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Effect of environment on seasonal dynamics of rat house mites at Pune

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Abstract
Mites are among the most diverse and successful of all the invertebrate groups. They have exploited an incredible array of habitat and act as ectoparasite. Animals with mite infestations have varying clinical signs ranging from none to mild alopecia to severe pruritus and ulcerative dermatitis. Mites have been shown to be important sources of indoor allergens associated with asthma and other allergic conditions in sensitive victims. Hence the monthly and seasonal study has been carried out in the intramural environment of rat house at Pune, for one year (for three seasons) from February 2015 to January 2016 to analyze the environmental impact on the incidence, population density, monthly variation, seasonal variation and dynamics of rat mite fauna by 'simple pickup technique' under stereo binocular dissecting microscope and Leica binocular research microscope.
Total 593 mite specimens have been screened, identified and classified into male and female under eight genera including five species and three unidentified species named as *Echinilaelaps echidninus* (Ee), *Haemolaelaps glasgowi* (Hg) belonging family Laelaptidae and *Gohieria fusca* (Oudemans) *(Glycyphagidae*, *Cheyletus eruditus* (*Cheyletidae*), *Dermatophagoides farinae* and Unidentified U1,U2,U3.
Echinolaelaps echidninus has been found dominant species during rainy 253 (42.66%) followed by summer season 92 (15.51%) and winter season 69 (11.64%). *Haemolaelaps glasgowi* contributed 51 (8.60%) in rainy season, 40 (6.74%) in summer season and 80 (13.49%) in winter season. Other specimens like *Gohieria fusca* (0.33%), *Cheyletus eruditus* (0.33%), *Dermatophagoides farinae* (0.16%) and Unidentified specimens (U_1, U_2, U_3) and one unidentified larva has been also observed. The Ee has been found to be dominant 414 (69.81%) species followed by Hg 171 (28.83%0 and very few specimens of other genera.

Keywords: Ectoparasite, Dermatitis, Allergy, *Echinolaelaps echidninus, Haemolaelaps glasgowi, Gohieria fusca, Cheyletus eruditus, Dermatophagoides farinae,* Dynamics.

1. Introduction
During the 1970s and 1980s there were tremendous efforts to eradicate infectious diseases from laboratory rodent colonies in order to eliminate their undesirable effects on research. Advances and refinements in science demanded high-quality animal subjects devoid of infectious diseases, commercial vendors, animal resource programs facilitated the eradication efforts through rederivation procedures and advances in bioexclusion caging and husbandry practices [1]. Animals that appear normal and healthy may be unsuitable as research subjects due to the unobservable but significant local or systemic effects of viruses, bacteria, and parasites with which they may be infected [2].
Mite infestation in laboratory animal is a common, but troublesome problem in animal facilities. They have exploited an incredible array of habitat and act as ectoparasites [3]. Recommended treatment regimens are frequently ineffective because of the short period of exposure to the control agent [4]. It is far better to prevent the introduction of pathogens than to account for their presence when interpreting experimental results. The drugs used to clear pathogens, will themselves alter the host physiology and interfere with research [5-7].
Efforts have been made to discover different species of mites and to keep their record, The American Scientist discovered *Dermatophagoides pteronyssinus,* a house dust mite as prominent cause of house dust mite allergy and not the proper dust in the homes as presumed earlier [8]. Accordingly, through further studies in all 36 species have been recorded world over as offending allergens [9], 29 species from West Bengal [10], 23 from Karnataka [11], 20 from Maharashtra [12] and 17 from Kerala [13]. Now the Indian record has been raised to 36 and record of Maharashtra to 27, updating the world record to 43 [14].
The dynamics of mite populations on a host is very complex and influenced by factors that include grooming, strain susceptibility and host immune responses. It was necessary to develop specific diagnostic assays and an understanding of their epidemiology and transmission routes before attempting eradication and then evaluate eradication methods for efficacy. Therefore, the present study has been carried out in the intramural environment of rat house to find the impact of an environment on the dynamics of rat house mites for exploring management of mite population in the rat houses and suspected allergic manifestations among researchers and workers handling rats in the rat house.

2. Materials and methods
Rat house has been considered as intramural detritus ecosystem comprising biotic components like microbes of bacterial and fungal or some times of viral origin from plant kingdom and mites, eggs of mosquitoes, cockroaches, lice, bed bugs etc from animal kingdom. Dusts in the houses constitute abiotic components including dandruff, cosmetics, debris, paints, colors etc. These biotic and abiotic components of detritus ecosystem constitute the material for the subject. Here we have selected study of mites from the husks of 'Rat House' at Pune.

Simple pickup method from husk samples under the stereo binocular dissecting microscope has been used for the separation of mite specimens from rat house husk. The fresh samples of rat house husk have been collected fortnightly regularly on the fixed dates for eg. 1st and 16th of every month at a fixed time for example at 8 a.m. sharply in sufficient quantity using paper envelope and immediately brought to the research laboratory.

2.1 Examination of Rat husk samples

Two grams of these fresh samples have been spread in a clean, dry and sterile petridish with a uniform thin layer and observed under a stereobinocular dissecting microscope with illumination device below, so that mites get exposed, agitated and illuminated when they start their movements and thus easily detected in the husk due to their prominent shape, size and color.

2.2 Isolation of mites

These mites have been manually picked up and collected using a moist needle dipped in 4% lactic acid and stored in lactic acid in a cavity block. Because on touching the mite in the husk by tip of moist needle, it sticks to needle and thus collected.

2.3 Clearing and mounting

Depending upon the extent of sclerotization of body cover they have been kept safe inside the lactic acid for one to three days and mounted when they become transparent, in the melted glycerin jelly, keeping ventral posture up. The cover slip is then gently pressed with a blotting paper to remove excess jelly and for proper spreading of body parts and leg-pairs. They get solidified on cooling to normal temperature and the slides are ready for microscopy and photography.

2.4 Identification

The mites have been identified according to the keys given scientists [15-18] and other authentic literature. These specimen slides have been screened under Leica binocular research microscope with camera, monitor and measurement facility in a special Leica room.

3. Results and Discussion

During the study period totally 593 specimens have been recorded comprising two genera/species *Echinolaelaps echidninus* (Ee) [19], *Haemolaelaps glasgowi* (Hg) [20] as dominant ones showing their association with rats and other six species/genera *Gohieria fusca*, *Cheyletus eruditus*, *Dermatophagoides farinae* and Unidentified specimens (U_1, U_2, U_3) including one unidentified larva as very rare specimens. Interesting findings have been recorded as briefly described and discussed here. Overall *Echinilaelaps echidninus* has been found to be dominant 414 (69.81%) throughout the study period comprising 208 (35.07%) males and 206 (34.73%) females. Out of 206 females of Ee 174 (84.47%) females have been found egg bearing and 32 (15.5%) non egg bearing. Overall Hg has been found to be 171 (28.83%), comprising 101 (17.03%) males and 70 (11.80%) females. Total number of males has been recorded (101) more than that of females (70). Out of 70 females of Hg 49 (70%) females have been found egg bearing and 21 (30%) non egg bearing. (Table No. 1 and 2)

The color of *Echinilaelaps echidninus* is reddish brown and attains a length of about (1137 X 580 µm) male and female (1122 X 594 µm). The genito-ventral plate is widely expanded posterior to coxae IV and extends nearly to the anal plate from which it is separated by a very thin strip of integument.

The percentage contribution of males and females of Ee during summer, rainy and winter season has been found to be 56 (9.44%), 102 (17.20%), 50 (8.43%) and 36 (6.07%), 151 (25.46%), 19 (3.20%) respectively. Highest load of Ee has been found in rainy season 253 (42.66%) as compared to summer 92 (15.51%) and winter 69 (11.64%). The highest contribution of males 102 (17.20%) have been observed during rainy season followed by summer 56 (9.44%) and winter 50 (8.43%). Maximum number females 151 (25.46%) has been observed during rainy season followed by summer 36 (6.06%) and winter 19 (3.20%). (Table No. 1, Fig. No. 2)

Ee has been prominently recorded and encountered in abundance during rainy season in accordance with meteorological parameters of June, July, August and September. (Table No.4) It has been also reported in good numbers during winter and summer conditions. From these findings it may be inferred that Ee has been reported in all the months round the year disobeying the usual rules of seasonal variation but has been found to show rhythmic typical dynamics of fluctuation exhibiting maximum incidence during rainy condition as compared to summer and winter conditions. (Table No.4)

Monthly variations have been observed on the percentage contribution of recorded species. Highest population of Ee has been observed during month of July 74 (12.48%) followed by month of August 72 (12.14%) while lowest in the month of January 05 (0.84%). The maximum load of males have been recorded in the month of September 35 (5.91%) and minimum in January 01 (0.17%). Similar number of males 19 (3.20%) have been encountered in the month of March, April and October. The females showed their highest contribution in the month of July 48 (8.09%) and lowest in the month of February 01(0.16%). (Table No. 1, Fig. No. 1)

The color of *Haemolaelaps glasgowi* is dark brown and attains an average length of about (508 X 315 µm) males and (1114 X 554µm) females. The genitoventral plate is separated from the anal plate by a distance distinctly greater than the length of anus.

Environmental parameters in different seasons affect mite population. Highest population of Hg has been observed during winter season 80 (13.49%) followed by rainy 51 (8.60%) and summer 40 (6.74%) as compared to other specimens. Out of total 171 (28.83%) Hg species, 101 (17.03%) males were observed which dominated over females. Maximum number of males 50 (8.43%) have been observed during winter season followed by rainy 31 (5.23%) and summer 20 (3.37%). (Table No. 2, Fig. No. 4)

Monthly variation in the dynamics of rat house mites has been observed, the highest load of Hg has been observed in the month of December 30(5.06%) followed by August 22 (3.71%) and October 21 (3.54%). Maximum contribution of males of Hg has been recorded in the month of December 22 (3.71%) and minimum in the month of March, May, June and January i. e. 03 (0.51%) each. The utmost population of females has been observed in the month of November 11 (1.85%) and the lowest contribution has been recorded in June 1 (0.17%). In March no any female specimen of Hg has been observed. (Table No. 2, Fig. No. 3)

With reference to meteorological data, Hg has been recorded maximum in the month of December at 22.2 ºC Temp., 38.7% relative humidity and lack of precipitation followed by sudden decline during January right up to June and slightly increased

during rainy months. Thus it does not exhibit much seasonal variation in relation with meteorological study. (Table No.4)

The monthly and seasonal observations of egg bearing and non egg bearing females of both dominant species of Ee and Hg have been recorded. Out of total Hg and Ee females, non egg bearing females of Ee have been found more 32 (11.59%) as compared to Hg 21 (7.61%).

In rainy season maximum egg bearing females of Ee have been encountered i. e. 131 (47.46%) as compared to summer 30(10.87%) and winter 13 (4.71 %). Highest load of egg bearing females of Ee has been recorded in the month of July 42 (15.22%) and lowest in February 01 (0.36%).

The highest contribution of Hg egg bearing females has been recorded during winter season 19 (6.88%) followed by summer season 16 (5.80%) and rainy season 14 (5.07%). Maximum number of females of Hg have been found egg bearing in the month of November 07 (2.54%) and minimum in the month of June 01 (0.36%). No females have been found egg bearing in the month of March. (Table No. 3, Fig No. 5)

The achievement of the present study was that, *Gohieria fusca* (2), *Cheyletus eruditus* (2), have been found for the first time from rat house during September (at 25.1 ºC Temp., 61.63% relative humidity and 10mm precipitation). Usually these are HDM and known to show seasonal variation, recorded maximum during rainy season [12] attaining peak during September hence shows impact of environment hence lacking in other seasons. Similarly three unidentified specimens U1 (02), U2 (01), U3 (01) and a larva have been reported during August 2015.Impact of environment including temperature, relative humidity and precipitation (24.5 ºC, 70.51% and 16mm) clearly indicate that these may be from the group of HDM and hence exhibit the effect of seasonal variation being absent during winter and summer conditions and found only in rainy condition. The house dust mite *Dermatophagoides farinae* has been observed in the beginning of October and obeying the rules of seasonal variation. (Table No.4, Plate No. 1)

Rat house mites may be considered as suspected allergens and need thorough investigations from clinical and parasitological point of view. They have been found to dwell as ectoparasites on the rats in the rat house sucking blood from them and turning blood red when fed, but have been found to cause harm on health of animal and these may exhibit “commensalism” type of association where animals are slightly harmed by the loss of blood. Through cross reference study, it has been observed that Jogdand reported rat and cat mites in the house dust [21].

It has been reported that Mesostigmatid blood sucking mites live in the environment adjacent to their rodent hosts and are freely mobile [22-23]. Rat mites can transmit several zoonotic diseases, *O. bacoti* and *L. echiddnina* bite humans. Permanent elimination of Mesostigmatid mite infestations requires a co-ordinated and sustained effort to eliminate mites and rodent vermin reservoir. Mites have been reported to survive two weeks to several months without feeding [24].

The general appearance of infested animal is not directly related to the size of the mite population. Infestations are commonly subclinical. Clinical signs include scruffiness, purities, patchy alopecia, self trauma, ulceration of skin, nodulation, splenic lymphoid hyperplasia and pyoderma. Close inspection often reveals bran like hyperkeratosis debris and mites on the skin around the base of hairs [25-27]. It has been observed that mice of the C57BL strains and their congeneic sublines are particularly susceptible to severe *M. musculi* related skin diseases (Companion guide to infectious diseases of mice and rats).

General observations have shown that house dust mites exhibit true seasonal variation every year during different seasons i.e. rainy (maximum load), winter (moderate) and summer (minimum)[14] while rat husk mites have been recorded in abundance during rainy and summer season also. Rat mite like *Echinolaelaps echidninus* and common rodent mite like *Haemolaelaps glasgowi* have been recorded during the study in abundance, with minimum fluctuations in the mites population dynamics irrespective of seasons and fluctuations in environmental factors thus disobeyed the concept of the seasonal variation.

The abundant availability of rat mites during the study period may be due to the constant favorable rat house environmental conditions like temperature, humidity and ventilation [28]. And most important is the body temperature of host animal (rat), moist condition of the husk due to presence of urine and faeces and leaked water from bottles. Thus it is the responsibility of the individuals in the laboratory animal science and research communities to work together to ensure the use of highest quality of laboratory rodent for specific research investigation.

Table 1: Monthly and Seasonal contribution of *Echinolaelaps echidninus* in the intramural environment at rat house, Pune.

Echinolaelaps echidninus						
Month / Season	**Total Load**	**Male**	**Female**	**% Total Load**	**% Male**	**% Female**
February	06	05	01	1.01	0.84	0.16
March	23	19	04	3.87	3.20	0.67
April	27	19	08	4.55	3.20	1.35
May	36	13	23	6.07	2.19	3.88
Summer	92	56	36	15.51	9.44	6.07
June	50	16	34	8.43	2.70	5.73
July	74	26	48	12.48	4.38	8.09
August	72	25	47	12.14	4.22	7.93
September	57	35	22	9.61	5.90	3.71
Rainy	253	102	151	42.66	17.20	25.46
October	25	19	06	4.22	3.20	1.01
November	18	13	05	3.04	2.19	0.84
December	21	17	04	3.54	2.87	0.67
January	05	01	04	0.84	0.17	0.67
Winter	69	50	19	11.64	8.43	3.20
Total	414	208	206	69.81	35.07	34.73

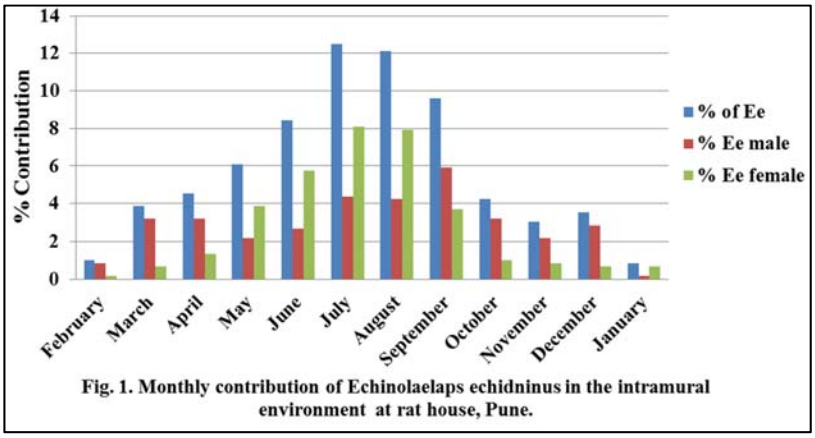

Fig. 1. Monthly contribution of Echinolaelaps echidninus in the intramural environment at rat house, Pune.

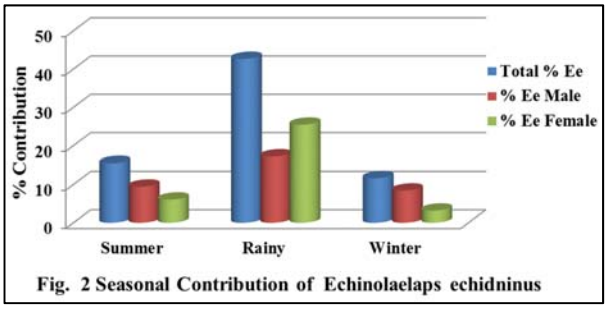

Fig. 2 Seasonal Contribution of Echinolaelaps echidninus

Table 2: Monthly and Seasonal contribution of *Haemolaelaps glasgowi* in the intramural environment at rat house, Pune.

Haemolaelaps glasgowi						
Month / Season	**Total Load**	**Male**	**Female**	**% Total Load**	**% Male**	**% Female**
February	13	06	07	2.19	1.01	1.18
March	03	03	00	0.51	0.51	00
April	16	08	08	2.70	1.35	1.35
May	08	03	05	1.35	0.51	0.84
Summer	**40**	**20**	**20**	**6.74**	**3.37**	**3.37**
June	04	03	01	0.67	0.51	0.17
July	09	04	05	1.52	0.67	0.84
August	22	14	08	3.71	2.36	1.35
September	16	10	06	2.70	1.69	1.01
Rainy	**51**	**31**	**20**	**8.60**	**5.23**	**3.37**
October	21	16	05	3.54	2.70	0.84
November	20	09	11	3.37	1.52	1.85
December	30	22	08	5.06	3.71	1.35
January	09	03	06	1.52	0.51	1.01
Winter	**80**	**50**	**30**	**13.49**	**8.43**	**5.06**
Total	171	101	70	28.83	17.03	11.80

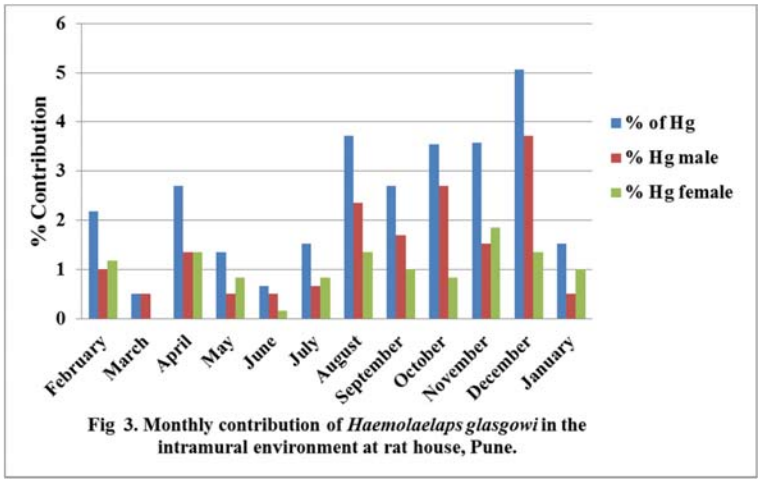

Fig 3. Monthly contribution of *Haemolaelaps glasgowi* in the intramural environment at rat house, Pune.

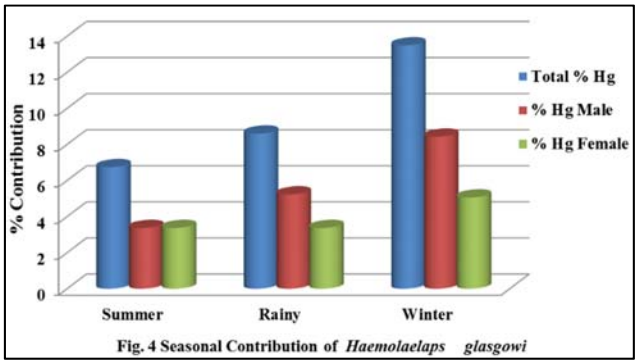

Fig. 4 Seasonal Contribution of *Haemolaelaps glasgowi*

Table 3: Monthly and Seasonal contribution of egg bearing and non egg bearing females in the intramural environment at rat house, Pune.

Month/ Season	Total Load of Egg bearing female		Total Load of Non Egg bearing female		% of Egg bearing female		% of Non Egg bearing female	
	Hg	Ee	Hg	Ee	% Hg	% Ee	% Hg	% Ee
February	06	01	01	00	2.17	0.36	0.36	00
March	00	03	00	01	00	1.09	00	0.36
April	06	06	02	02	2.17	2.17	0.27	0.72
May	04	20	01	03	1.45	7.25	0.36	1.09
Summer	16	30	04	06	5.80	10.87	1.45	2.17
June	01	30	00	04	0.36	10.87	00	1.45
July	03	42	02	06	1.09	15.22	0.72	2.17
August	06	39	02	08	2.17	14.13	0.72	2.90
September	04	20	02	02	1.45	7.25	0.72	0.70
Rainy	14	131	06	20	5.07	47.46	2.17	7.25
October	03	03	02	03	1.09	1.09	0.72	1.09
November	07	04	04	01	2.54	1.45	1.45	0.36
December	05	03	03	01	1.81	1.09	1.09	0.36
January	04	03	02	01	1.45	1.09	0.72	0.36
Winter	19	13	11	06	6.88	4.71	3.99	2.17
Total	49	174	21	32	17.75	63.04	7.61	11.59

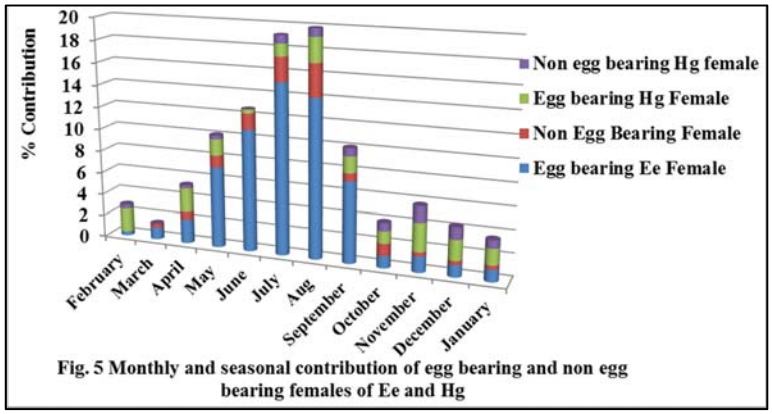

Fig. 5 Monthly and seasonal contribution of egg bearing and non egg bearing females of Ee and Hg

Table 4: Meteorological Study from February 2015 to January 2016

Month/ Season	Temperature (°C)	Relative humidity (%)	Precipitation mm	*Echinolaelaps ecidninus* (%)	*Haemolaelaps glasgowi* (%)	Other specimens (%)
February	22.0	26.82	0	1.01	2.19	
March	25.6	31.67	0	3.87	0.51	
April	28.8	26.8	1	4.55	2.70	
May	29.7	34.45	3	6.07	1.35	
June	27.4	61.6	10	8.43	0.67	
July	25.5	71.09	17	12.48	1.52	
August	24.5	70.51	16	12.14	3.71	U1,U2 and U3
September	25.1	61.63	10	9.61	2.70	*Gohieria fusca, Cheyletus eruditus*
October	25.0	53.16	5	4.22	3.54	*D.farinae*
November	22.3	43.1	2	3.04	3.37	
December	20.2	38.7	0	3.50	5.06	
January	21.0	30.41	0	0.84	1.52	

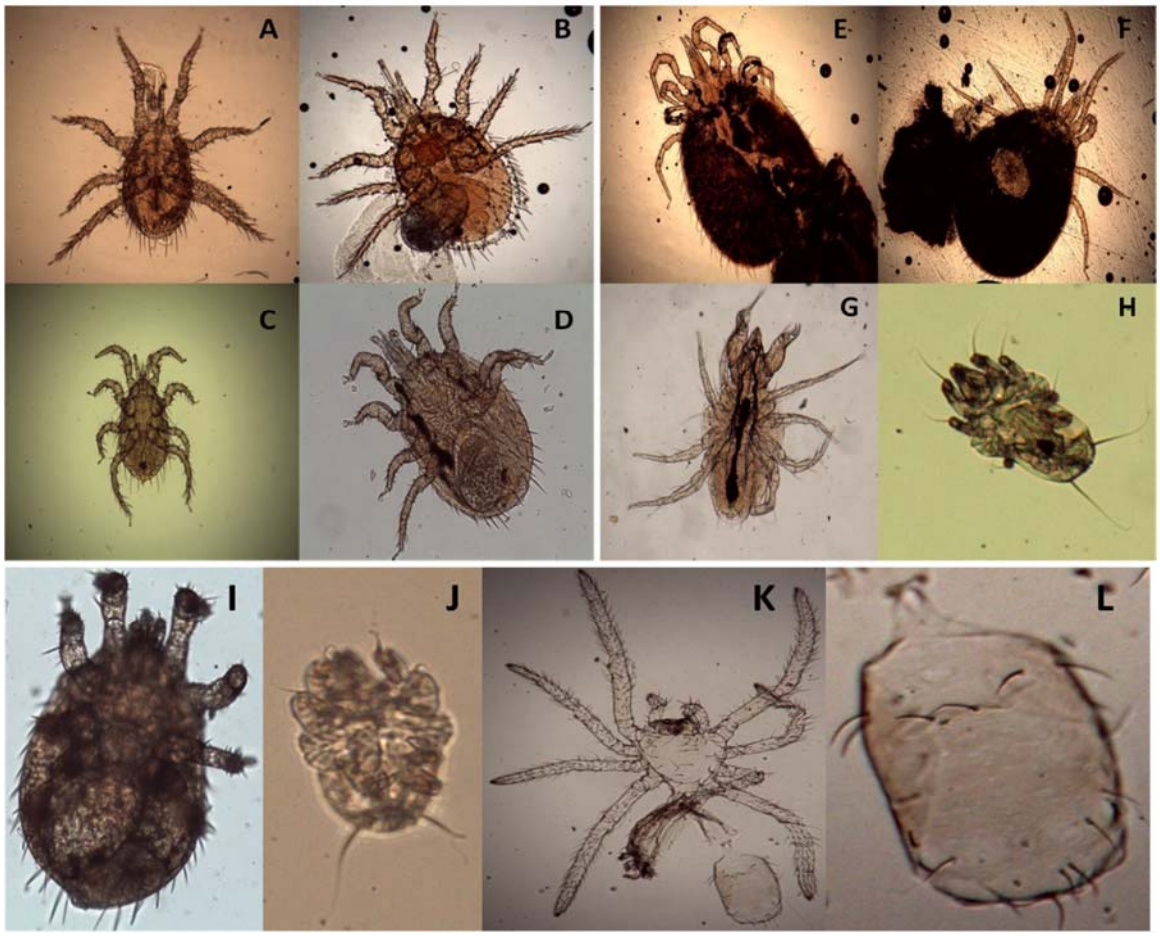

Plate 1: Various species observed during intramural environment study at rat house.

A) *Echinilaelaps echidninus* Male **B)** *Echinilaelaps echidninus* Female
C) *Haemolaelaps glasgowi* Male **D)** *Haemolaelaps glasgowi* Female
E) *Gohieria fusca* Male **F)** *Gohieria fusca* Female
G) *Cheyletus eruditus* **H)** *Dermatophagoides farina* **I)** Unidentified U1 **J)** U2 **K)** U3
L) Unidentified Larva.

4. Conclusion

This study has opened a new thrust area from intramural environment for further detailed exploration of rat house husk mites. Environmental factors play detrimental role in the HDM exhibiting clear seasonal variation but here in rat mites the impact of environmental parameters is not so pronounced as in HDM. Hence it appears to be insignificant and rat mite load has been found in good numbers during all the seasons including in summer season also. It may lead to management of mite population by various methods like starvation, chemical control, thermal control etc. and may help to solve the problem of mite allergy. Therefore the preliminary care should be taken when working in a research laboratory. The appropriate protective equipment should be wear when working with animals. Immunotherapy is well known method in majority of allergy cases to cope up with the allergy problems. So far antigen testing of rat mites has not been done, so this study may be found useful to supply specimens for antigen testing, manufacture of vaccines and give relief to allergy patients by immunotherapy.

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