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An Integrated Study on Effect of Climate Change on Biodiversity and Visit of Insect Pollinators and the Seed Germination in *Clitoria Ternatea*, An Ornamental and Medicinal Plant

Author
Girish Kumar. E
P.G. Dept. of Botany, Mahatma Gandhi Govt. Arts College, Mahe, U.T. of Puducherry, India

ABSTRACT
Clitoria ternatea (Sankhu pushpam, in Malayalam) plant population is dwindling day by day and various anthropogenic pressures like Climate change, Pesticide usage, Urbanization etc. cause their decrease in number. A study was conducted to analyse the biodiversity of insect pollinators of the plant along with a correlation study of insect visit to the day temperature. Keen observation was made on daily basis to selected five flowers for 6 days and the time period of observation was from 6: a.m to 2: p.m. From the primary data the average frequency of visit and time spent within the flower by the pollinator per day per flower is calculated. The study showed that there are 7 insect pollinators mainly and the blue banded bee, Amegilla cingulata, was the most important pollinator. The other insect pollinators were Jamides celano, Xylocopa latipes, Zygaenidae moth, Udaspus folus, Xylocopa aestuans and Spoladea recurvalis. The pollinators included 3 bees, 2 butterflies and 2 moths. The correlation study between insect visit and the day temperature showed a strong negative correlation value (r = ˉ 0.7097).A comparative study of germination of normal seeds and climate change affected seeds showed a decreased germination percentage of the latter. Climate change induced vivipary was also reported during the study.
Keywords: *pollinators, average visit, seed germination, vivipary.*

INTRODUCTION

According to IPCC (Inter Governmental Panel for Climate change) report (2007), climate change is the prime factor that would endanger the biodiversity of Earth. Global warming which is the main cause of climate change is the product of multiple activities of human origin. Non-judicious use of fossil fuels, deforestation, invasive species, pesticide usage, intensive agriculture and urbanization have caused much damage to the life of biosphere and the survival of all organisms including man is at peril. Elevation of temperature at a higher rate has direct effect on biodiversity, as heat energy is a potent factor that causes mutation. Though heat energy and the resultant temperature is useful to living organisms for maintaining their homeostasis, uncontrolled increase of temperature will affect the general growth, metabolism and reproductive features of all organisms including insects .The behavioral changes in the life pattern of insects in relation to elevated temperature is an interesting topic of environmental biologists. Hence a study on insects and day to day temperature variations warrants much importance.

Insects are one of the major pollinators of flowering plants and pollination entomology is one of the latest topics that have caught the attention of scientist all over the world.

Pollination biology is unique in that it is interdisciplinary in nature and represents the essence of plant-animal interaction. This association between plant and animal through pollination is an important mechanism in the field of biology because the suppression of pollination leads to the non-development of viable seeds and hence the future generation of plants. At the same time the animals are also deprived of food as nectar, pollen grains etc. are the major attractions for insects. This symbiotic interaction is very much balanced and important for the sustainable development of biosphere. Animal pollination of both wild and cultivated plant species is under threat as a result of multiple environmental pressures acting in concert (Schweiger *et al*. 2010). Gordo and Sanz (2005) have examined the nature of phenological responses of both plants and pollinators to increasing temperature. It was found that there occur mismatches in pollination interactions among species and regions due to direct temperature response (Hegland *et al.*2009). Any change in the environmental factors has its direct effect on this interaction and hence a study to decipher how the environmental factors like temperature, intensity of sunlight, wind etc. affect the visit of pollinators is the need of hour.

Clitoria ternatea of family Fabacea is a perennial, ornamental climber with many medicinal properties. There are two varieties of Clitoria - white flowered and blue flowered. Flowers are usually axlliary in position and are solitary or rarely in cymes. The corolla is typical, papilionaceous type with a prominent and attractive single standard petal, two wing petals which is the landing place of insects and two keel petals which are joined together and enclose the essential organs – androecium and gyneocium. Fruit is a typical pod or legume.

In traditional Ayurvedic medicine, *Clitoria ternatea* has been used for centuries as memory enhancer, anti-stress, antidepressant, tranquilizing and sedative agent. The extract from the plant contains tannins, resins, starch, taraxerol and taraxerone. Recently several biologically active peptides called cliotides have been isolated from the heat stable fraction of Clitoria ternatea extract. Cliotides belong to the cyclotides family and activity studies show that cliotides display potent antimicrobial activity against Escherichia coli and cytotoxicity against Hela cells. These peptides have potential to be the leading compounds for the development of novel antimicrobial and anti-cancer agents.

The population of Clitoria is dwindling now a days and this decrease can be attributed to various factors like climate change and anthropogenic pressures. The availability of plant has been scanty as ornamental plant and for the medicinal purpose. So a study has been conducted to ascertain various insect pollinators of Clitoria ternatea, pattern of insect visit including frequency and time and influence of daily temperature on frequency of visit, the nature of seed dispersal and seed germination in normal seeds and climate change affected seeds and the structural modifications in seeds to cope up with climate change.

The locations selected for study are three semi urban areas-Azhchavattom, Beach road and Rajiv nagar, situated nearly 3 km east of Kozhikode city in Kerala. Kozhikode (latitude 11° 15' 0" N & longitude75° 46' 0" E) is one of the major cities of Kerala and is situated in the Northern part of Kerala. Arabian Sea occupies the western part of Kozhikode and Eastern part is Wayanad district which is very much the part of Western Ghats, one of the “hot spots” of biodiversity. The general topography of Kozhikode district shows high land, mid land and coastal areas. The study areas belong to mid land area. The soil of the locations was alluvial in nature which promotes profuse growth of vegetation. Two major rainy seasons are South West-monsoon and North-East monsoon.

MATERIALS AND METHODS

Five healthy flowers were selected from the group of flowers and they were brought under observation since anthesis. The duration of observation was from 6: am to 2: pm, for a period of 8 hours. A preliminary study showed that the visit by insect pollinators in afternoon and during

night hours was scanty and negligible in Clitoria plant and hence the time period of study was restricted between 6: am to 2: pm which was found to be more favourable time for visit by the pollinators. The keen observation was made to analyze the insects. The observation was made under strict vigil without tampering their body and the niche. The morphology of the insect, frequency of visit, duration of time spent within the flower etc. were recorded. A stopwatch (Emkay-Deluxe) was used to record the time and daily temperature was recorded in every hour using a thermometer. Photographs were taken by using a digital camera (Nikon-coolpix).Behavioral pattern of the insect pollinators and other noticeable biotic changes of the surroundings were recorded in a field notebook. A cello tape trap was placed near the flowers to check any chance of wind pollination. The average of visit and the time spent by the pollinator per day per flower was calculated using the following formula.

Frequency of average visit by the pollinator/ hour/ flower =

$$= \frac{\text{Frequency of visits in total}}{\text{No. of flowers studied X Duration of the time of study (in hours)}}$$

Similarly, frequency of average time spent by the pollinator/hour/flower =

$$= \frac{\text{Total time spent by the Pollinator in seconds}}{\text{No. of flowers studied X Duration of the time of study (in hours)}}$$

The primary data generated were tabulated and brought under statistical analysis to correlate the insect visit to the day temperature. Using photographs the pollinators were identified with the help of scientists from zoological survey of India, Western Ghats field research station, Kozhikode, Kerala.

Normal, healthy and mature fruits and climate change affected fruits were collected numbering 50 each from three selected locations to study the morphology, seed dispersal and germination strategies. Seeds of both normal and climate change affected fruits were kept for germination in special sterilised Petridishes in wet cotton and the germination was noted by observing the emergence of radicle.

RESULTS AND DISCUSSION

The study showed that mainly there are 7 insect pollinators in the case of C*litoria ternatea*. They are *Jamides celano* (Common Cerulean butterfly), *Xylocopa latipus* (black carpenter bee), *Zygaenidae* moth, *Udaspes folus* (grass demon butterfly), *Amegilla cingulata* (blue banded bee), *Xylocopa aestuans* (yellow spotted black carpenter bee) and *Spoladea recurvalis* (Moth). Bees are the most important pollinators of worldwide (Kearns *et al.* 1998).The present study have endorsed this concept. The plant also supported other types of insects which were the part of the microclimatic system. Of the seven pollinators identified, 2 were butterflies, 2 were moths and 3 were bees. The important salient features of the pollinator are as follows:

1. *Jamides celano* (Common Cerulean butterfly), Order – Lepidoptera, Family – Lycaenidae: This butterfly visited the flower during time period of 7-8 a.m mainly. The average number of visits per day per flower is 0.12. The average time spent per day per flower is 1.33 seconds.

2. *Xylocopa latipus* (black carpenter bee), Order – Hymenoptera, Family – Apidae: This bee was mostly visiting the flower in the morning hours of 8-10 a.m. The average number of visits per day per flower is 1.7. The average time spent per day per flower is 16.06 seconds

3. *Zygaenidae* moth. Order – Lepidoptera, Family – Zygaenidae: This moth was present in the flower in the morning and the average number

of visits per day per flower is 0.16 and the average time spent per day per flower is 2.36 seconds

4. *Udaspes folus* (grass demon butterfly). Order – Lepidoptera, Family – Hesperiidae: This butter fly was visiting the flower in late morning and the noon. Average number of visits per day per flower is 0.06 and the average time spent per day per flower is 0.73 seconds.

5. *Amegilla cingulata* (blue banded bee). Order – Hymenoptera, Family – Apidae: This was the most efficient pollinator as it was visiting the flower almost at will irrespective of day temperature. The average number of visits per day per flower is 4.7. The average time spent per day per flower is 35.15 seconds

6. *Xylocopa aestuans* (yellow spotted black carpenter bee). Order – Hymenoptera, Family – Apidae: The bee prefers mostly morning hours of 10-11 hours for the visit. The average number of visits per day per flower is 0.2 and the average time spent per day per flower is 2.33 seconds.

7. *Spoladea recurvalis* (Moth). Order – Lepidoptera, Family – Crambidae: The average number of visits per day per flower is 0.07. The average time spent per day per flower is 2 seconds. Above findings were recorded in Table-1

The plants were also supporting other animals like Crab Spiders, Ants, Grass hoppers, Garden Snails, Garden Lizard and Odonates. They were not pollinators and the Ants were "stealers" as they robbed only the nectar and did not affect the pollination. The Spiders, Lizards and Odonates were well known carnivores and were trapping the insects which approached the flower for pollination. Cello tape trap study indicated no presence of tri-porate pollen and hence no chance of wind pollinaton. A comparative study was conducted between white flowered and blue flowered varieties and it was found that there is no significant variation among the type of pollinators. A correlation study was conducted by correlating the average number of visits by insects to the day time temperature and a negative correlation (r value = ˉ 0.7097) was observed. It was found that when the temperature increased the number of visits by the insects decreased and from this it was evident that the insect visit is influenced by the rising temperature (Table-2). Hence it was assumed that global warming and the related climate change can influence the insect visit and insect pollination. Any slight deviation in the temperature has direct effect on the behavior of insects. As we know, any hindrance to pollination will lead to deformed seed set and animals depending on them will be in danger. This endorsed the importance of maintenance of optimum temperature for the insect pollination.

Normal fruits were creamish brown in colour and dehisced from both edges to release the seeds. Climate change affected fruits were blackish in colour with some ash tinge. Its dehiscence was irregular and this is caused by non-seasonal rain. The most important discovery was the development of Viviparous seeds in climate change affected fruits. Vivipary is the germination of the seeds within the fruit itself, where fruit is still attached to the mother plant. In Clitoria, this is induced to overcome the stress causes by the non seasonal rain on the fruits. This has not been reported earlier in this plant. Sachin et al. (2016) have reported vivipary in *Memecylon umbellatum* Burm. due to non-seasonal rain. Vivipary is usually seen as an adaptation in Mangrove plants for their survival in salty water conditions

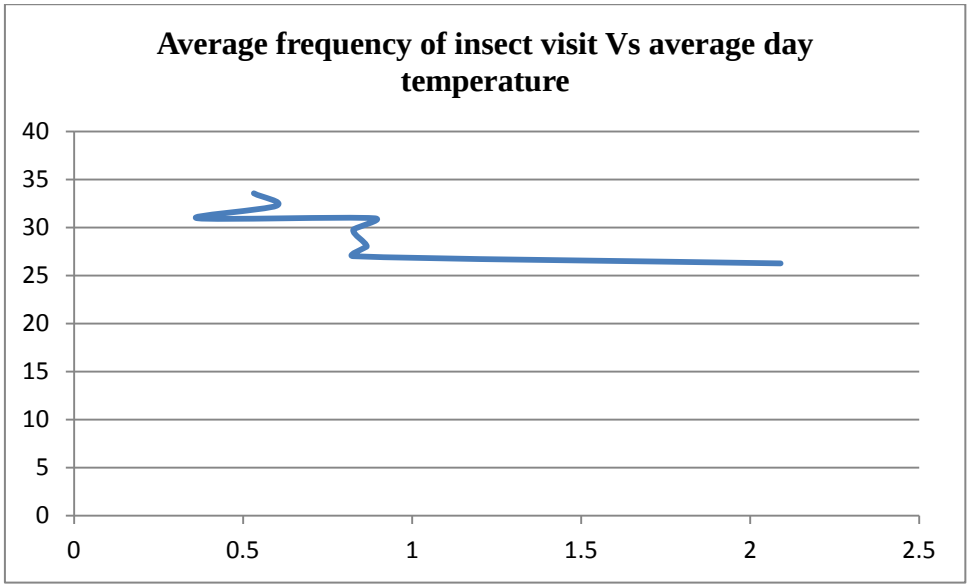
Both normal seeds and Climate change affected seeds, numbering 50 each were germinated and there were 98 % germination in the case of normal seeds. The seeds from climate change affected fruits showed only 18 % of germination and from this it was concluded that the climate change had profound inhibitory effect on seed germination and survival of the plantlets. More over it was noted that climate change affected seeds were infested with some fungal growth while germinating in the petridishes. The fungus was later identified as *Penicillium* species, a member of Ascomycetes class. Danilo et al. (1996) have mentioned the infestation of paddy seeds by Aspergillus fungus as a result of high moisture content. Such type of fungal association was absent in the case of normal and healthy seeds while germination.

Table-1: Average of Frequency of Visit and the Duration of Time Spent by Pollinators / Flower / Day

Time of observation	6-7 am 26.25° C		7-8 am 27° C		8-9 am 28° C		9-10 am 29.75° C		10-11am 30.95° C		11-12am 30.95° C		12-1 pm 32.25° C		1-2 pm 33.55° C	
Pollinator	x	y	x	y	x	y	x	y	x	y	x	y	x	y	x	y
Jamides celeno	0	0	0.06	0.33	0	0	0	0	0.03	0.83	0.03	0.17	0	0	0	0
Xylocopa latipes	0.27	1.02	0.2	1.06	0.4	3.73	0.46	6.12	0.2	2.4	0.03	0.47	0.07	0.9	0.07	0.36
Zygaenidae moth	0	0	0	0	0.1	1.43	0	0	0.06	0.93	0	0	0	0	0	0
Udaspes folus	0	0	0	0	0	0	0.03	0.66	0	0	0	0	0.03	0.07	0	0
Amegilla cingulata	1.76	14.66	0.5	3.56	0.37	1.66	0.33	1.73	0.47	3.77	0.3	2.47	0.5	3.73	0.47	3.57
Xylocopa aestuans	0	0	0.07	0.33	0	0	0	0	0.13	2	0	0	0	0	0	0
Spoladeous recurvalis	0.07	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	2.1	17.68	0.83	5.28	0.87	6.82	0.82	8.51	0.89	9.93	0.36	3.1	0.6	4.7	0.54	3.93

(X= Frequency of visit & Y = Frequency of time duration)

Table-2: Graph depicting insect visit (X-axis) and temperature (Y-axis)



SUMMARY

The study was conducted to analyse the important insect pollinators of *Clitoria ternatea* plant and also to correlate the insect visit to the day temperature. The study showed that there are mainly seven insect pollinators in Clitoria plant, of which blue banded bee is the main pollinator. The correlation study showed the existence of negative correlation between frequency of insect visits and the increase of temperature which is an evidence to hypothesize that any increase in temperature will affect the insect population and hence it is the prime duty of every individual to reduce global warming and the related climate change. A comparison of normal and healthy seeds with climate change affected seeds provided decreased germination percentage in the case of latter and it also showed fungal infestation of the seeds which may be another reason for the decreased vigour and vitality of these seeds. The climate change affected seeds showed certain degree of vivipary as a result of non-seasonal rain fall due to climate change.

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