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EVALUATION OF PUPAL AND ADULT LIFE SPAN OF THE SELECTED TWO VOLTINE GROUPS OF THE SILKWORM *BOMBYX MORI* L.

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ABSTRACT
The present study was carried out to understand the longevity/life period of selected four bivoltine races/breeds namely, CSR_2, NB_4D_2, APS12 and APS45 and four multivoltine races/breeds like, Pure Mysore, C. nichi, APM1 and APM3 were drawn from the germplasm banks of Department of Studies in Sericulture Science, University of Mysore, Mysuru and APSSRDI, Hindupur. They were maintained and observed under the standard laboratory conditions with preparation of layings and silkworm rearing by utilizing the standard procedures. The healthy spinning silkworm larvae were mounted on the mountages to spin the cocoon. From the third day after spinning (chrysalis stage) the male and female pupae were separated and along with cocoon shell the separated male and female pupae were kept in container. They are hundred males and hundred females pupae were selected for the experimentation and after the emergence of silkmoths, they are twenty five virgin female and twenty five mated female and twenty five unmated male and twenty five mated male moths were separated and darken covered with cellules and temperature and humidity was maintained. The observation was carried out at intervals time of 6.30 AM 12.30 AM, 6.30 PM. The death of an adult silkmoth was identified when they did not show any biological response to being pocked by the point of a pencil. The life period of pupae and moth were recorded in hours. among the four bivoltine the pupal longevity from the 3^{rd} day of spinning stage ranges from 9 to 10 days, similarly, in the four multivoltines races/breeds the pupal duration was range from 6 to 9 days, whereas, the bivoltine and multivoltine sexes exhibits the significant differences. The unmated male and virgin female moths exhibited the longest life span than those of mated male and mated female moths.

KEYWORDS: Longevity/life span, Bivoltine, Multivoltine Silkworm *Bombyx mori* L.

INTRODUCTION
The domesticated silkworm is monophagous lepidopteron insect feeds only on mulberry leaf for the growth, development and reproduction of the organism and an important laboratory model system. It is very interesting to understand its wonder mechanism of lifespan, which are tend to grow older and older finally dies due to the intricate aging process of complexities and study of aging process is overall defined as gerontology. Different groups of terrestrial insects in general and silkworm, *Bombyx mori* in particular are immensely provide a unique biological system for aging investigation under developing scientific world of 21^{th} century. Based on the inferences derived by various gerontologist on different phenomenon *i.e.*, physiological, biochemical, ecological, cytological, genetical and molecular studies. It seems like that, the striking longevity plays an important role over all the benefits/income from economical important insect like, silkworm, wherein aging relevant aspects/phenomenon are very crucial in the silkworm for the benefit of silk industry.

MATERIALS AND METHODS
Totally four bivoltine races/breeds namely, CSR_2, NB_4D_2, APS12 and APS45 and four multivoltine races/breeds like, Pure Mysore, C.nichi, APM1 and APM3 were drawn from the germplasm banks of Department of Studies in Sericulture Science, University of Mysore, Mysuru and APSSRDI, Hindupur. They were maintained and observed under the standard laboratory conditions with preparation of layings and silkworm rearing by utilizing the standard procedures described by Tazima, (1978) and Krishnaswami, (1978) respectively. The healthy spinning silkworm larvae were mounted on the mountages to spin the cocoon. From the third day after spinning (chrysalis stage) the male and female pupae were separated and along with cocoon shell the separated male and female pupae were kept in container. They are hundred males and hundred females pupae were selected for the experimentation and after the emergence of silkmoths, they are twenty five virgin female and twenty five mated female and twenty five unmated male and twenty five mated male moths were separated and darken covered with cellules and temperature and humidity was maintained. The observation was carried out at intervals time of 6.30 AM 12.30 AM, 6.30 PM. The death of an adult silkmoth were identified when they did not show any biological response to being pocked by the point of a pencil. The adult mean lifespan for each races/breed for male and female will be calculated by following the Murakami *et al.,* (1989a) method. The life span of pupae and moth were recorded in hours. Four bivoltine races/breeds *viz*, CSR_2, NB_4D_2, APS45 and APS12, four multivoltine races *viz.*, Pure Mysore, C.nichi, APM1 and

APM3 were selected for the experimentation. The pupal and adult moth duration was calculated by following formula Murakami (1989a) and Anantha, R (2010)

Total duration from spinning to moth emergence
$[\sum nD] = \sum nS + \sum nC + \sum nP$
Pupal duration $= \sum nP = [\sum nD]- [\sum nS + \sum nC]$
Where,
$\sum nS$ = Duration of the spinning larva
$\sum nC$ = Duration of pre-pupa (chrysalis)
$\sum nP$ = Duration of pupae

RESULTS

The data pertaining to the mean values of pupal duration of four bivoltine and four multivoltine races/ breeds are represented in tables 1.1 and 1.2 with statistical analysis *viz*., F- value, CD @5% and CV level. The adult life spans of virgin female and mated female and unmated male and mated male of four bivoltine and four multivoltine races/breeds are presented in the tables 1.3 and 1.4. Figures 1.1 to 1.4 represented the mean values of male and female pupal duration and virgin and mated female and male adult life span are as follows.

Table 1.1 represents the mean value, F value and CD@5% and CV level in four bivoltine breeds in relation to male and female pupal duration and the figure 1.1depicted the same. The highest pupal duration among the four bivoltine were observed in female CSR_2 breed 241.70 ± 13.28 h followed by APS45, NB_4D_2 and APS12 (235.33±10.2 h, 232.67±8.570 h and 232.33±7.51 h) respectively and the F value was recorded 4.202 and CD@5% was 20.924. The lowest pupal duration was observed in the APS12 breeds of female pupae. The statistical data revealed significant differences ($P<0.05$) in all the selected breeds. Whereas, in case of male pupal duration, the lowest duration of 217.67±5.04 h was noticed in APS12 breeds and highest pupal duration of 233.33±8.74 h was recorded in CSR_2 breed followed by 229.67±11.41h and 227.30±7.31 NB_4D_2 and APS45 and the F value was 4.319 and CD@5% was 23.901.

Table 1.2 depicted the mean value, F value and CD@5% and CV level of four multivoltine races/breeds in relation to the male and female pupal duration and same as represented in figure 1.2. The highest female pupal duration was noticed in Pure Mysore (216.00±5.20 h) and shortest life span was recorded in case of female pupae of C.nichi (165.00±3.79 h) and the F value was 7.232 and CD @5% was 26.089. The intermediatory life duration was recorded in APM1 and APM3 breeds with respect to female pupae (198.33±8.09 h and 195.67±11.89 h). The F value was 6.499 and CD @5% was 29.097 was exhibited for the male pupae of all the four races/breeds. The lowest pupal duration of male pupae was recorded in case of C.nichi race of 153.67±7.62 h and the Pure Mysore, APM1 and APM3 race/breeds were recorded the mean value of 206±67.5.81 h, 190.67±8.69 h and 189.67±11.89 h respectively. The significance differences ($P<0.05$) were observed for all the selected male and female pupal duration of selected silkworm races/breeds.

The calculated data with regard to the mean value of adult life span of virgin and mated female, unmated and mated male of four bivoltine breeds were presented in table 1.3 and figure 1.3. Among the virgin females the highest life spans of 254.33±7.81 hours in case of CSR2 breed. Whereas, the lowest adult life span was exhibited in virgin female of 233.00±4.36 h of APS12 breed. The significant difference among the breeds of virgin female was depicted with the F value (4.181) and CD@5% (20.445). Statistically insignificant difference (($P\geq0.05$) was clearly indicated between the selected mated female. Among the mated female adult life span ranges from highest of 227.7±8.84 hours was recorded in CSR_2 breed, whereas, a lowest of 215.3±3.48 hours was observed in APS12 breed and the intermediatory adult life span was exhibited in APS45 and NB_4D_2 breeds of 225.30±5.81 and 218.0±3.61 hours and F value was 1.002. The data clearly indicated the unmated male moth that, among the four bivoltine breeds, the NB_4D_2 and APS45 breeds exhibited the intermediatory mean values of 224.30±7.97 hours and 227.70±6.33 hours. The lowest and highest adult life span of 218.30±11.67 hours and 232.30±5.19 hours were observed in case of APS12 and CSR_2 breeds respectively. Whereas, the statistically significance difference was observed clearly in unmated males of selected bivoltine breeds. The result indicates that, the adult life span of mated male moths of bivoltine breeds namely, CSR_2, NB_4D_2, APS45 and APS12 with the F value, CD@5%, CV values and statistically significance difference. Among the four races an highest adult life span was recorded in case of CSR_2 (199.73±10.81 hours) when compare to the other breeds *viz*., NB_4D_2, APS45 and APS12 with 188.33±7.45 h, 193.30±6.43 h and 185.67±3.18 h respectively.

Table 1.4 and figure 1.4 present the mean value, F value, CD and CV value of mated adult lifespan expressed in hours in selected multivoltine races/breeds namely, Pure Mysore, C.nichi, APM1 and APM3. Among the virgin female and unmated male showed increased longer longevity in APM1 breed of 236.33 ±6.84 h and 225.67±7.64 h when compared to the other race/breeds *viz*., Pure Mysore, C.nichi and APM3 (221.67 ±5.78 h, 217.67±4.33 h and 228.03 ±7.57 h and 207.00 ±10.54 h, 202.33 ±9.45 h and 222.30 ±9.84 h) respectively. In case of mated male and female moths revealed lowest life span of 168.67±13.57 hours and 202.33± 9.45 hours in C.nichi race and followed by adult duration of 182.00±8.15 h and 225.67±7.64 h in APM1 breed, 172.33±10.17 h and 207.00±10.54 hours in Pure Mysore, 189.30±16.90 h and 222.30±9.84 hours in APM3 respectively. The F value of 5.126, 4.453, 4.311 and 4.274 and CD @5% of 17.481, 26.402, 26.065 and 36.098 were observed for all the virgin and mated female and unmated and mated males between the race/breeds and statistically significant difference was observed between the breeds in all the crossed and virgin silk moth.

TABLE: 1 & 2. Average pupal duration of bivoltine and multivoltine silkworm races/breeds (Each observation are expressed in hours)

Sex Races/ breeds	Male pupae	Female pupae
CSR2	233.33±8.74	241.70±13.28
NB4D2	229.67±11.41	232.67±8.570
APS45	227.30±7.31	235.33±10.21
APS12	217.67±5.04	232.33±7.51
F-value	4.319	4.202
CD @5%	23.901	20.924
C.V. (%)	5.466	4.64

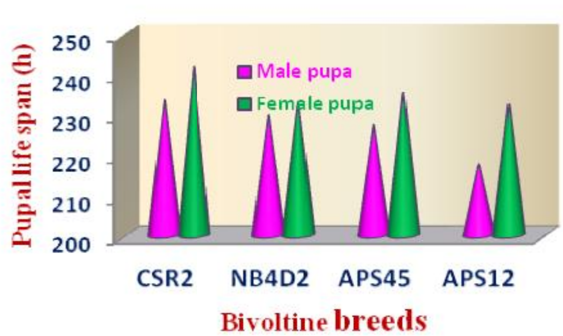
Sex Races/ breeds	Male pupae	Female pupae
PM	206.67±5.81	216.00±5.20
C.nichi	153.67±7.62	165.00±3.79
APM1	190.67±8.69	198.33±8.09
APM3	189.67±11.89	195.67±11.89
F-value	6.499	7.232
CD @5%	29.097	26.089
C.V. (%)	8.218	7.042

TABLE: 3 & 4. Average adult life span of bivoltine and multivoltine silkworm breeds (Each observation are expressed in hours)

Sex Races/ breeds	Virgin female	Mated female	Unmated male	Mated male
CSR_2	254.33±7.81	227.7±08.84	232.30±5.19	199.73±10.81
NB_4D_2	239.00±1.53	218.0±03.61	224.30±7.97	188.33±7.45
APS45	251.00±6.35	225.3±05.81	227.70±6.33	193.30±6.43
APS12	233.00±4.36	215.3±03.48	218.30±11.67	185.67±3.18
F- value	4.181	1.002	4.195	4.97
CD @5%	20.445	N/A	18.88	19.662
C.V. (%)	4.345	4.575	4.359	5.265

Sex Races/ breeds	Virgin female	Mated female	Unmated male	Mated male
PM	221.67±5.78	207.00±10.54	193.67±6.12	172.33±10.17
C.nichi	217.67±4.33	202.33±9.45	206.33±5.04	168.67±13.57
APM1	236.33±6.84	225.67±7.64	212.67±7.69	182.00±8.15
APM3	228.03±7.57	222.30±9.84	207.33±11.80	189.30±16.90
F- value	5.126	4.453	4.311	4.274
CD @5%	17.481	26.402	26.065	36.098
C.V. (%)	3.992	6.385	6.491	10.015

Fig: 1.1. Mean values of pupal duration of four bivoltine silkworm breeds.



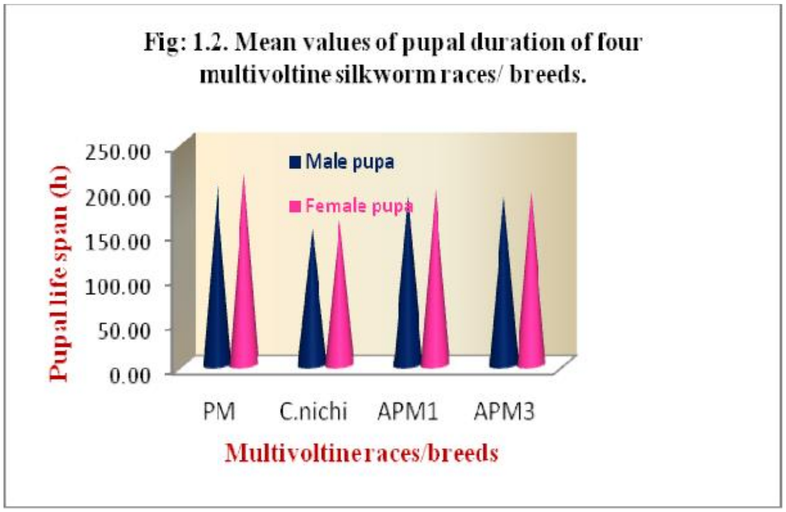


Fig: 1.2. Mean values of pupal duration of four multivoltine silkworm races/ breeds.
Male pupa
Female pupa
Pupal life span (h)
250.00
200.00
150.00
100.00
50.00
0.00
PM
C.nichi
APM1
APM3
Multivoltine races/breeds

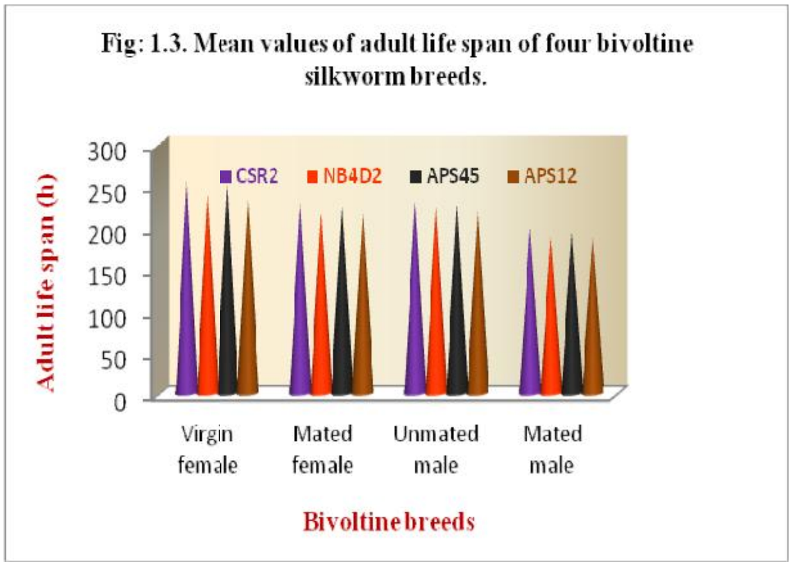


Fig: 1.3. Mean values of adult life span of four bivoltine silkworm breeds.
CSR2
NB4D2
APS45
APS12
Adult life span (h)
300
250
200
150
100
50
0
Virgin female
Mated female
Unmated male
Mated male
Bivoltine breeds

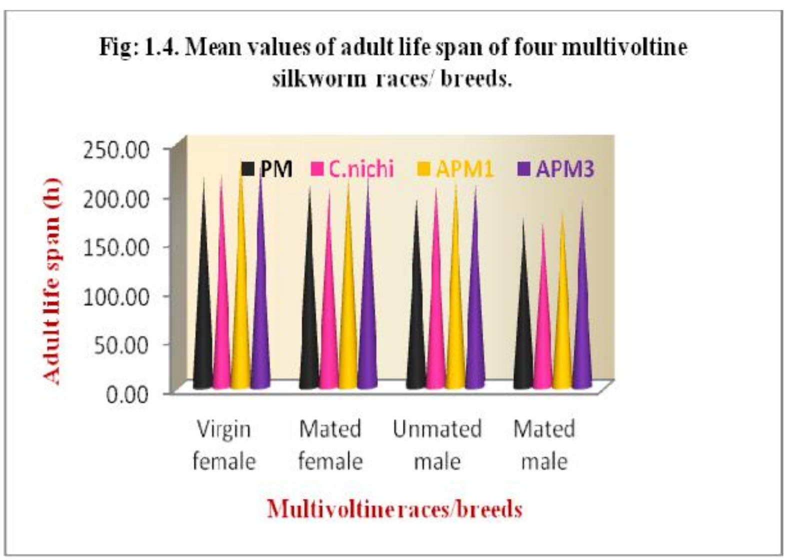


Fig: 1.4. Mean values of adult life span of four multivoltine silkworm races/ breeds.
PM
C.nichi
APM1
APM3
Adult life span (h)
250.00
200.00
150.00
100.00
50.00
0.00
Virgin female
Mated female
Unmated male
Mated male
Multivoltine races/breeds

DISCUSSION

The term aging is commonly used by gerontologists and public as a synonym to the word as senescence. Attempts to develope a fundamental quantitative theory of aging, mortality and life span have deep historical routes. Several research reports mentioned that, the development of genetic and pharmaceutical therapies can extend their healthy lifespan in *Drosophila* with single gene mutation extends aging (Lin *et al.,* 1998 and Rogina *et al.,* 2000) and life extension through feeding single drug (Kang *et al.,* 2002) have already been identified that can dramatically extended the longevity period and Stephen *et al.,* (2003) has reported that, during a time when normal flies are decrepit or dead, long-lived flies that have mutations in the "*Indy"* gene are still leading a healthy active life while maintaining sexual and physical activity. Kumral *et al*., (2007) and Shehata *et al.* (2003) reported that, adult longevity period of *P. unionalis* varied 9.92 to 11.64 days for females and 9.00 to 10.57 days for males and 11.4 days for females and 11.9 days for males under laboratory conditions in lepidopteran and the differences on adult longevity could be due to adult diet (Yilmaz, C and Genç, H, 2012).

In silkworm, generally the average adult lifespan of the female bivoltine races have longer longevity period than compare to the male bivoltine races and this phenomenon occurs in multivoltine races too *(*Murakami, 1989a, 1991 and Doddaswamy and Subramanya 2007). Among two sexes of the silkmoths, the unmated male and virgin female moths exhibited the longest life span than those of mated male and mated female moths (Anantha, 2010). Thus, several research workers emphasized gerontological research work utilizing insect species because of high degree of reproductability of experimental data (Medawar, 1946 and Charlesworth, 2001), short life span like *Drosophila melanogaster* and other *Dipteron* insects and relatively limited space requirement and low cost maintenance. From the present studies of the author, among the four bivoltine the pupal longevity from the 3rd day of spinning stage ranges from 9 to 10 days, similarly, in the four multivoltines races/breeds the pupal duration was range from 6 to 9 days, whereas, the bivoltine and multivoltine sexes exhibits the significant differences. In this regard, a detailed research investigation the long lived pupae showed dominant over the short lived pupae. The present observation also records the data where in the females live longer than the males in both the voltinistic races/breeds the finding of Murakami (1989a), in his studies on temperate bivoltine races/breeds showed that, virgin female moths have highest longevity and proposed that, adequate supply of diet plays an important role in longevity and hence the bivoltine races which consumes maximum nutrient leaf compare to multivoltine found to be longer losting in the adult stage.

Bloem *et al.,* (1994) noticed that, the large pupal weight has been associated with greater longevity and pupal length increases as pupal weight increases. The silkworm Chinese strain and Japanes strain have more pupal length than normal (Alimurong *et al.,* 1986). Positive relationship between pupal length, width or size and weight of female pupae of the silkworm were noticed by (Rithinam *et al*., 1991). On the basis of the present studies and above information, it may be concluded that, the utilizing of four bivoltine and four multivoltine races/breeds of both virgin and mated moths indicates the sexwise differences as well as racial differences and it is evident that, in the table 24 and 25. The unmated male and virgin female a lived longer life of 8 to 10 days, whereas, in case of mated male and female silkmoth lives shorter duration of 7 to 9 days. Apart, in some organisms longevity effects due to restricted feeding on lifespan studied in rodent for more than 60 years (McCay *et al.,* 1935, Harrison *et al.,* 1984, Bertrand *et al.,* 1980 and Weindruch and Walford, 1998). Moreover, few researchers were also conducted on biochemical/molecular biology work suggested that, longevity depends on protein profiles and adult life span (Kang *et al.,* 1999) and between species and their role of DNA (Ames *et al.,* 1993 and Richter *et al.,* 1988).

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