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Seasonal climatic influence on the leaf biochemicals of Sal (*Shorea robusta*) flora and *in situ* breeding behaviour of Laria ecorace of tropical tasar silkworm *Antheraea mylitta* Drury

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Abstract
The voltinism and breeding behaviour of Laria ecorace was studied *in situ* in relation to the climatic factors and foliar biochemicals of the *Shorea robusta (*Sal) leaves. Meteorological data was recorded with higher average temperature during May and June (30.77 ^{0}C and 29.81 ^{0}C) whereas higher average humidity (%) recorded during rainy season from July to September (82.93, 84.50 and 81.66) and a corresponding higher rain fall (mm) from July to October (195, 255, 185 and 205). Linear correlation between the leaf moisture and biochemical contents with the abiotic factors was studied. It revealed a positive correlation between leaf moisture (Av. temp.0.673; Av. humid 0.246), while, proteins and carbohydrates of leaf have positive correlation with humidity but negatively correlated with maximum temperature (-0.538). On contrary, leaf phenol and tannin showed negative correlation with the abiotic factors. The suitability of leaf nutrition and the ecological factors are prominent for the Laria silkworm breeding and subsequent cocoon production on Sal. A peak moth emergence was observed during second and third week of June at the onset of rainy season with coupling rate of 45% over total emergence. Larval population was found more during August to October. At the same time, higher survivability and cocoon production was recorded.

Keywords: Laria, *Shorea robusta* (Sal), *Antheraea mylitta,* Climatic factors, leaf nutrition, *in situ* breeding

1. Introduction
Among the forty four ecoraces of *Antheraea mylitta* identified with diverse phenotype, behavioural traits and adaptability to various host plants, Laria being an important wild ecorace of economic importance distributed in various parts of Jharkhand and adjoining regions of Chhattisgarh [1, 2]. Primarily, Laria ecorace adapted on *Shorea robusta* (Sal) and this flora covers 86.9% of the total tasar flora in India as against 13.1% of *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan). In nature the voltinism of this ecorace is uni-, bi-, and trivoltine. It was estimated that about 10% populations is univoltine, 60% bivoltine and rest 30% is tri-voltine. The breeding behaviour of this ecorace in nature has not been studied so far [3, 4].

Due to various reasons the Laria population on Sal flora is declining alarmingly. This reduction in population size may cause low genetic variability which in turn reduces the adaptive potential of the species thus increasing the chance of extinction. To comprehend this scenario, the ecological factors and the nutritional dynamism in Sal flora was examined for its persuasion on the *in situ* breeding behaviour of the Laria tasar silkworm. Hence, a suitable strategy can be developed to protect and sustainably utilize the wild ecorace Laria particularly to harness the utilization of vast Sal flora.

Plant nutrition and climatic conditions play important roles on the growth and development of the herbivore insects. However, the nutritional dose is strongly governed by the soil properties and climatic conditions of the growing region [5]. The seasonal changes in the plant nutrients have major role on the population dynamics and breeding behavior of the phytophagous insects. The phenology of plant has imminent influence on the nutritional quality of the leaves and in turn on the lifecycle behavior of phytophagous insect, voltinism and other meta-physiological activities such as diapause *etc*., [6].

Contrary to the deciduous tree species which are generally summer-flushing, *Shorea robusta* (Sal) is a spring-flushing species in Indian dry tropical forests. The period around spring equinox is important because of the overlap of leaf fall, leaf flushing, and flowering/fruiting in Sal [7]. Hence, the present study was undertaken to elucidate the influence of ecological factors on nutritional variability of Sal flora and subsequently on the *in situ* breeding & population dynamics of the Laria silkworm.

2. Materials and Methods

The present study was carried out during March 2012 to January 2015. Two Sal forest patches namely Hashim and Kedla of Peterbar forest area were selected. The experimental site was located at 23.04^{0}N, 85.40^{0}E with altitude of 613mASL. The eco-zone experiences average maximum temperature 28.08 ^{0}C, average minimum temperature of 17.66 ^{0}C and annual precipitation of 1100 mm (Ministry of Water Resources, 2009). Climatically, three well defined seasons are experienced in this ecological niche. The seasonal variation in the population of Laria ecorace of tasar silkworm in relation to climatic factors such as temperature, humidity and rainfall was recorded. Also the Sal leaf biochemicals were analyzed by standard procedures. Data obtained were composited to correlate the ecological factors, leaf nutrition with the breeding behaviour of the Laria ecorace (total population).

2.1 Meteorological Data: The atmospheric temperature and relative humidity was recorded with the help of automatic thermo-hygrometer whereas data of rainfall obtained from Meteorological observatory of Bokaro, Jharkhand.

2.2 Observation and recording of different life stages of Laria silkworm population: Data collected from periodic survey included the collection of number of eggs, different larval instars, cocoons and observation of moth emergence pattern, coupling pattern and egg laying behaviour on Sal plants.

2.3 Nutritional Analysis of Sal leaves

The analysis of leaf samples of tender, medium and mature was carried out over different seasons for which following parameters have been considered:

2.4 Extraction and estimation of Sal leaf biochemical

Extraction of biochemical samples was carried out from the dried Sal leaves and using appropriate solvents to dissolution and isolation of biochemical compound for further estimation.

2.5 Protein: Protein sample was extracted [8] using TCA for precipitation of proteins and estimation of total proteins was carried out using Folin-Ciocalteau reagent and BSA as standard [9].

2.6 Carbohydrates: total carbohydrate content of Sal leaves was extracted using hot ethanol and estimated using anthrone reagent [10].

2.7 Total Phenols: The phenolic content in the leaf sample was extracted using 80% ethanol and were estimated by Folin-Ciocalteau reagent (FCR). The concentration of phenol was quantified comparing standard Catechol solution (1mg/ml) [11].

2.8 Total Tannins: Tannin content of Sal leaves was extracted and estimated using Folin-Dennis reagent. The concentration of tannin was quantified comparing standard tannic acid (0.5mg/ml) [12].

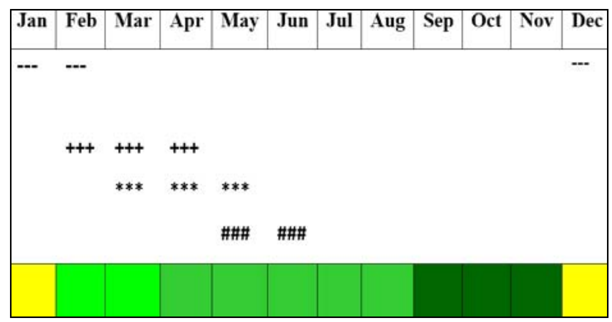
2.9 Statistical Analysis: The data generated were the average of the three years observation and presented in the mean with standard deviation. In some cases the data also presented in mean with Minimum value and maximum value. The data analyzed with Microsoft excel statistical package.

3. Results

The observations made for some parameters at two Sal forest patches of natural ecological niches were recorded as follows.

3.1 Phenology of *Shorea robusta* (Sal)

The phenology of Sal was recorded and it was found that the leaf shedding starts from the month of December and continues up to February. New leaves sprout started in the month of February till March. Flowering takes place from the month of March to May and fruiting in the month of May and June (Fig.1).



--- leaf fall, +++ new leaf, *** flowering, ### fruiting: Intensity of colour represents the maturity of leaf

Fig 1: Phenology of *Shorea robusta* at two Sal forest patches namely Hashim and Kedla of Peterbar forest area

3.2 Meteorological Studies

Meteorological data were recorded throughout the period (Table 1) which includes minimum and maximum temperature, average humidity, temperature and rainfall. Higher average temperature was recorded during May and June (30.77 ^{0}C and 29.81 ^{0}C) whereas average humidity was recorded higher during rainy season from July to September (82.93, 84.50 and 81.66%) and a corresponding higher rain fall was recorded from July to October (195, 255, 185 and 205mm).

3.3 Observation of different Life stages of Laria silkworm in relation to the climatic factors

Laria larval populations were observed from May to February. Different stages of larvae were found feeding on Sal leaves during the period *in situ* at the two locations of Peterbar Sal forest. Though there was emergence of moths during March and April, no larva was found in this period. This may be due to very high temperature and also due to poor hatching recorded during the period. 1st stage larvae were found in the month of May and subsequent month's different stages of Laria larvae were observed until next February. More number of larvae with all five stages of larval life was recorded during July to November. Concurrently higher cocoons were also found during these periods. Larval life span in rainy as well as autumn season was recorded in the *in situ* condition this period is concurrent with the commercial tasar silkworm rearing season (Table.1)

Table 1: Abiotic factors and different life stages of Laria silkworm during different seasons

Month	Temperature (^{0}C)		Humidity (%)	Rainfall (mm)	Number of individuals			
					Egg (clutches)	Larva	Cocoon	Moth
Apr	Mean	27.76 ± 4.58	46.45 ± 12.95	36	5	0	65	0
	Min-Max	19.3-36.6	24.0-86.0					
May	Mean	30.77 ± 5.18	41.73 ± 14.73	3	6	3	15	6
	Min-Max	18.8-40-9	19.0-83.0					
Jun	Mean	29.81 ± 4.88	59.72 ± 19.01	91	25	14	23	8
	Min-Max	21.6-40.9	25.0-89.0					
Jul	Mean	25.14 ± 2.01	82.93 ± 6.37	195	42	10	31	12
	Min-Max	22.5-33.5	56.0-89.0					
Aug	Mean	24.58 ± 1.87	84.50 ± 5.12	255	2	>3000	65	0
	Min-Max	21.8-30.8	66.0-90.0					
Sep	Mean	24.28 ± 1.13	81.66 ± 4.13	185	5	450	5	1
	Min-Max	20.9-29.9	66.0-90.0					
Oct	Mean	23.26 ± 3.53	66.0 ± 11.15	205	15	305	35	3
	Min-Max	18.5-26.5	54.0-87.0					
Nov	Mean	20.77 ± 4.18	61.73 ± 12.22	5	5	25	15	0
	Min-Max	14.8-25.9	46.0-75.0					
Dec	Mean	17.15 ± 3.58	59.22 ± 11.71	0	25	15	5	11
	Min-Max	10.6-24.9	40.0-65.0					

3.4 Moth emergence and Coupling behavior of Laria silkworm

Emergence of moths from Laria cocoons was observed under *in situ* condition. A sporadic emergence of moths was recorded during the month of March and April. But there was hardly few moth couplings were recorded and subsequently hatching was very poor. After a gap of short period a regular moth emergence was started from third week of May and continued till July. A peak emergence pattern was observed during second and third week of June at the onset of rainy season with coupling rate of 45% over total emergence (Male & female moth emergence). During August to September, two peaks of emergence were recorded with adequate coupling over 65% and hatching of more than 70%. Moth emergence (10-15%) was also observed during November to January and peak value was recorded in the month of December with a coupling of about 25-30% in spite of cold weather (12 ^{0}C to 22 ^{0}C). The emergence patterns of Laria silk moth in different seasons are presented in Fig 2-4.

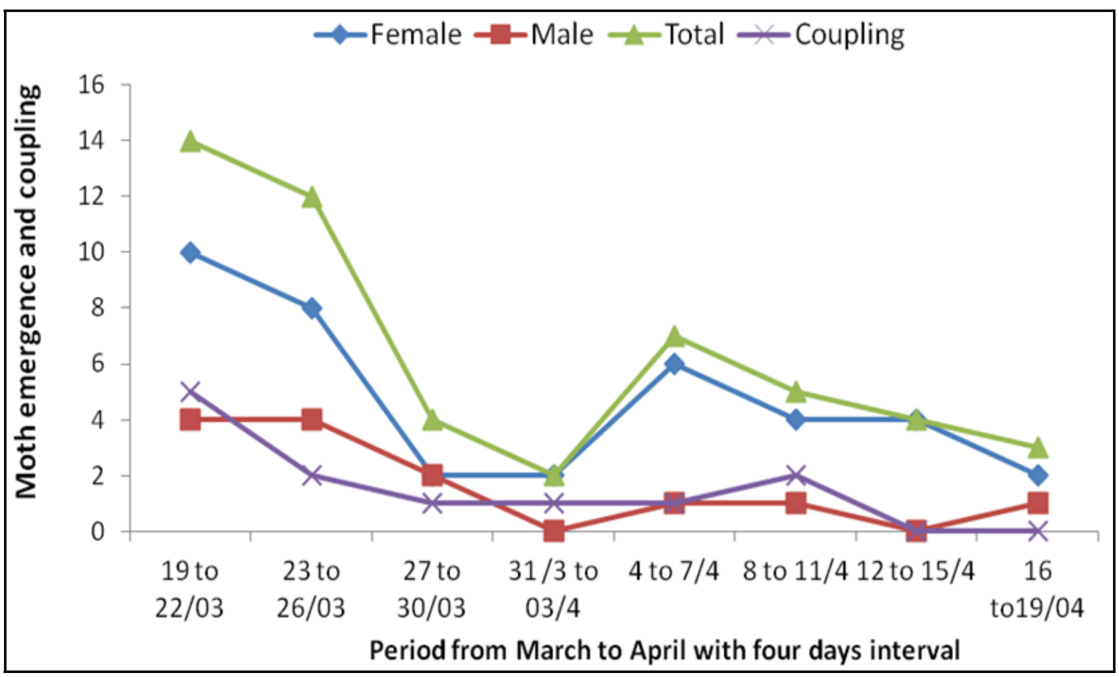


Fig 2: Moth emergence pattern and coupling *in situ* condition at Peterbar Sal forest During March to April

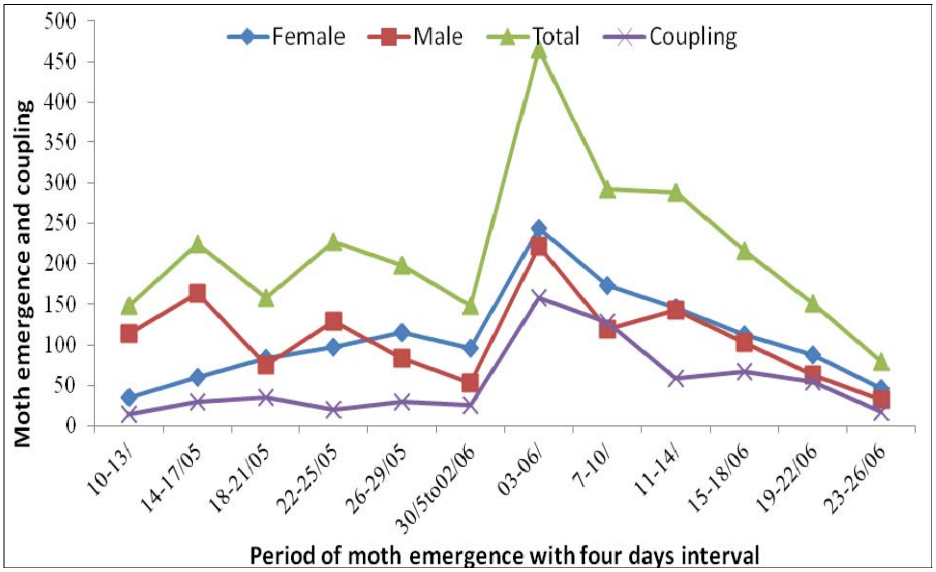


Fig 3: Moth emergence pattern and coupling *in situ* condition at Peterbar Sal forest during May to June

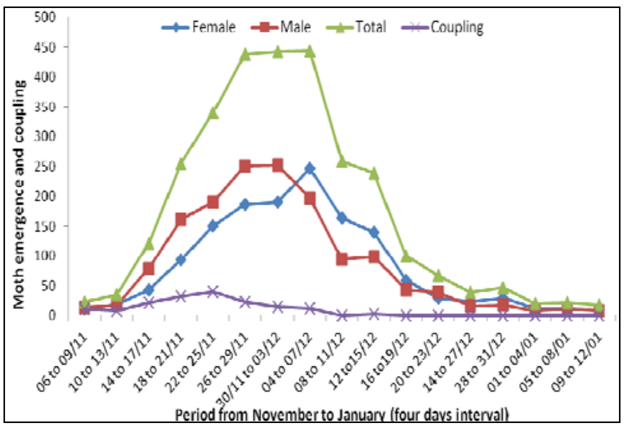


Fig 4: Moth emergence pattern *in situ* condition and coupling at Peterbar Sal forest during November to January

3.5 Nutritional Analysis of Sal leaves

Month-wise collection of Sal leaf samples was done from April to December and analyzed for the biochemicals (Table 2). Primary metabolites such as protein (101.0±11.5 mg/g) and carbohydrates (59.73 ± 8.95 mg/g) showed an increase in concentration from April to September (protein: 132.3 ± 6.3 mg/g, carbohydrate: 63.97 ± 13.45) followed by a declining trend. However, secondary metabolites, phenols and tannins increased initially till July but declined afterwards during August and September. Further there was increase in the phenol and tannin concentration with the age of the Sal leaves. Coarse leaves contained more phenol and tannin while the moisture content was low in comparison to tender and medium leaves. The linear correlation studies were also made between the leaf moisture and biochemical contents with the abiotic factors (Fig. 5).

Table 2: Nutritional analysis in the Sal leaves collected month-wise from new leaves formation to onset of leaf fall at natural ecological niches of Laria ecorace

		Leaf moisture (%)	Leaf Protein (mg/g)	Leaf Carbohydrate (mg/g)	Total Phenols (mg/g)	Leaf Tannins (mg/g)
April	mean	70.83±6.12	101.0±11.5	59.73 ± 8.95	38.88 ± 12	27.2 ± 4.4
	Min-max	64.3- 74.12				
May	mean	69.22± 5.34	108.7± 10.1	57.37± 7.11	39.27± 4.8	29.88 ± 3.9
	Min-max.	59.3 -71.5				
June	mean	68.83 ±5.98	115.0±9.8	55.72± 8.50	43.61 ± 5.6	32.33 ± 3.33
	Min-max.	63.93-75.3				
July	mean	69.89± 4.98	121.5 ± 9.6	58.13± 5.16	45.2 ± 9.6	34.82 ± 6.3
	Min-max.	62.33-74.98				
August	mean	70.66±5.77	125.3 ± 8.1	60.33± 7.12	40.08 ±8.1	33.12 ± 6.1
	Min-max.	65.67-76.01				
September	mean	70.13± 3.98	132.3 ± 6.3	63.97 ± 13.45	36.77 ± 8.8	28.15 ± 5.6
	Min-max.	65.66 - 75.6				
October	mean	69.65± 5.88	127.7± 13.3	62.26 ± 8.90	39.56 ±9.2	27.5 ± 6.1
	Min-max.	66.1 - 74.5				
November	mean	66.88±6.23	120.2± 12.3	58.38 ± 7.10	46.81± 15.2	31.00 ± 3.2
	Min-max.	59.38 - 70.5				
December	mean	64.52± 5.72	114 ± 13.45	50.22± 6.60	52.67 ± 12.3	35.11 ± 4.8
	Min-max.	55.0 - 70.8				

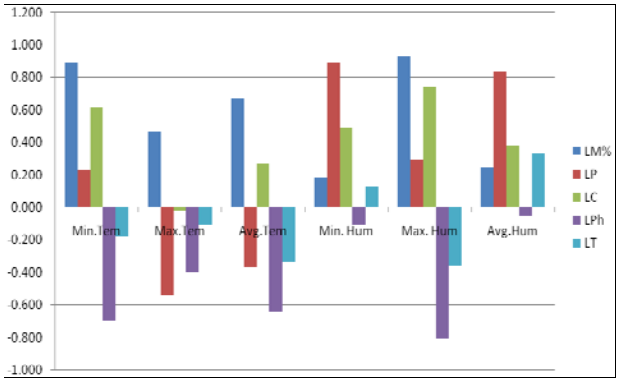


Fig 5: Correlation of abiotic and biotic factors (leaf biochemicals)

4. Discussions

The life cycle of Lepidopteran phytophagous insect depends on the availability of foliage on the food plants. Hence, the study of Sal leaf phenology is important to understand the dynamics of Laria silkworm population in the Sal flora. Leaf phenology reflects the influence of evolution and environment on plant characteristics, and in turn has substantial implications for plant functioning. Contrary to the deciduous tree species which are generally summer-flushing (vegetative bud breaks in hot-dry summer, May–June), *Shorea robusta* (Sal) is a spring-flushing species (vegetative bud breaks around spring equinox, March–April) in Indian dry tropical forests. The period around spring equinox is important because of the overlap of leaf fall, leaf flushing, and flowering/fruiting in Sal [7, 13]. This aspect is also evident from the present observation Sal phenology at Peterbar Sal forest where leaf flush started from last week of February till April. Flowering takes place from the month of March to May and fruiting in the month of May and June.

Due to very high temperature during summer period, there was very less survivability of silkworm larvae. Regular moth emergence was started from third week of May and continued till July. A peak emergence pattern was observed during second and third week of June at the onset of rainy season with coupling rate of 45% over total emergence (male and female moth emergence). Larval population was found more during August to October *in situ*. This has showed the most convenient period for the Laria perpetuation on Sal flora. Also abiotic factors play a major role in population dynamics in Laria. Though there was good moth emergence during April and May, the survivability was very low. Due to gradual decrease in temperature and increase in the moisture from June/July due to onset of monsoon, it became quite congenial for the silkworms.

Leaf moisture was positively correlated with abiotic factors while, protein and carbohydrates of leaf has positive correlation with humidity but negative correlate with maximum temperature. On contrary, leaf phenol and tannin had negative correlation with the abiotic factors. The suitability of leaf nutrition and the ecological factors are prominent for the Laria silkworm growth and development and subsequent cocoon production on Sal. Dietary nutrition mainly proteins are converted into silk proteins in sericigenous insects. Nearly 70% of protein content of raw silk namely fibroin and sericin are directly biosynthesized from leaf protein and remaining 30% is derived from silkworm body tissue and haemolymph protein, emphasizing the importance of leaf protein in silkworm nutrition [14, 15, 16]. The quality and quantity of leaf nutrition is highly influenced by varieties, geographical locations, rainfall and other atmospheric factors [17] and this has direct bearing on the growth and development of phytophagous insect.

It is inferred from the present study that, role of ecological factors and leaf nutritional properties of *Shorea robusta* (Sal) is pertinent to the *in situ* breeding behaviour of Laria silkworm on Sal flora which was evident from the Laria population size observed during different seasons. Based on the information from the study there is intriguing possibilities in creation of artificial niches for the Laria population provided with appropriate climatic conditions so as to improve the ailing Laria population from the various anthropogenic activities and narrowing of natural habitats. The information also holds good for the development of *in situ* conservation strategies for dwindling wild ecorace of *A. mylitta* on natural Sal flora. There are apparent possibilities of standardization of Laria silkworm rearing and improvement in cocoon production on Sal plants.

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