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IMPACT OF ENVIRONMENTAL FACTORS ON OUTBREAK OF DISEASES IN THE MUGA CROPS, *ANTHERAEA ASSAMA*, WESTWOOD (LEPIDOPTERA: SATURNIIDAE), IN KAMRUP DISTRICT (ASSAM)

RASHMIMALA KAKOTI
Department of Zoology, B. Borooah College,
Guwahati - 781 007
E-mail: kakoti.r@gmail.com

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ABSTRACT
Population growth of the Muga crops , *Antheraea assama* Westwood (Lepidoptera:Saturniidae), an important bioresources of Assam, were analysed with respect to various agro-climatic conditions during the year Dec.2006-Dec.2009. Experimental data showed that the intensity of diseases became high at winter season (Dec.-Feb.). Two microbial diseases caused by protozoa and bacteria were seemed to be appear in devastating manner in winter crops, which were identified as 'Pebrine' and 'Flacherie' respectively. Less frequently, autumn (Sep.-Nov.) crops were also attacked by flacherie. Rate of larval mortality was found to be highest (92.66%) in winter crops infected with bacterial flacherie. Pebrine has claimed 61.3% of the larval life in winter crops. Death related to the impairment of larval moulting was identified in winter crops. However, mortality rate was minimum in spring (March-May) and summer (June-Aug.) seasons. No incidences of viral and fungal infections were recorded during the experimental periods.

INTRODUCTION

Antheraea assama Westwood commonly known as Muga silk worm is a multivoltine species, endemic to Assam and adjoining regions (between 90-97ºEL and 22-29ºNL).It feeds on *Persea bombycina, Litsea polyantha* and some other plants belongs to the family Lauraceae. There are many factors that affect the success of commercial silk cocoon production, the menace of diseases being the prime one (Sridar *et al.,* 2000). Yearly, large number of muga crop loss occurs due to these diseases (Kakati, 2002). As a result, it becomes a major constraint in the progress of muga silk industry in Assam. Being reared outdoor, the worms have adjusted themselves to a certain degree against all kinds of selection forces of nature.

Four major seasons prevalent in Assam are winter (December-February), spring (March-May), summer (June- -August) and autumn (September-November). As the region is situated on the subtropical zone of India, temperature, relative humidity and rainfall differ by seasons. Change of climatic factors, specially temperature and relative humidity affect almost every aspects of the life cycle of silkworm including their development and survival (Gohain and Borua, 1983). Even, the change of season can influences on consumption and utilization of food in muga worms (Das *et al*., 2002), which in turn affects on their physiological state. Therefore, although muga silkworms are reared in all the seasons of the year, their susceptibility to diseases differ in different seasons.

In the present work, attempt has been made to elucidate the major environmental factors operating at four different seasons of the year that influence on outbreak of diseases.

MATERIALS AND METHODS

The present investigation was carried out on muga silkworm, *Antheraea assama* W reared under natural state on the host plant *Persea bombycina* in the Central Muga Seed Farm, Guwahati (District Kamrup). Experiments were performed at all the four seasons of the year from Dec.2006 to Dec.2009. In each season of the year, batches of 100 DFLs replicated 3 times were reared. To avoid loss of larvae by predators they were reared under nylon net. Temperature, relative humidity and rainfall were recorded by standard procedures. Larval growth and physical condition of the hostplants were kept under constant supervision. Mortality rate at different instars were recorded separately.

Diseased worms were collected before death for microscopic examinations. Pebrinized larvae were detected after examination of spores present on the gut tissues and faecal pellets by the method adopted by Patil *et al.,* (2001). Flacherie infected larvae were detected by observation of flaccid condition of the internal organs. Moulting impaired larvae were carefully monitored at their natural state.

For statistical test, mean performance data were derived from the primary data. Recorded data were analysed. Comparisons of percentage of mortality at four different seasons were obtained by ANOVA.

RESULTS

Effects of temperature (Temp.), relative humidity (RH) and rainfall (RF) on population growth of the muga crops in terms

of larval mortality and larval durations were recorded at four different seasons of the year. Mean value of the rate of mortality at different instars along with percentage of total larval mortality are presented in the Table 1.

Effect on winter crops

In winter season, range of Temp., RH and RF are found to be confined within 19 ±2ºC-20±2ºC, 58-68%, 0.0 mm-8.1 mm respectively. In expt. no.1, larval duration was 50.75 days, within these 61.3% mortality was recorded. From 3rd instar onwards, appearance of black spots on the body surface and spindle-shaped spores in the midgut tissues and excreta indicated that the larvae suffered from 'pebrine'. The growth of the larvae became rhythm irratic. They refused to take food and frequently came down to the lower part of the hostplant and wondered aimlessly. It is evident from the data that rate of larval mortality in the late instar larvae were significantly higher (23.66, 20 and 13.33 nos. in 3rd, 4th, 5th instars respectively).

In expt. No. 2, within 53.00 days of larval life, 92.66% larvae died. Mean value of larval mortality were significantly higher in 4th and 5th instars (31.66 and 41.33 nos. respectively). The body of the larvae become soft and dull in appearance. Frequently they vomited brownish colour (Space for-Table 1 and Table 2)

Liquid. They shrunken lengthwise. Microscopic examinations showed flaccid conditions of the internal organs. They were found to be hanged from the branches of the hostplant and died at this state. The whole culture was disrupted before attaining larval maturity. The disease was identified as 'flacherie'.

In expt. no.3, percentage larval mortality was 63.63 and total larval duration 55.6 days. The cause of death was identified as impairment of moulting. At 3rd and 4th moults, larvae failed to recover from moulting. Although, new integument was formed, the old exuvia attached firmly on the body surface. They excreted some sticky faecal matter which sealed the anal lips completely. The larvae remained in this condition for 12-48 hr. and died thereafter.

Effect on spring crops

Spring seasons were specified by Temp. 26±2ºC-28±2ºC., RH 70-80%, RF 372.3-541.1mm. In expt. no. 1, 2, 3 of Table 1B, larval mortality at three consecutive years was recorded as 4.63%, 3.32% and 7.33%, which can be considered as quite insignificant. Larval durations were recorded as 31.25, 30.50 and 28.75 days. The larvae were quite active and moulted uniformly. They fed voraciously. No diseased worms were recorded during the observation periods.

Effects on summer crops

Summer seasons were characterized by heavy rain with wind and thunderstorm, high temperature and high humidity. Table 1C presents range of Temp.34±2ºC-35±2ºC, RH 80-92%,RF 783.1 mm- 871.1 mm. Larval mortality was recorded as 15.33%, 9.99%, 10.69% and durations 23.00, 25.50, 21.30 days in expt. no. 1,2,3 respectively. Larvae were quite normal and active. Death of the larvae might be accidental as heavy shower of rain inflicted mechanical injury to the developing larvae. Rate of larval mortality was uniform at different instars. No sign of diseases were observed in summer crops.

Effects on autumn crops

Effect of environmental factors on autumn crops are presented in the Table 1D. The range of Temp., RH and RF recorded at this season are 29±2ºC-34±2ºC, 65%-75%, 222.2 mm -

Table 1: Incidence of larval mortality of muga crops in four seasons of the year (from Dec. 2006-2009)

Table 1A: Percentage of larval mortality of Winter crop (Dec - Feb)

No. of Expt.	*Temp.	*RH	*RF	*LD(days)	Rate of larval mortality /instar (mean value) I	II	III	IV	V	Percentage of larval mortality
1	20 ± 2ºC	60 –65%	0.0mm	50.75	1.67	2.66	23.66	20.00	13.33	** 61.3%
2	20± 2ºC	62-68%	1.6mm	53.00	0.66	1.33	17.68	31.66	41.33	***92.66%
3	19 ± 2ºC	58-65%	8.1mm	55.6	1.00	1.66	11.67	20.66	18.64	****63.63%

Table 1B: Percentage of Larval mortality of Spring crops : (March-May)

No. of Expt.	Temp.	*RH	*RF	*LD (days)	Rate of Larval mortality /Instar (mean value) I	II	II	IV	IV	Percentage of larval mortality
1	28 ± 2ºC	70-75%	541.1mm	31.25	1.00	1.33	1.67	0.66	-	4.63%
2	27 ± 2ºC	72-78%	441.4mm	30.50	-	1.32	1.00	1.00	-	3.32%
3	26 ± 2ºC	75-80%	372.3mm	28.75	1.00	2.00	1.67	2.33	0.33	7.33%

Table 1C: Percentage of larval mortality of Summer crops : (June – August)

No. of Expt.	Temp.	*RH	*RF	*LD(days)	Rate of larval mortality /instar (mean value) I	II	III	IV	V	Percentag e of larval mortality
1	35 ± 2ºC	80-90%	783.1mm	23.00	4.00	4.00	4.33	1.67	1.33	15.33%
2	34 ± 2ºC	82-92%	871.1mm	25.50	2.67	2.33	3.66	0.66	0.66	9.99%
3	35 ± 2ºC	80-85%	840.0mm	21.30	2.33	2.00	2.70	2.66	1.00	10.69%

Table 1D: Percentage of larval mortality of Autumn crops (Sept. – Nov.)

No. of Expt.	Temp.	*RH	*RF	*LD(days)	Rate of larval mortality /instar (mean value) I	II	III	IV	V	Percentage of larval mortality
1	32± 2ºC	65-72%	222.2.mm	38.00	1.70	3.67	5.33	6.70	3.00	20.4%
2	29 ± 2ºC	70-75%	241.5mm	33.30	0.33	2.67	1.66	4.66	2.33	11.65%
3	34 ± 2ºC	65-75%	385.1mm	35.50	1.33	3.00	9.00	28.66	46.33	***88.32%

* RH-Relative humidity, RF- Rainfall, LD-Larval duration, Temp.- Temperature; ** death due to pebrine *** death due to outbreak of flacherie, **** death due to moulting impairment

Table 2: Comparison of larval mortality (%) of muga crops in four different seasons for 3 consecutive years (Dec.2006-2009) of experiment

Periods year –wise	Winter	Spring	Summer	Autumn
Year –I	61.3%	4.63%	15.33%	20.40%
Year –II	92.66%	3.32%	9.99%	11.65 %
Year – III	63.63 %	7.33%	10.69%	88.32%
Toatl Value	217.59	15.28	36.01	120.37

Let,our 'Null' Hypothesis: Mortality rate at 4 seasons are homogenious; From Table 2, we can find – Raw SS (RSS) = 25523.2583,Correction factor (CF) = 12626.29688; Total sum of square (TSS) = RSS- CF = 12896.96142(Between season) sum of square = 8495.217625; (Within season) sum of square = 4401.743795

385.1mm respectively. In expt. no.1, larvae showed irregular growth. They became slow and sluggish. Mortality rate was recorded as 20.4% in 38.00 days of larval life.

In expt. no. 2, larval mortality was 11.65% and larval duration 33.30 days. Differences of rate of larval death at various instars were found to be insignificant. Larvae became active and no diseased forms were identified. In expt. no.3, cultures were incomplete, as 88.32% larvae died before attaining maturity. In 4th and 5th instar stage, mortality was recorded as 28.66% and 46.33% respectively. The disease was identified as flacherie. Space for Table 3 and Fig 1

Statistical test

Comparison of data from four seasons of the year and 'Analysis of Variance' (ANOVA) shown in the Table 2, 3 and Fig.1 revealed that population growth of the muga crops were quite heterogeneous. Larval death was highest in winter crops, followed by autumn crops. It was found to be minimum in summer crops. It was quite negligible in spring seasons.

DISCUSSION

As evident from the results, larval mortality of outdoor rearing of muga crops in four different seasons of the year is quite heterogeneous. It indicates that there is surely certain environmental impact on the experimental insect. Aruga, (1994) stated that, although several environmental factors operate in unison during rearing of silkworm, some of them may be suitable, but others may not. He further opined that occurance of silkworm diseases is also closely related to different environmental conditions. On the otherhand, fluctuation of humidity (to some extent temperature) too is the resultant affect of amount of rainfall prevailing at the season (Chaudhuri *et al*., 1999).

Analysis of several batches of winter crops, it is evident that the larvae reared in winter seasons are prone to diseases. The result shows outbreak of deadly disease 'pebrine' in winter crops. Thangavelu *et al*., (1988), also reported infectivity of pebrine in muga crops at winter. Sukla and Upadhyay (2008) suggested that the tendencies of the infestation of pebrine

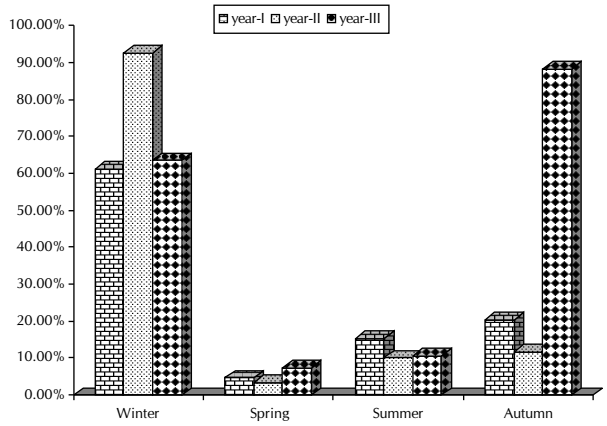

Figure 1: Percentage larval mortality at four seanons for 3 consecutive years

spores are more when silkworms are reared to dry and cool conditions. They also claimed that longer larval periods in winter enhanced the infectivity of pebrine. Although, pebrine spores transmitted transovarially, oral feeding of contaminated leaves is also one of the important factor of pebrine infection (Prasad *et al*., 2000).

Observation shows that several batches of muga crops in winter seasons are completely destroyed by 'flacherie'. Same result has been reported by Raj, (2000) for spreading of bacterial diseases of tasar silk, *Antheraea mylitta* in India. According to Goel, (2000), the predisposing factors for bacteriosis include low temperature, low humidity along with overmatured leaves. In case of mulberry silkworm, Subbaswamy *et al*., (2001) opined that poor leaf quality is the major constraint for outbreak of flacherie. Same is also true in case of muga culture in Assam (Kakati, 2002). In winter, due to reduction of moisture contents, leaves become hard and overmatured. It becomes dirty and polluted due to absence/ scarcity of rain water.

Flacherie was also evident in autumn crops. In this season, due to irregularity of rainfall, temperature and humidity fluctuate to a great extent. Excessive fluctuation of environmental factors might be responsible for their susceptibility to diseases.

The reasons for larval death due to impairment of moulting at 3rd and 4th moults in winter crops are not clear. Bardoloi and Hazarika (1992) and Hazarika *et al*., (1994) stated that moisture content of the hostplant is directly related to the body water content of the mugaworms, which may inturn influence the haemolymph volume in larval body. It can be presumed that reduced moisture content of the leaves as well as reduced humidity of the environment in winter season may adversely effect on larval moulting. As moulting is a serious physiological event for silkworm larvae (Prasad, 2002), perhaps both environmental and hormonal factors are responsible for

Table3: Larval mortality level at four seasons of the year: Analysis of variance (ANOVA)

Source of variation	*df	Sum of square	Mean square	F
Between season	4-1 = 3	8495.217625	2831.739208	F = 2831.739208 / 550.21797
Within season (error)	11-3 = 8	4401.743795	550.21797	= 5.1466
Total	12-1 = 11	12896.96142		

*df: degree of freedom; From Table 3, we can find-Tabulated F (0.05) for 3 and 8 df = 4.76. Hence, calculated value of F is greater then tabulated value of F for 3and 8 df at 5% level of significance; So, it can de concluded that mortality level at different season are heterogeneous. Here, we reject our 'Null' Hypothesis.

impairment of moulting. In fact, it needs further physiological investigations.

After first shower of rain, food plants in spring season flourished with new soft and tender leaves suitable for young age larvae. Availability of stage specific leaves matching to each larval instar is one of the important factor for population growth of the muga crops. Our findings showed lowest mortality rate in spring crops followed by summer. Barton- Browne (1964) reported that increased water content of the leaves act as a stimulant to phytophagous insect. On the otherhand, growth and development of insect larvae are directly related to food quality (Scriber and Slansky, 1981). Similar expectations can be made in case of mugaworms, as they feed voraciously during spring and summer seasons and become healthy.

Researchers claimed that during summer season, silkworms are susceptible to fungal diseases due to increased humidity. But our findings showed no incidence of mortality due to fungal infections. Patnaik (2008) also stated that fungal infection is a rare occurrence in muga crops.

It is now evident from the present study that incidence of outbreak of diseases in muga silk worm is related to environmental factors. Hopefully, this findings helps the rearer to take appropriate steps to contain diseases.

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