Asian J. Exp. Sci., Vol. 35, No. 2, 2021; 27-32

Online available on : www.ajesjournal.com, ISSSN : 0971-5444

Effect of Temperature and Relative Humidity on Viability of *Campanulotes bidentatus compar* (Phthiraptera: Ischnocera)

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Received: May 2, 2021; Revised: June 8, 22, 2021; Accepted: June 15, 2021

Abstract : Small pigeon louse, *Campanulotes bidentatus compar* were studied *in vitro*. They were subjected to different temperature i.e. 20˚C, 25˚C, 30˚C, 35˚C, 40˚C and 45˚C and relative humidity i.e. 33%, 50%, 75% and 90% to determine lice longevity, an incubation period of egg, daily egg production and hatching of an egg. The optimum temperature range between 30-40˚C was found essential for normal survival of *C. bidentatus compar*. The best suitable temperature and humidity were found to be 75±1˚C and 75±1%. Temperature above 40˚C was lethal to adult as well as developing stages of *C. bidentatus compar*. Egg laying, survival time, incubation and hatching period dropped as temperature and relative humidity increased or decreased mention range (i.e. above 40 ˚C and 75% and below 30 ˚C and 50%).

Key Words: adult longevity, *Campanulotes bidentatus compar*, incubation period, temperature and relative humidity.

Introduction

Various environmental factors influence the physiology of insects in many ways. For them, temperature and relative humidity are the most important ones (Wigglesworth, 1950). Phthirapteran ectoparasites prefer to live on constant environment condition made of host feather/hairs, even though they are unable to escape from the impacts of environmental factors.

Many workers have tried to investigate the effect of various temperature and humidity on development and hatching of eggs of a certain phthirapteran louse (Saxena and Agarwal, 1982; Rodriguez *et al*., 1987; Abbas and Kumar, 1991; Kumar *et al*., 1993a and b and Surman *et al*., 1998) Moreover, Ash (1960) cited that various stages in the life history of four mallophagan species, require slightly different conditions which was proven by large mortality amongst incubator breed nymphs. It has been found that ischnoceran dependent upon temperature in the 30-40˚C range for survival and reproduction (Kellogg, 1896; Barber, 1923; and Scott, 1952). It has been expected that anoplurans exhibited wider latitude than mallophagans with rather low preferred temperature.

Phthirapteran ectoparasites show a high degree of specificity and niche specialization. They are unable to survive for a longer time when removed from their host body. Moreover, these species can survive only for short time after the death of their host. Petryszak *et al*. (1996) observed the vitality of lice from dead pigeons. It was assumed and proved that this dependence on the host is based on temperature (Barber, 1923; Martin, 1934; Wilson, 1934 and 1939; Matthysse, 1944; Conci, 1952, 1956a and b; Arora and Chopra, 1959). By providing lice with proper nutritional diet and an artificial heat source, it has therefore, proved possible to prolong survival of these ectoparasites and even to rear them. Yet, temperature requirement for the growth, longevity and oviposition are not always the same for all. One species of lice may survive for a longer period at a particular temperature which might not suit for its oviposition but temperature slightly higher or lower than that may be suitable. Williams (1970a) noted that at 30˚C survival of *Goniodes colchici* was maximal but few eggs were laid while egg production was maximal at 35 ˚C at high humidity. Similarly, relative humidity is another important factor for phthirapteran rearing. Though, humidity requirement for these ectoparasites is less well known. It seems that there was a threshold relative humidity in the region of 60% above which the lice survive but below it, death rapidly occurs. It was reported by Williams (1970b) that the threshold humidity for the survival of an adult *Goniodes colchici* follows an identical pattern. It was also reported that humidity preference was more marked during oviposition. Murray (1957) reported that sheep biting louse, *Damalinia ovis* prefers a dry atmosphere for oviposition while Heath (1973) noted that none of the egg of *D. bovis* hatched at 90% R.H. Furthermore, Moyer *et al.* (2002) established positive correlation evidence between louse loads and ambient humidity and demonstrate experimentally that low ambient humidity reduces the abundance of lice.

Herein present study, an attempt has been made to demonstrate the effect of different relative humidity and temperature on viability of *Campanulotes*

bidentatus compar.

Materials and Methods

Healthier adult of unknown age and different instar nymphs of *C. bidentatus compar* was sorted out from host birds and colonized in glass beakers (without spout)/jar (having bottom lined with black paper) (both adults and nymphs were colonized separately). Layers of suitably chopped feather which are devoid of eggs were laid at the bottom to facilitate the lice. Then the lice were transferred to a cultural vial with the help of fine hair brush. For fulfilling the nutrient requirement, a feather was plucked from the fore part of a body (e.g. side, vent and back) and placed in a cultural vial for feeding, as *C. bidentatus compar* prefer to feed on fluffy parts of body feathers.

Furthermore, in another set of experiment, feathers having freshly laid eggs were obtained either from cultural stock or host birds are used for oviposition were placed in separate rearing container. For *C. bidentatus compar*, suitably trimmed feather of back, breast and abdomen were placed as lice prefer to lay eggs on under converts and have a tendency of resting in furrows.

All of them (adult, nymphs and feather with eggs) were made to fit in rearing container (small petridish/beaker) lined with black paper. The mouth of the container was covered with muslin cloth and tied with the help of a rubber band and finally, they were placed in desiccators/battery jar. The saturated solution of salts was placed in a beaker (plus extra salt at the bottom) in desiccators/battery jar to obtain the intended R.H. by the method given by Winston and Bates (1960). Desiccators/battery jar was then transferred to digital mammert type incubator (BOD incubator) maintained at a designated temperature. These containers were examined daily to record the mortality of adults and nymphs, a number of an egg laid, developmental status of eggs and to remove exuviae and dead lice.

Results

In the first set of experiment, colonies of freshly looking healthier adults and nymphs were maintained at the temperature of 45˚C, 40˚C, 35˚C, 30˚C, 25˚C and 20˚C and at a relative humidity of 33%, 55%, 75% and 90%. After careful examination, feather normally used for feeding and oviposition were

Table- 1: Showing mean survival time and mean egg laying in relation to temperature and relative humidity.

Temp.(˚C)	R.H. (%)	Mean Survival Time (in Days)					Mean Egg Laid	Range (in days)
		M	F	IN	IIN	IIIN		
45	33	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	75	0	0	0	0	0	0	0
	90	0	0	0	0	0	0	0
40	33	1.9	2.37	0.13	0.26	0.65	0.13	1-5
	50	1.86	3.8	0.33	0.64	0.97	0.99	2-11
	75	5.2	7.8	0.91	1.2	1	0.84	1-10
	90	2.46	2.76	1	0.81	1.2	0.31	2-5
35	33	1.9	3.34	0.78	0.84	0.87	0.48	1-5
	50	5.9	6.87	0.9	1.9	1.99	0.87	3-18
	75	8.36	12.15	1.9	2.21	3.2	1.65	4-22
	90	4.56	4.39	1	1.8	2	0.76	3-12
30	33	6.9	4.8	0.16	0.86	0.82	0	1-8
	50	7.3	10.7	0.96	0.99	1.11	0.26	2-20
	75	8	11.5	2.1	2.52	2.23	0.53	2-20
	90	4.2	3.92	0.72	0.67	0.75	0.26	1-9
25	33	3.2	3.7	0	0	0	0	2-5
	50	2.71	2.81	0	0	0.18	0	2-6
	75	4.63	3.25	0	0	0.51	0	3-4
	90	3.16	3.86	0	0	0	0	1-2
20	33	0.19	0.53	0	0	0	0	0-2
	50	1.57	1.46	0	0	0	0	1-2
	75	1.21	1.8	0	0	0	0	1-2
	90	0.2	0.91	0	0	0	0	0-1

Abbreviations: Temp. (˚C)- Temperature in Degree Celsius; R.H.- Relative Humidity; — Male; F- Female; IN- First Instar Nymph; IIN- Second Instar Nymph; IIIN- Third Instar Nymph.

placed in a cultural vial. One colony consists of 10 females and 10 males while others have 12 third instars, 10-second instars and 8 first instar nymphs in each batch. Each batch of colonies was examined daily to record the number of survivors and egg laid and to remove excreta, mounted skins and dead lice. Feather having fresh eggs were replaced by one which is devoid of an egg. The effects of various temperature and relative humidity condition on longevity and egg production were measured. Mean survival time (in days) and mean egg lay (in days) for lice maintained under these various conditions are shown in Table - 1.

Mean lifespan of male *C. bidentatus compar* varied between a maximum of 8.36 days at 35˚C and 75% R.H. and a minimum of 0.19 days at 20˚C and 33% R.H. while female had maximal average survival time of 12.15 days and minimal 0.53 days at same temperature and R.H. At 20˚C, lice were remained in still state (chill coma), only sign of life is an occasional crop or gut movement. As the temperature increases from 20˚C to 30˚C, mean survival time increase from minimum level to about 8 in male and 11.5 in a female at 75% R.H.

The average lifetime for *C. bidentatus compar* were recorded at 35˚C and 40˚C however it also dependent upon R.H. In 75% R.H., the mean life span was in the region of the maximal value of 8.36 and 12.25 days for male and female respectively at 35˚C and 5.2 (male) and 7.8 (female) days at 45˚C. The mean survival time of lice held below and above 75% R.H. however, decreases rapidly as temperature roses from 30˚C to 40˚C (Table-1). High temperature (45˚C) was found to lethal for both male and female.

Furthermore, the mean survival time of different instar nymphs was also recorded. Maximal mean survival time for different instars were found to be 2.1 and 2.52 days for first and second nymph at 30˚C and 75 % R.H. and 3.2 days for third nymph at 35˚C and 75% R.H. On the other hand, instar nymphs are failed to overcome or survive too low and high temperature irrespective of levels of R.H. as no one survives at 25˚C, 20 ˚C and 45˚C, except third nymph which however able to live at 25˚C for only 0.18 days at 50% R.H. and 0.51 days at 75% R.H. At 75% and 50% R.H. and temperature between 30˚C to 35˚C, mean survival time of all nymphs was in vicinity of maximal value. An average life span of all instar nymphs decreases rapidly as temperature and R.H. increases and decreases from 30 to 35˚C and 50% to 75% R.H.

Moreover, the rate of eggs production per day by female has also been observed. Maximum 1.05 eggs were laid by *C. bidentatus compar* held at 35 ˚C and 75% R.H. It might be due to fact that the maximum number of female survived at the same condition. At 50% R.H., mean survival time for adults become half which results in the reduction of a total number of eggs to 0.87. Mean number of an egg laid by female held at 40˚C and 75% R.H. was lower the number of

Table- 2: Showing effect of different temperature on hatching of eggs at 75%±2 R.H.

Temperature (˚C)	Number of Hatched eggs (%)	Incubation Period (in days)	Range (in days)
45	-	-	-
40	44.76	4.77±1.09	3-6 n=56
35	67.12	5.8±1.06	3-8 n=58
30	32.25	6.0±0.78	5-8 n=26
25	4.66	6.5±0.74	3-8 n=26
20	-	-	-

Table- 3: Showing effect of different relative humidity on hatching of eggs at 35˚±1C.

R.H. (%)	Number of hatched eggs (%)	Incubation Period (in days)	Range (in days)
33	49.47	4.81±1.02	2-7 n= 47
50	53.26	5.32±0.96	4-8 n=49
75	78.1	5.85±1.07	4-8 n=25
90	55.81	4.98±0.97	5-8 n=24

eggs laid at 35˚C. Reduction in a mean life span of adults at 55% R.H. and 40˚C results in further reduction in the total number of eggs laid (0.91). A low number of eggs was obtained at 30˚C despite a high mean survival time of female. As the temperature goes down (20˚C), female failed to lay eggs at any R.H may be because adult undergoes in chill coma state at this temperature.

In another set of experiment firstly, batches of fresh eggs of *C. bidentatus compar* were incubated at 45˚C, 40˚C, 35˚C, 30˚C, 25˚C and 20˚C and 75% R.H. to record the impact of different temperature on hatching of eggs. The maximum number of eggs hatched at 35˚C (67.12, n= 49) with an average incubation period of 5.6±1.06 days (range 3-8, n=49) while eggs failed to hatch at 20˚C. As the temperature decreases from 30 to 35˚C, a percentage of hatching (32.25, n= 20) also reduces but the incubation period increases (6±1.26 days, range, 5-8 days) (Table 2). Further lowering of temperature causes a rapid reduction in the percentage of hatching as only 2 eggs hatched (4.66, n=21) on 6.3 days. However, an increase in temperature (40˚C) cut down the hatching percentage to 44.76 (n=47) but reduces the incubation period to 4.77±1.09 days (range, 3-6 days). Higher temperature (45˚C) appears to be lethal for the eggs of *C. bidentatus compar* as no hatching occurred at 45 ˚C (Table-2).

Moreover, the second batch of eggs was reared at a fixed temperature of 35˚C and at different R.H. of 33%, 50%, 75% and 90% to record the effect of R.H. on eggs (Table-3). At 35˚C, hatching percentage of eggs was 49.47, 53.26 and 55.81 at 33%, 50% and 90% whereas 78.0 % eggs were hatched at 75% R.H. However, the incubation period shows a slight difference (4.81±1.02, 5.32±0.96, 5.85±1.07 and 4.98±0.97 respectively) at different R.H. taken into consideration.

Discussion

Through analyses of data indicates that those lice species which rely purely on skin derivatives (feathers in case of avian lice and skin scruf in case of mammalian lice) has been successfully reared. Most of them belong to suborder Ischnocera whilst those which feed on blood (haematophagus species) are difficult to rear *in vitro* condition. They mostly belong to Amblycera.

This study has displayed that *C. bidentatus compar* show similarity with all other ischnoceran lice which have been studied so far in temperature requirement range (30-40˚C) to sustain its normal life. Maximum survival time and egg production are all geared to the temperature in this range. On the other hand, *Goniodes colchici* show maximal survival at 30˚C and egg production at 35˚C at high humidity (Williams, 1970b). He also reported that fecundity, egg-laying and survival dropped as humidity falls and temperature increases or decreases about 35˚C. While optimum cultural temperature for *Cuclutogaster heterographus* was recorded in the neighborhood of 42˚C. Matthysse (1946) showed that optimum condition for the survival of *Damalinia bovis* was geared at 35˚C and 70-80% R.H. However, Heath (1973) reported that adult lice of *D. bovis* and *D. caprae* live longest at 25˚C.

Only a few workers have examined the life span of different nymphal instars at a different temperature. Matthysse (1946) noted that *D. bovis* first, second and third nymphs with food and under favorable condition had a life span of 7, $5^{1/2}$ and 6 days mean duration respectively. Whereas, longevity was reported approximately 6, 5 and 6 days duration for first, second and third instar nymphs of *D. bovis* respectively when kept at 30˚C without food (Heath, 1973). He also recorded that nymphal instars of *D. caprae* had a duration of 4, $4^{1/2}$ and $4^{1/2}$ for first, second and third instars respectively. Newly emerged first instar nymphs lived no longer than 12 hours in both *D. bovis* and *D. caprae*. Most probably the lack of food and energy necessary for moulting contribute to death of lots of nymphs during ecdysis. Thorold (1968) noted that *D. caprae* nymphs lived only 5-6 hour after hatching at 18-25˚C. In present study also nymphal instar lives only for a few hours to a few days in different temperature and R.H.

Furthermore, the impact of temperature on incubation period remained unclear due to a lacuna of knowledge on concerning topic. Impact of lower temperature on a prolongation of the incubation period of phthirapteran eggs had been noted by Agarwal and Gupta (1970), Kumar *et al*. (1993 a & b) and Surman *et al*. (1998). The incubation period of eggs of most of the phthirapteran species varies considerably from 4-17 days (Matthysse, 1946; Murray, 1960 and 1963; Nelson and Murray, 1971; Rodriguez *et al*. 1987 and Abbas and Kumar, 1991). It has been found that lowering of temperature diminishes the hatching percentage and extends the incubation period. On the other hand increase in temperature above optimum reduces the life span and cut down the incubation period. Relative humidity does not seem to have a profund effect on hatching percentage and incubation period. Nearly similar results have obtained in case of *Lipeurus lawrensis tropicalis* (Agarwal and Saxena, 1982; Saxena and Agarwal, 1982), *Menopon gallinae* (Surman *et al*., 1998), *Colpocephalum turbinatum* and *Columbicola columbae* (Singh, 1999) and also in case of mammalian lice species (Murray, 1960 and 1963a

and Abbas and Kumar, 1991). *C. bidentatus compar* also show similar results. Matthysse (1946) shows that under optimum condition (35˚C and 75% R.H.) eggs of *Damalinia bovis* took 6-9 days to hatch with an average of over 7 days while Heath (1973) reported that eggs of *D. bovis* hatched at approximately 11 days at 35˚C and 10 and 50% R.H. None of egg hatched at 90% R.H. For *D. caprae* hatching occurred only at 30˚C. Hence, it appears that for egg hatching *D. caprae* had an average optimum temperature of 35˚C while *D. caprae* preferred 30˚C whilst both species laid more eggs at 30˚C. Murray (1963a) reported that most of the eggs of *D. equi* were laid at 36˚C whereas *Goniodes colchici* laid their eggs at 35˚C (Williams, 1970a).

Thus, it appeared that temperature and humidity are one of the important factors affecting the biology of phthirapteran species. It seems that low temperature may not kill the lice as quickly as temperature which is too high but may have adverse effects on the population. It shows that lice are able to tolerate fairly high humidity but the adverse effects of low optimum humidity have yet not been clearly understood. Higher humidity (above 90%) creates other problems as they cause moisture which may affect louse behaviour. In general, most of the phthirapteran species are able to survive within a temperature range of 30-38˚C and R.H. 50-90% (provided dietary requirement and fulfilling oviposition site/feather/hair) made available.

Acknowledgment

Authors are thankful to Principal, Govt. P.G. College, Ranikhet, Almora for providing necessary facilities required for this investigation.

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