of insect brain and midgut are suggested to have important regulatory role in digestive processes like, enzyme secretion, epithelial tissue regeneration, absorption of nutrients, working of gut musculature and maintenance of gut pH (Prabhu and Sreekumar, 1994 and Sunitha *et al.,* 1999). In *Apis cerana indica* remodeling of gut occurs through histolysis and histogenesis during metamorphosis but no information is available regarding biomolecule concentration and protein profile in the midgut cells during metamorphosis. Therefore, present work has been undertaken to know the changes in biomolecules, enzymes and protein profile in the midgut epithelial cells of larvae and pupae of *Apis cerana indica.*

Materials and Methods

Biochemical Techniques

Extract Preparation:

The midguts were dissected out from the

larval and pupal stages, in ice-cold saline/ Ringer's solution. The dissected midguts were homogenized using pestle and mortar for 5 minutes at room temperature in the Ringer's solution. 25 mg tissue/ml volumes of Ringer solution was added, sample was homogenized at 3000 rpm and the supernatant after centrifugation was used for estimation of total concentration of protein according to the Lowery *et al.,* (1951) method. The total carbohydrate was estimated by Phenol-Sulphuric acid method of Dubois *et al.,* (1962), DNA and RNA by Bruton's Diphenylamine and Dische-Orcinol methods of Searcy and MacInnis (1970a and 1970b).

Biochemical Estimation of Digestive Enzyme Activity

Preparation of enzyme extract:

The enzyme extract preparation was carried out according to the method of Applebaum *et al.,* (1964) with some modification. Only midgut was taken from both larvae and pupae, kept in ice-cold insect Ringer's solution, accurately weighed and homogenized for 3 minutes at 0°C in ice-cold citrate phosphate buffer (pH–6.8) using pestle and mortar. The midgut was suspended in ice–cold buffer and made up to 1 ml, the homogenate was centrifuged at 10,000 rpm for 15 minutes and the supernatant was used as an enzyme for estimation of digestive enzyme activity. The enzyme activity of amylase and invertase were estimated by the methods of Ishaaya and Swirsky (1970), while protease activity by Snell and Snell (1971) method and lipase activity by the method of Cherry and Crandel (1932).

Preparation of midgut for bioassay experiment:

Alimentary canal was dissected out in insect saline, the midgut was separated, contents of the midgut were removed by injecting the insect saline into the open midgut tube and contents were flushed out. The epithelial tissue was washed in insect saline and transferred to fresh saline. The extract of the midgut epithelia was prepared, the homogenate having a concentration equivalent to two midgut epithelia / 10 ml saline was bioassayed for its effect on in vitro digestive enzyme secretion in preparation of the midgut. The midgut preparation was incubated with 2 ml of incubation solution (midgut epithelial extract) in the bioassay apparatus for 30 minutes with a bubbling gentle stream of oxygen. After incubation, the midgut preparation was taken out and washed in insect saline, the gut was opened and contents were collected in 0.5 ml distilled water for estimation of protease and amylase activity. In control experiments, ligated midgut tubes were incubated in insect saline.

SDS-PAGE Electrophoresis

Midgut extraction was carried out by the method of Laemmli, (1970) with some minor modifications (Barsagade, 1998). The molecular weight of the protein bands was estimated with the help of Gel.Doc.

Results

Biochemical Analysis

Concentration of the biomolecules present in the midgut epithelial cells of larvae and pupae were found as-

Total DNA concentration

The total midgut DNA concentration in fifth instar was about 0.58 ± 0.08 µg/mg, it decreased to 0.3 ± 0.06µg/mg in early pupa. The total DNA concentration thereafter increased gradually up to 0.45 ± 0.04 µg/mg and 0.66 ± 0.08 µg/mg in mid pupa and late pupa respectively (Fig.1).

Total RNA concentration

The total RNA concentration in the fifth instar larvae was estimated as 12.2 ± 0.99 µg/mg while it was 7.12 ± 0.59 µg/mg in early pupa, increased to 9.12 ± 1.01 µg/mg in mid pupa and was found to be 18.6 ± 1.01 µg/mg in the late pupal stage (Fig-2).

Total Protein concentration

The total midgut protein concentration in fifth instar was about 4.5 ± 0.29 µg/mg. It decreased to 2.04 ± 0.22µg/mg in early pupa, the total protein concentration thereafter increased gradually up to 3.73 ± 0.31 µg/mg and 6.5 ± 0.14 µg/mg in mid pupa and late pupa respectively (Fig-3).

Total Carbohydrate concentration

The total carbohydrate concentration in the fifth instar larvae was estimated as 0.70 ± 0.0046 µg/mg while it was 0.67 ± 0.0023 µg/mg in early pupa, increased to 0.68 ± 0.0021 µg/mg in mid pupa and was found to be 0.72 ± 0.0041µg/mg in the late pupal stage (Fig-4).

Midgut Digestive Enzyme

The digestive enzymes, amylase, invertase, protease, and lipase in the midgut have been demonstrated qualitatively and quantitatively. Enzyme estimation in the larval and pupal stages is summarized in table 1. and fig.5.

Table-1: Enzyme estimation in larval and pupal stages of *Apis cerana indica*. (Mean ± SD)

S. No.	Enzyme	V-instar µg/mg	Early Pupa µg/mg	Mid Pupa µg/mg	Late Pupa µg/mg
1.	Amylase	0.19± 0.0054	0.53± 0.0187	0.028± 0.022	0.32± 0.014
2.	Invertase	0.34± 0.015	0.44± 0.0122	0.77± 0.015	0.80± 0.055
3.	Protease	0.75± 0.0057	0.78± 0.01	0.43± 0.0291	0.82± 0.021
4.	Lipase	4.5± 0.05	5.5± 0.291	2.9± 0.212	3.2± 0.254

Effect of Midgut Extract

Bioassay experiment was conducted to study the effect of midgut extract on the midgut amylase and protease activity in the fifth instar larvae of honey bee *Apis cerana indica* (Fig.6). During the bioassay experiment the midgut extract showed the significant ($P<0.0001$) effect elevating amylase and protease activity in fifth instar.

Amylase activity:

The midgut amylase activity was measured about 0.71 ± 0.089 mg glucose/midgut /minute after the incubation in midgut extract for 30 minutes while, it was observed 0.51 ± 0.071 mg glucose/midgut/minute in the normal condition.

Protease activity:

The midgut protease activity was measured about 0.94 ± 0.091 mg protein/midgut/minute after incubation in midgut extract for 30 minutes while, it was noticed about 0.79 ± 0.083 mg protein/midgut/minute in normal condition.

Electrophoretic Analysis

The SDS PAGE electrophoretic analysis of midgut extract demonstrated about eleven bands of protein among which six bands were predominant ranging from 26-65 kDa molecular weight in first to fifth instar larvae, early, mid and late pupae of *Apis cerana indica* .While an additional band of 12KD protein was found only in late pupa (Fig. 7).

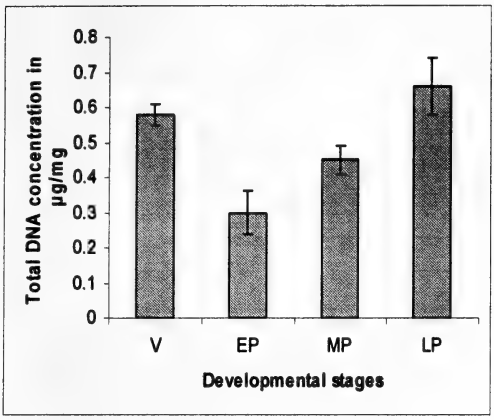

Fig. 1 Total DNA concentration during post-embryonic development

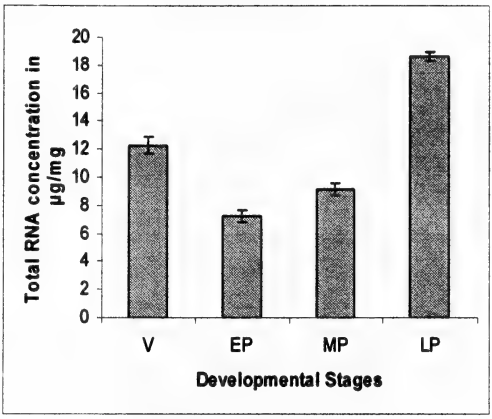

Fig. 2 Total RNA concentration during post-embryonic development.

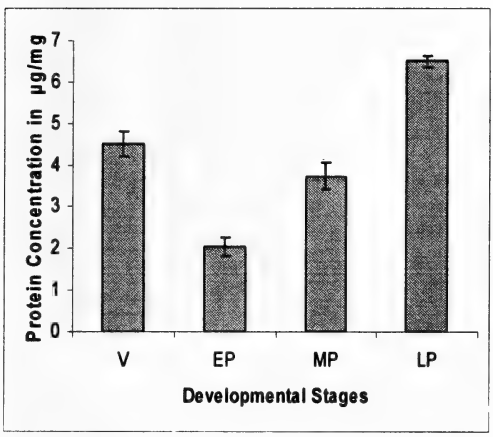

Fig. 3 Total protein concentration during post-embryonic development.

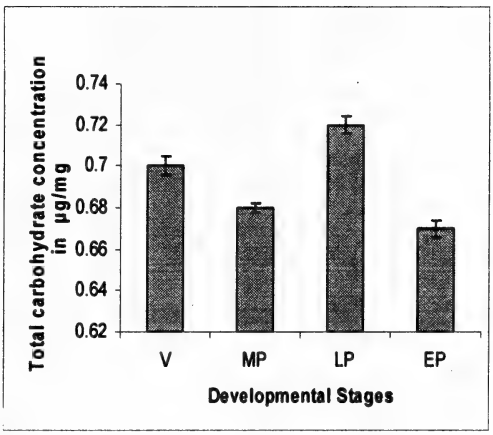

Fig. 4 Total carbohydrate concentration during post - embryonic development.

Abbreviations : V – Vth Larval stages ; EP- Early pupa ; MP – Mid pupa ; LP –Late pupa

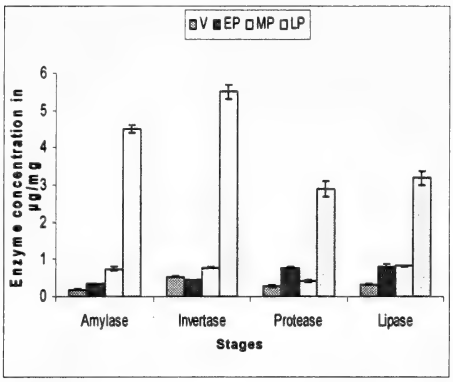

Fig. 5 Estimation of enzymes in larval and pupal stages during post-embryonic development.

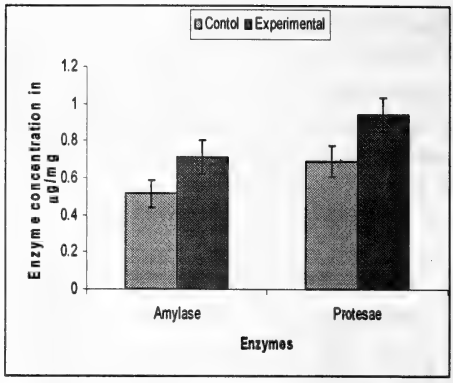

Fig. 6 Effect of midgut extract on midgut activity in V-instar larva.

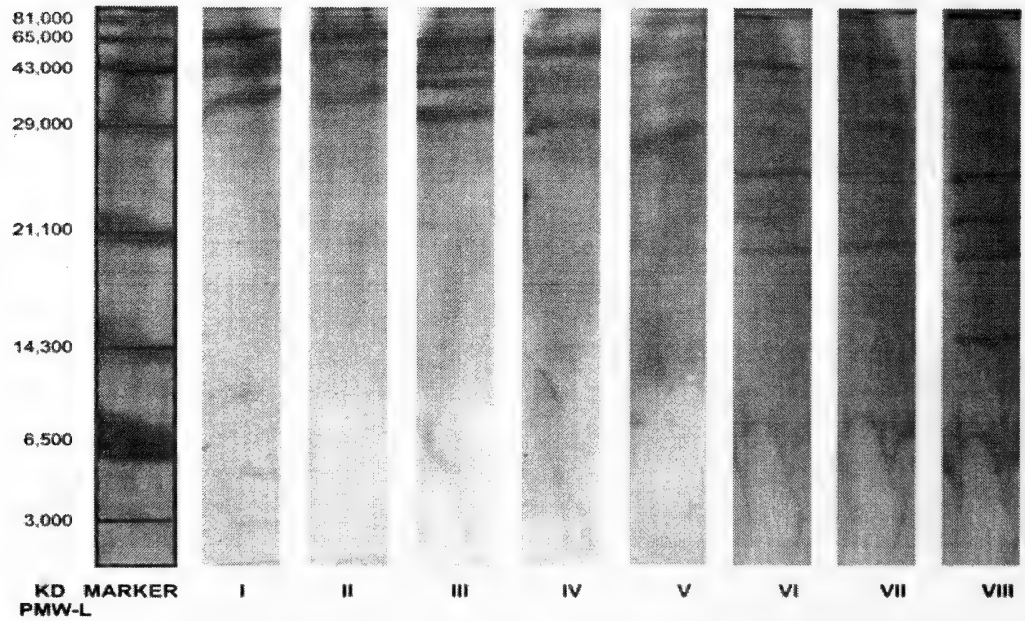

Fig. 7 : SDS-PAGE analysis of the midgut extract of larvae of *Apis cerana indica* showing the presence of protein pattern.

Abbreviation: I- first instar, II- second instar, III - third instar, IV - fourth instar and V- fifth instar, VI-early pupa, VII- mid pupa, VIII-late pupa.

Discussion

During the post embryonic development, the differentiation of midgut epithelial cells occurs in honey bee (Chapman, 1985a and 1985b and Cruz-landim and Cavalcante, 2003) while in the successive development, replacement of larval midgut epithelial cells to adult by formation of new epithelium is found in *Apis cerana indica* (Barsagade and Kelwadkar, 2008). In the last larval instar the midgut epithelial cells, produce various digestive enzymes that help for the food digestion in *Apis mellifera* (Cavalcante and Cruz-Landim, 2004). During larval development midgut cells are replaced by the other cells depending upon death of larval digestive cells, proliferation of regenerative cells occurs in order to constitute a new digestive epithelium in the adult, *Apis mellifera* (Geogorc and Bowen, 1997) and in *Apis cerana indica,* (Barsagade and Kelwadkar, 2008).

The midgut columnar epithelial cells containing the biomolecules such as DNA, RNA and Proteins are actively engaged in the protein synthesis in order to secure various digestive enzymes during the larval–pupal metamorphosis (Fuji, 1979, Wigglesworth, 1972 and Chapman, 1998).

In *Apis cerana indica,* total DNA, total RNA, total proteins and total carbohydrates were intensely demonstrated in the nuclei and perikarya of columnar epithelial cells, as found in *Apis florea* and *Apis mellifera,* described by earlier workers showing their role in protein synthesis (Pearse, 1968; Ban and Prezlec, 1974). The similar role of these biomolecules may be played in the protein synthesis during metamorphosis in *Apis cerana indica.*

Various biochemical tests reveal the presence of amylase, invertase, protease and lipase activity in the midgut of larvae of *Apis cerana indica* which are very specific in their activity (Horie, 1970, Banergee and Saxena, 1983, Wajiro *et al.,* 1984 and Zufelato *et al.,* 2004). The higher level of enzyme acid phasphatase activity in the larval peritrophic membrane in *Apis mellifera* is mostly used for digestion (Cavalcante and Cruz-landim, 2004). Similarly, activity of other fourteen enzymes was determined in the midgut of *Apis mellifera* and these enzymes were alfa-amylase, 4 alfa-glucosidase, 3 protease, 5 amino peptidase, lipase etc. (Banerjee and Sexena,1983). According to Mahmoud (2007), the high food metabolism level depends on the protein concentration in the midgut and haemolymph, it may reflect the digestion level with acid phasphatase and other digestive enzymes. The present investigation reveals that the concentration of biomolecules viz. total DNA, total RNA, total protein and total carbohydrates in the fifth instar larvae of *Apis cerana indica*, shows initial depletion and then a significant rise because of involvement of total protein consumption in the midgut for synthesis of digestive enzymes.

The study also supports the findings of earlier works, showing the amylase, invertase, proteases and lipase activity during the larval-pupal metamorphosis in *Apis cerana indica.* Cavalcante and Cruz-landim (2004), noticed the mass range of different proteins varying form 19 to 142 kDa, increased greatly from larvae to pupae and tends to decrease during pupation, until the phase of brown eye pupae and increased again in the pupae with pigmented body (pharate adult). In *Apis cerana indica,* 11 bands were observed, among which six bands were predominant ranging from 26–65 kDa, found in the midgut extract of developing larvae and pupae, supporting the earlier studies.

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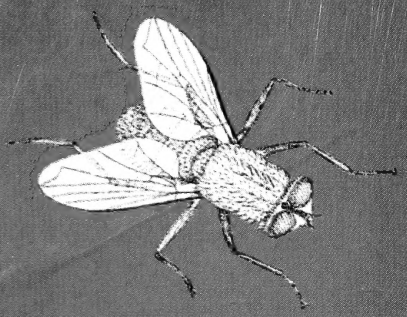

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