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Discovery that Matters

Biological Studies on the Wild Silkworm, *Epiphora bauhiniae* (Guerin-Meneville): (Lepidoptera: Saturniidae) in Gedarif State; Sudan

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***Abstract*--** This study was conducted in Gedarif town, Gedarif State, Sudan for the three rainy seasons 2003, 2004 and 2005. The objective was to determine the presence of the wild silkworm of *Epiphora bauhiniae* in Sudan. The biology of the insect was studied on a *Ziziphus* spp. Cultivar under laboratory and field conditions, to determine the effect of rainy season's period on larval mortality, larval and pupal duration, insect viability, egg hatchability, adult fecundity, emergency, longevity and ability to regenerate naturally and artificially. The study confirmed the presence of *Epiphora bauhiniae* in Sudan and showed that the species is bivoltine. Diapause occurs in the pupal stage. Temperature and relative humidity are known to be important factors that affect survival of the insect and production of cocoons. The mean number of eggs laid per insect was 297.7 during life span; the mean incubation period was 7.90 – 8.11 days and was not significantly different during the rainy season. The eggs hatched 96% under temperature 32–33.6º C and relative humidity (RH) 65–88%. The larva passed through five instars. The mean larval period ranged between 19-20 days. The pupal period ranged between 18–20 days during favourable conditions while it ranged between 170-226 days during adverse conditions. The copulation period was 13:19:28 hrs, while ovi-position rate was significantly different within the period of the rainy season. The adult male longevity was 4.2 days and that of the female was 5.2 days. This study claimed to be the first report of biological study on *Epiphora bauhiniae* in Sudanhas good gum properties close to those of gum Arabic.

Index Terms: *Biological studies, silkworm, Epiphora bauhiniae*

I. INTRODUCTION

All the true silkworms belong to the order Lepidoptera. The famous super family of silkworm is *Bombycoidea*, under which the two families *Bombycidae* and *Lasiocampidae* are classified. *Bombyx mori* is a member of the family *Bombycidae* [1]. However, *Saturniidae* is the largest silkworm family. Members of this family are brightly coloured moths. The Larvae are large, characterized with spine tubercles and pupated in silken cocoons. The family *Saturniidae* world wide has nine subfamilies, 165 genera, and 1480 species [2]. This includes the genera *Actias*, *Antheraea*, *Attacus*, *Philosamia* and *Saturnia* [1]. Nevertheless, Gashe and Mpuchane [3] added *Epiphora*, as an important genus to this family. Species under the genus *Antheraea* are known as Tasar silkworms and Japanese oak silkworms [4]. However, species under *Epiphora*, are known as Emperor moths. They are classified under the sub order Ditrysia and the family *Saturniidae* [5; 3]. So far species under *Epiphora* are *bauhiniae*.

The subject species, *Epiphora bauhiniae*, was found and known in South and Central Africa [5], and hosted on important fodder trees *Ziziphus* spp. The leaves are used for rearing Tasar silkworm for commercial uses in tropical regions [6]. The biology of *E.bauhiniae* is not known. However, scanty information about the biology of *E.mythimnia* was reported by Billoelke [5], it was stated that the larval period is 24:09:08 days and pupal period is 28:10:08 days. Beside the production of silk (cocoons) as a commercial material, The silkworm *Bombyx-mori* have nutritional values as reported by Calvert and Frye [7].There are several types of produced silk, these are Mulberry silk, Muga silk and Eri silk. Mulberry silk account for more than 90% of silk production in the world [8]. Gashe and Mpuchane [3] reported that *Epiphora bauhiniae* produced cocoons of a great commercial value and the silk producing moth has good potential for being used in community based projects.

With good management policies this species could be used as candidate for the sustainable use of natural resource and lend itself for being incorporated into conservation project. It is called emperor moths; edible moth larvae. However, Data on silkworms and their biology in the Sudan is scanty. Therefore, the aim of this study was to support sericulture by

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enhancing and improving silkworm industry in the Sudan by identifying a local, or indigenous, form of worms to produce silk by potential utilization of the local *Ziziphus spini-christi* trees (indigenous trees) as feed sources.

The ultimate goals of this study are to investigate the influences of rearing period at the rainy season on the biological traits of *Epiphora bauhiniae* such as productive and reproductive behaviour of the insect.

II. MATERIAL AND METHODS

This study was conducted in the area of Gedarif State, Eastern Sudan which lies between latitudes 12º 45` N, and 14º 15` N, longitudes 34º E and 37º E, (altitude 600m above sea level). The rainfall varies from north to south. The average annual rainfall is ranging between 175 mm in Gerba (143 km from Gedarif), to 570 mm at Galabat (150 km South Gedarif). It is markedly seasonal in character. The length of the rainy season fluctuates during July to October, and reaches peak in August (Gedrif metrological office 2003). Records on relative humidity, and temperature during the study were recorded. Some specimens of subject insect were sent to State Natural History Collections Museum of Zoology, Dresden, Germany for classification (done by Matthias X. (2006) and were then sent back to Sudan and recorded.

Biological studies

This study was conducted both in the field and under laboratory conditions. Experiments conducted in the lab. included biology of *Epiphora bauhiniae*. The field surveys were carried out during seasons 2003,2004, and 2005 covered twelve states in the Sudan Viz Gedarif, Kassala, Red Sea, Sinnar, Blue Nile, Gezira, Southern Darfur, White Nile, Kordufan, Northern, Nile, and Khartoum State. The aim of the survey was to confirm the presence of the silkworm *Epiphora bauhiniae* (*Lepidoptera*: *Saturniidae*) in some localities of these states; where the silkworms *E.bauhiniae* were expected. A map showing the sites where these worms were expected was drawn (**figure 1**).

Different instars and/or healthy cocoons of *Epiphora bauhiniae* (total 100 from each geographical region) were collected and preserved for laboratory studies to determine the biological studies. Effects of rain during the rainy season on the biological traits were also determined. Biological studies covered the productive and reproductive periods of *E. bauhiniae*, these included the incubation period, percentage hatchability of the eggs, number and duration of instars, the larval and pupal periods, larval mortality, percentage pupation and pupal weight, the percentage adult emergence, the adult longevity and total life cycle as well..The activities of the moths i.e. mating time, pre-oviposition period, oviposition period, post ovi-position period, oviposition rate, six ratio of the progeny, temperature, and relative humidity beside the observations of its major enemies were all recorded throughout the rainy seasons of the three years (2003, 2004 and 2005).

Culturing of the eggs and larvae of Epiphora bauhiniae

The leaves containing the prolific eggs deposited on the same day were used. Fifty eggs were collected, transferred to a cup and kept in the lab. Five replicates i.e. ten eggs in each cup were used. When the eggs hatched, the incubation period, and % of eggs hatched were recorded monthly during the rainy season for the three seasons. Weights and lengths of the eggs were also recorded. Ten of the hatched larvae were transferred, by means of brush, to feeding trays. Food was supplied to larvae three times a day, at morning, midday and at night. Tender small leaves of *Ziziphus spina -christi* were fed to i–iv instar larvae while mature leaves were fed to fifth instar larvae. Description of the different instars and their behavior, length, weight, and % survival during each period (month) of the rainy season were recorded. When the larvae pupated, the duration of each instar and the total larval period were recorded.

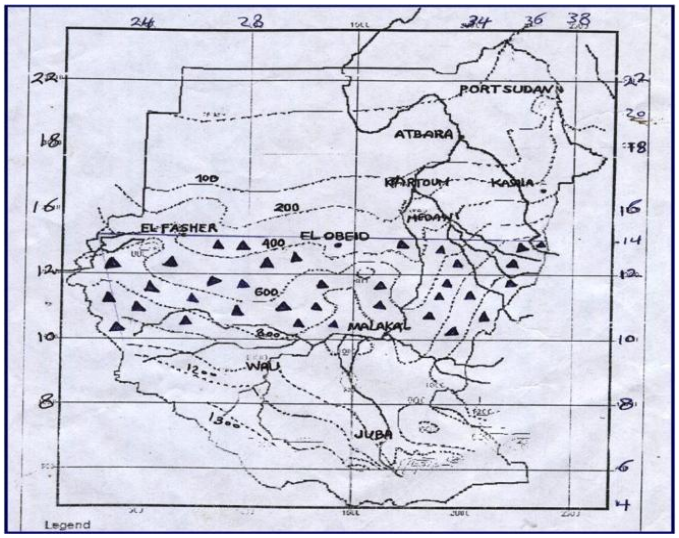

Figure 1: Mean annual rain fall (mm) and distribution of insect in sudan

Data analysis

Statistic analysis system (SAS) was used for data analysis and the least significant difference (LSD) was used to determine the significance of difference between means. Transformation of data was carried before using the ANOVA to test for significant differences, to determine the significant effect of period of rainy season (July, August, September and October) on productive and reproductive behavior of the insect.

III. RESULTS AND DISCUSSION

According to the classification of the insect under study, Um khiwait (local name), by State Natural History Collections Museum of Zoology, Dresden, Germany, the insect was classified as follows; Class: Insecta, Order: Lepidoptera. Family: *Saturniidae*, Genus: *Epiphora* and Species: *bauhiniae* (Guerin Meneville).

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The results obtained in this study for the insect *Epiphora bauhiniae* showed high success in survival and yield of cocoons in Sudan's conditions during the rainy season (Autumn) at different regions, or areas (**App. Plate 1**). The study, besides confirming the presence of the insect in Sudan, it also suggested to be the first biological report. It showed the importance of the wild insect (silk moth) and the possibility of application of silkworm rearing for silk production in Sudan using wild silk moth of the family *Saturniidae*. This is also supported by Peigler [9], who reported that many *spp* from *Saturniidae* silkworm had been knowledged for silk production from ancient time.

Plate (1): Cocoons of *E. bauhinae* produced during rainy season

Effect of temperature and relative humidity conditions on survival, growth and larval period:

The study showed significant variation in survival percentage and all growth parameters (weight and length), pupal weight and % pupation in relation to temperature and the relative humidity (RH) during the period of the rainy seasons. The high values were obtained in August and September, at average temperature ranged between 32º – 35º C, and relative humidity ranged between 65 - 88%. The low values were obtained in October at average temperature 37.4º C, and relative humidity < 60% **(Figures 2 and 3).** From the above result the relative humidity and temperature showed significant effect on growth parameters, this result are agreed with Shaarawy et al [10].

The high survival percentage of *E.bauhiniae* occured in the first month (Periods) of the rainy seasons showed that mortality of larva is minimal at low temperature 32-35º C and high relative humidity 65-88% . However, the effect of temperature was pronounced in October (37.4º C) where survival percentage was 60 and 70% compared to it in low temperature when high survival % (100%) was observed in July, August and September (100%). This agrees with Madras [1] statement, who reported that the combined influences of temperature and relative humidity on insects is best observed by the effect on mortality.

Similarly, the growth (weight, length) of larvae and the yield of insect was started to be affected by RH in July (the first month of the rainy season) with clear effect observed in October. High survival %, length, weight of larvae, fecundity of insect and cocoon (pupa) weight were obtained at high relative humidity than at low relative humidity. This indicates the effect of relative humidity on survival, growth of larvae and yield of insect (**Figure 4**).

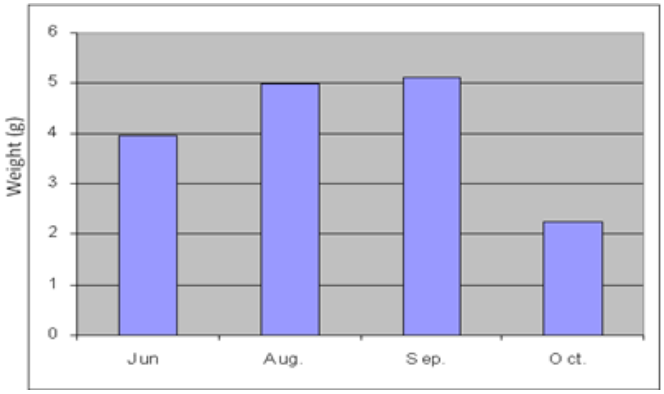

Figure 2: Effect of period of rainy season on larval growth (weight) of *E. bauhiniae* reared during season 2003-2005

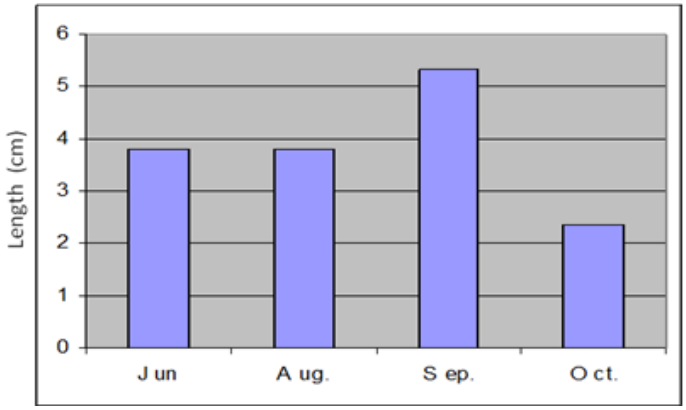

Figure 3: Effect of period of rainy season on larval growth (Length) of E. bauhiniae reared during season 2003-2005

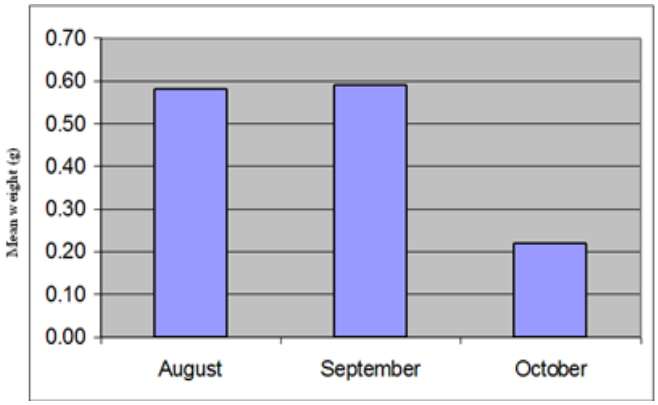

Figure 4: Effect of period of rainy season on pupal (weight) of *E.bauhiniae* reared during rainy season (2003 – 2005).

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The same result was stated by Wali *et al*. [11] who reported that the mean high temperature of 30º C with low RH decreased larval weight by 13%, cocoon weight by 24%, fecundity by 48% in *Bombyx mori*, also he showed an effect of RH, and temp. on economic characters of silkworm, which is in agreement with this result. Lee [12] mentioned that the male larvae produced less silk than the female in *Bombyx mori* which in agreement with this result where the female larva of *E. bauhiniae* produced largest cocoon than male. The same result was reported by Shaarawy *et al* [10].

Adult fecundity and life cycle compared to other silk moths: This study showed that the life cycle of the insect was short (58-59.2 days) in favorable conditions **(Figure 5)** compared to tussah and *Anaphe* silkworm (77-85 days) as stated by Mauersberger [13] and Bergmann [14].

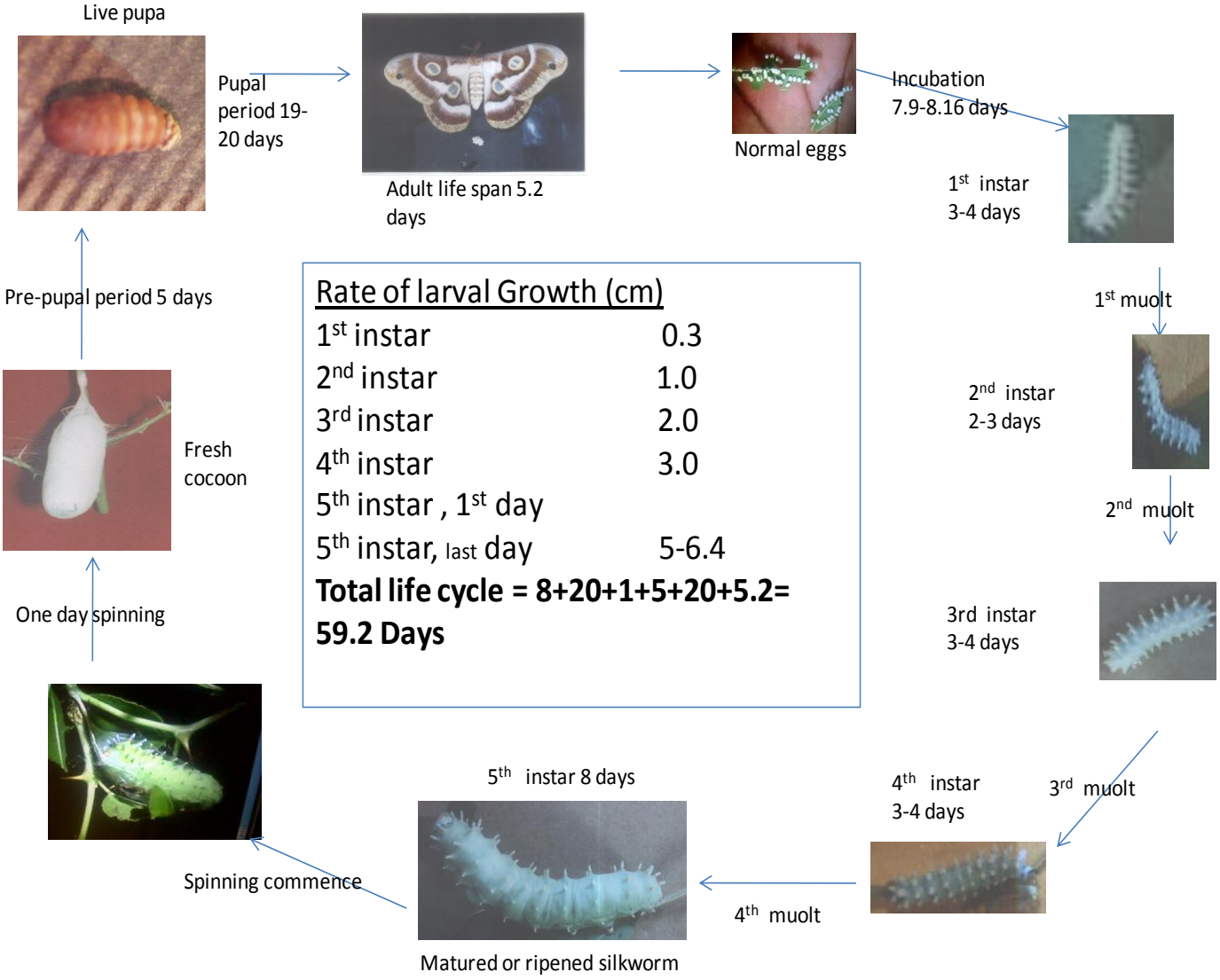

Figure 5:.Life cycle of silkworm, *Epiphora bauhiniae* (*Lepidoptera: saturniidae*)

Tussah larva required 47-75 days feeding but that of *E.bauhiniae* larva required 19-20 days only while *Anaphe* larva reguired 198-280 days as reported by [15]. Larva of *E.bauhiniae* remained inside cocoon for five days before transformed into pupa (Pre pupal period) the larvae passed through 5 instars during larval period, the same result was reported by Himanrigshu et al [16] (**Table 1**), these days represented the period required before harvesting cocoon.

This result is in agreement with the results of Lee [12] who reported for harvesting cocoon in tropical countries, and is also inline with the results reported by Choi [17] on *Bombyx mori* reared in Sudan. The insect *E. bauhiniae* has high fecundity (296.8 eggs) per insect (**Table 2 & Figure 6**) than that of Tussah insect (150 eggs) as Mauersberger [13] comparison reported on Tussah silk moth.

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Table 1
Preoviposition period, oviposition period, post oviposition period, oviposition rate and fecundity of *Epiphora bauhiniae* reared during the rainy months (season 2003-2004-2005).

Season	Period	Reproductive parameters				
		Preoviposition period (days)	oviposition period (days)	Post oviposition period (days)	oviposition rate (eggs)	Fecundity (eggs)
2003	July	1.09 a	3.00	1.24 a	57.23 bc	201.2 c
	August	1.09 a	3.00	1.14 a	94.23 a	284.7 a
	September	1.09 a	3.00	1.10 a	81.13 ab	243.8 b
	October	1.04 a	3.00	1.00 a	54.53 c	160.6 c
	G. M	1.081	3.00	1.20	74.45	222.59
	M.S.E.	0.006	0	0.082	158.05	983.173
	C.V.%	7.38	0	25.61	16.73	14.09
	S.E.±	0.0356	0	0.1283	3.9858	9.9155
	L.S.D.	N.S	0	N.S	16.20	40.30
2004	July	1.084 a	3.00	1.20 a	98.80 a	286. 9 0 a
	August	1.080 a	3.00	1.20 a	92..76 a	279.20a
	September	1.080 a	3,00	1.30 a	91.36 a	283.10 a
	October	1.05 a	3.00	1.00 a	36.50 b	104.50 b
	G. M	1.076	3.00	1.175	82.10	245.40
	M.S.E.	0.003	0	0.163	177.72	1876.44
	C.V.%	5.23	0	0.163	16.24	17.65
	S.E.±	0.0252	0	0.1803	4.215	13.90
	L.S.D.	N.S	0	N.S	17.16	55.76
2005	July	1.028 a	3.00	1.50 a	73..90 b	221.80 b
	August	1.068 a	3.00	1.50 a	94..85 a	283.70 a
	September	1.034 a	3,00	1.40 a	939.15 a	278.30 a
	October	1.056 a	3.00	1.40 a	46.66 c	140.40 c
	G. M	1.047	3.00	1.475	77.04.	231.10
	M.S.E.	0.004	0	0.188	349.6	140.56
	C.V.%	5.68	0	32.68	16.18	16.20
	S.E.±	0.0266	0	0.1936	5.9127	11.84
	L.S.D.	N.S.	0	N.S	24.07	48.19

Means in the same column followed by the same letter are not significantly different at 0.05 level of significance using LSD.

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Table 2

Life cycle of wild silkworm E.bauhiniae reared during the rainy months of (2003, 2004 and 2005).

Stages	Period (days)							
	Incubation	Feeding of larval instars	Sleeping of larva before spinning	Sleeping of larva after spinning (prepupal period)	Bulding of cocoon shell	Pupation	Longevity	Total life cycle
Egg	8	0	0	0	0	0	0	8
I	0	2	1	0	0	0	0	3
II	0	2	1	0	0	0	0	3
III	0	2	1	0	0	0	0	3
IV	0	2	1	0	0	0	0	3
V	0	8	0	5	1	0	0	14
Chrysalis	0	0	0	0	0	20	0	20
Moth	0	0	0	0	0	0	5.2	5.2
Total Period	8	16	4	5	1	20	5.2	59.2

Adults do not feed at all (App. Plate 2), a result which is close to that reported on emperor moth that has no mouth parts to feed (http:11 trittaway tripod. com /silk/s cyn .htm 08/02/1424.*Websites maintained by Billoehlke*) [18]. The study showed that the species can be generated twice a year (bivoltine) which reflects the importance of the insect in terms of the cocoon quality as bivoltine races are superior quality compared to multivoltine silkworm species Lee [12]. The results showed that two crops are possible during the rainy season (Autumn), where the first crop was spun at the beginning of August and the second crop at the end of September and early in October. This result was the same as that reported by Huber [19] for Tussah worm on bivoltine race. The dormancy period occurred at the pupal stage and could be broken by moistening the pupa several times only in November-December-January-February-March-April-May and June. This result agrees with what Madras [1] reported. Mohammed *et al*. [20] reported on *Bombyx mori* diapause occurring at the egg stage. Temperature and the RH have a significant effect on % emergence of adult (Table 3).

The study revealed that bio cycle of the insect was affected by the combined effects of temperature and relative humidity **(Figure 7 & 8),** a result which is in line with that of Madras [1] who reported that the long period of different pupal stage was obtained at the highest temperature and lowest relative humidity. Climatic factors profoundly influence the biocycle of the insect, the study recommended to use this species for rearing in August and September to obtain a high survival percentage and the heaviest cocoon weight. This was also explained by Lee [12] who stated that weight is the most significant commercial factor of cocoon. So the result showed a reasonable weight of cocoon produced by *E.bauhiniae* (> 2.9 g) and this support a recommendation for rearing this species during the rainy season (Autumn) because the produced cocoon is heavier than that reported by Lee [12] for pure breed (1.5 to 2.2 g), and hybrid weight of cocoon (1.8 to 2.5 g) for *Bombyx mori*.

Plate 2: Adult (Female) of *E . Bauhiniae* stretched wings

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Table 3

Percentage adult emergence, mating time, and incubation period and percentage hatchability of eggs of *Epiphora bauhiniae*, reared on the rainy season during (2003, 2004 and 2005 seasons.

Season	Period	Biological indicis			
		(%) adult emergence *	Mating time (hrs)	Incubation period (days)	Percent (%) hatchability *
2003	July	4.3 ab (18.8)	13:08:16	7.56 a	9.84 a(94.4)
	August	5.70 a (38.8)	13:08:33	8.32 a	9.84 a(98.4)
	September	5.80 a (33.8)	13:45:00	7.96 a	9.80 a(97.6)
	October	2.30 b(5.3)	13:45:00	7.98 a	9.88 a(95.6)
	G.M	4.50 (24.1)	13:26:17	7.96	9.84 (96.5)
	M.S.E	1.301	0	0.379	0.020
	C.V%	52.20	0	7.74	1.400
	S.E ±	0.570	0	0.2753	0.063
	L.S.D	2. 620	0	N.S	N.S
2004	July	4.530 b(20)	13:08:00	7.6 9a	9.768 a(95)
	August	6.548 a(42.5)	13:08:00	8.16 a	9.806 a(95.8)
	September	4.613 b (26.25)	13:18:00	8.10 a	9.88 a (97.2)
	October	2.485c (5.75)	13:16:00	8.40a	9.766a (94.8)
	G.M	5.54 (23.63)	0	8.155	9.805 (95.5)
	M.S.E	0.279	0	0.356	0.025
	C.V%	11.62	0	7.31	1.62
	S.E ±	0.264	0	0.2667	0.0712
	L.S.D	1.214	0	N.S	N.S.
2005	July	4.513 b (20)	13:30:00	7.94 a	9.674a (93.2)a
	August	6.421 a(41)	13:30:00	8.06 a	9.818 a(96.0)
	September	6.093 a(33.76)	13:20:00	8.10 a	9.860a (96.4)
	October	2.205 c (4.5)	13:10:00	8.36 a	9.767 (94.0)
	G.M	4.808 (24.8)	13:22:05	8.115	9.716 (94.9)
	M.S.E	0.692	0	0.091	0.029
	C.V%	17.31	0	3.71	1.75
	S.E ±	0.4161	0	0.1347	0.0766
	L.S.D.	1.912	0	N.S.	N.S.

Means in the same column followed by the same letter are not significantly different at 0.05 level of significance using LSD.

* Means between perenthesis are transformed to $\sqrt{(x + 0.5)}$

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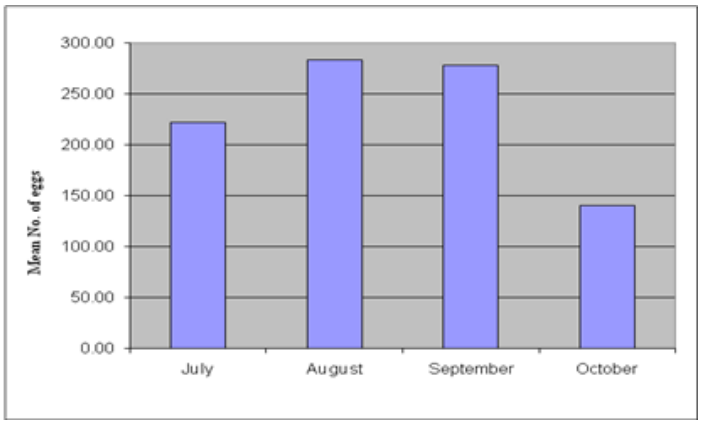

Figure 6: Mean fecundity of silkworm *E. bauhiniae* during July – October, season (2003 – 2005).

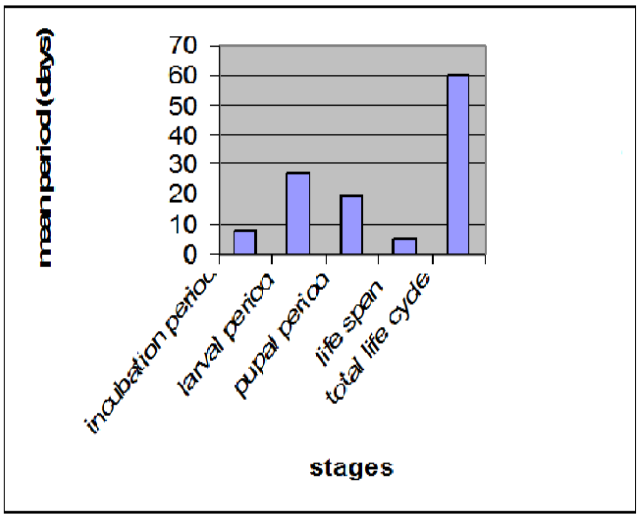

Figure 7: life cycle of silkworm *E. bauhiniae* (female) reared during the favourable conditions July- September, season 2003.

Potentiality of silkworm rearing in Sudan

Dookia [21] reported that the presence of silkworm in any area indicated the possibility of rearing this worm in this area. Therefore, results obtianed in this study indicated the potentiality of rearing this worm species for silk production in Sudan. The importance of this species selection is due to the fact that it is an indigenous insect to Sudan However; this species is a candidate if survival and cocoon weight are included in selection criteria for silk production in future. Because of its commercial value as stated by Raina [22] and Delport [23] who reorted an African wild silk fibers from *Saturniidae: Epiphora* genera produce silk of commercial value and with significant economic importance due to their interesting mechanical properties the same result reported by Addis *et al* [24]. This is in agreement with Gashe and Mpuchane [3] who reported that *E. bauhiniae* produced silk of commercial value and has good potential for being used in community based projects, as candidates for the sustainable use of natural resource and for its edible larva. So this makes it one of the most important resources of income in the future because the produced silk would have wide industrial uses, good marketing quality, important export value, as well as it is a source of rural employment and is an alternative to costly imports.

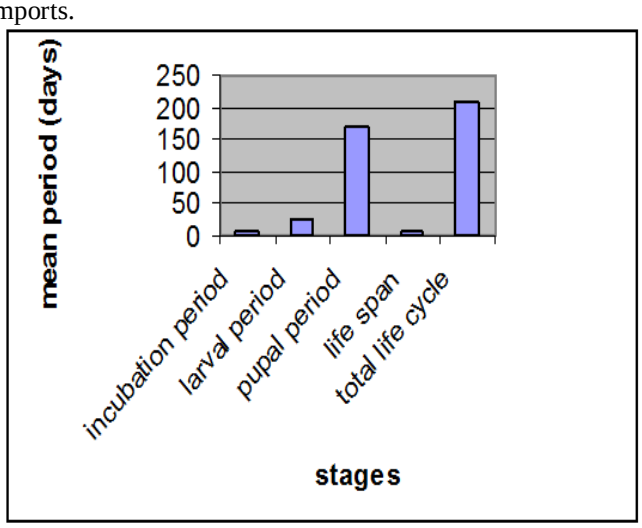

Figure 8: The life cycle of silkworm *Epiphora bauhiniae* during adverse condition. October –June.

Natural enemies of E.bauhiniae and its defence

According to observations in the field, and during the experiments, some enemies were observed attacking the insect during different stages of its life cycle; the eggs are attacked by ants, while the larvae are fed on by lizards, birds, spiders, camels and goats attacked them during feeding on the plants, the pupae are attacked by the parasitoid wasp (**App. Plate 3**) and rats. The same observation was recorded A. Tikader [25] about parasitoid attack *Lepidoptera: Saturniidae* eats the pupae inside cocoons. The adults of *E.bauhiniae* are attacked by cats, salamanders and cockroaches.

Also human beings were observed as another enemy of the insects, some tribes in Gedarif and Sinar States use the cocoons for spell bound as what is called mascot, particularly by astrologers, but some tribe use it as medicine or cure for kids cough and coryza. Insects have different types of defences, the insect laid their eggs under leaves well tighten with gummy substance to avoid enemy. Larvae live in groups under leaves during the first stages (i, ii, and iii), this is the time of exposure to birds where the worms white in colour particularly stages ii, and iii. In stage iv and v the worms green in colour resembling their self to the surrounding not to be seen by birds, and animals. Mature worms spewed green spittle or fluid as defence tools. Adults exclude white fluid or fly away to avoid enemies, pupa is enclosed in a silken cocoon and tied strongly to the tree branches and hence protected from wind.

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Plate (3): prasatoid (wasp) of the wild silkmoth (saturnidae) *E. bauhiniae*

IV. CONCLUSIONS

The study confirmed the presence of the silk moths *Epiphora bauhiniae* in the Sudan, and the possibility of rearing silkworm in different areas in the Sudan. The study also showed that there is inter- specific variation as well as variation due to environmental factors (Temp. and RH.) in growth, survival % and yield of the silkworm during different periods of rainy seasons. The inter-specific variation was significant in survival, length, weight and yield of insect. August and September showed high survival, growth and yield. October showed low values, indicating the importance of the selection period before introducing any sericulture activities using new silkworm species. The period August, September was found to be the suitable one, between the tested periods, for high survival, growth and yield of cocoons. The harvest of crops twice a year is possible and the duration between the first, and second harvest depends on the temperature and relative humidity where the high productivity obtained at proper temperature and relative humidity at that period (32 – 35.1º C, 70 – 80%, respectively).

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