Monitoring of Reproductive Biology of *Cassia fistula L.*

THESIS

Submitted in partial fulfillment of the requirements for the award of the degree of

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BY

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ABSTRACT

The **"Monitoring of Reproductive Biology of *Cassia fistula*"** was carried out in and around **Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, (U.P) India**.

The vegetative characters was observed in five phenotypically superior trees of *Cassia fistula* showed that the maximum of days taken for leaf bud swell was 62.50 days in T_5 and minimum in T_2 with 58.20 days. For the leaf bud burst the maximum duration was seen T_3 (32.80 days) and minimum in T_5 (30.90 days). Leafing period was noted to be highest in T_4 (103.20 days) and minimum in T_1 (92.90 days). The final vegetative character recorded was leaf fall which was longest in T_1 (52.20 days) and shortest in T_4 (45.80 days). Reproductive characters were also observed and the days taken for flower bud swell was maximum in T_1(37.60 days).Flower bud burst was recorded to be maximum in T_2 (44.4 days) as compared to the other trees. Flowering was observed to be longest in T_5 (42.3 days) and fruiting was maximum in T_5 (45.5 days). Flower bud development took twelve stages to complete among which the longest stage was VII-VIII and shortest was XI-XII. Anthesis occurred from 8:30-10:30 a.m. and continued throughout the day. Anthesis dehiscence took place around 12:30-14:30 hours. Pollen grain viability percentage was seen to be highest in T_5 (99.00%) on the day that pollen was collected which later decreased to (46.70%) after 15 days. The in vitro pollen germination percentage at room temperature was maximum at 25% sucrose (95.67%) on the first day which then decreased to 40.33% at about 60 days and it was completely dead after around day 70.In 4±1°C and -18±1°C the mean germination percentage was 82.78% and 85.28% . The most common pollinator was Honey Bees which were active from 6:00 a.m to 6:30 p.m.

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CHAPTER-I

INTRODUCTION

India is the seventh largest country in the world with a total area of 3,287,263 sq. km. It lies in the Northern Hemisphere between latitudes 84°N and 37°6′ N and longitudes 68°7′E and 97°25′E. The climatic conditions of India is extraordinary, ranging from tropical in the south to temperate and alpine in the Himalayan north, with the elevated regions receive snowfall. The climate is strongly influenced by the Himalayas and the Thar Desert. Forest covers about 21.54 percent of the total area of India, i.e., about 708,273 sq. km. India has also added 6,778 sq. km of forest cover to the nation and also extended 1,243 sq. km of tree cover. There are mainly six major groups of forest in India.

In a country like India where most of the population depends on agriculture, forests play a very important role as it helps in transpiration and humidifies the air which helps in the rainfall. But much of the forest areas are being cleared for agriculture with the ever increasing population of the nation. Forests are also an important form of natural wealth as they provide a wide and diverse range of products. India has claimed to increase its forest cover to 33 percent but has been struggling to go above 22 percent.

This is where tree breeding comes into play. It holds the key to help the forest and also to receive the best from the forest in return. But it should be kept it mind that the most gain can be made only when proper seeds and sources are used otherwise there will be loss in the productivity or at worst, complete failure of the plantation.

Reproductive biology is a branch of tree breeding that helps in studying the mechanisms and process of sexual and asexual reproduction in plants. It also sheds light on fixing or describing the limits of the species as well as the classification of species and infraspecies. Hence, reproductive biology of a species must be studied in detail so as to develop effective strategies for their conservation and sustainable utilization.

Cassia fistula belongs to the Leguminosae or Fabaceae family of the plant kingdom. It is more commonly known as Golden Shower tree or ‘Amaltas’ in Hindi. *Cassia fistula* is a medium sized tree of about 10-20m. It is most famously and commonly recognised for its yellow flowers which covers the tree during its flowering period. This tree is a native of India, naturalized in Africa, West Indies and South America. It has attained the title of being an ornamental tree. *Cassia fistula* is the national tree of Thailand and its flower is the state flower of both Thailand and Kerela.

Cassia fistula thrives well in the dry to moist tropics and subtropics with annual rainfall of about 500-2,700 mm range and an average annual temperature of 18-29^{o}C. The plants are vulnerable to frost. It prefers well drained, moderately fertile sandy loam soil with a pH of 5.5-8.7. The plants when established are fairly resistant to drought.

The *Cassia fistula* tree is most well-known for their flower that heavily covers the entire canopy. The flowers are bisexual meaning, each of the flowers have both male and female reproductive structures. *Cassia fistula* wood is hard, heavy and durable, and is well suited for making cabinets, agricultural implements, buildings and can also be used to make good quality charcoal. In India, the flowers are sometimes eaten. While the leaves are fed to cattle, sheep and goats together with low-quality forages. The bark of *Cassia fistula* is used for dyeing and tanning. The leaves of *Cassia fistula* are deciduous and pinnate and each leaflet is about 7-21 cm long and 4-9 cm broad. They are bright green colored but are paler on the underside. These leaves will drop from the tree for a short period of time and are quickly replaced by new leaves. The leaves are used widely for skin problems while the bark is used in the treatment of boils, leprosy, ringworm, etc.

The flowers are of raceme inflorescence, 20-40 cm long and each flower is 4-7 cm in diameter with five yellow petals of equal size and shape. After blooming period the production of cylindrical pods or fruit takes place. The flowers are bright yellow in color and attract pollinators like bees and butterflies. According to experiment conducted by Robert Scott Troup, Golden jackals also help in dispersal of seeds by feeding on the fruit.

The pods of *Cassia fistula L*. are straight, cylindrical. The pods are 20-60 cm long and 1.5-2 cm in diameter. They are dark green when young, turning dark brown to black when mature. The ripe pods contain dark color sweetish pulp and numerous yellowish-brown seeds. The pods are collected when ripe and must be carefully dried. The best ones are those which do not rattle when shaken. These possess the most pulp. This pulp is extracted by first bruising the pods and then boiling them in water and after which the decoction is allowed to evaporate. Pulp can also be obtained from fresh pods by opening them at the edges and removing the pulp with the help of a spatula.

The pod of *Cassia fistula* has been used as a laxative drug in Thai traditional medicine for a long time. The pods and leaves contain Anthraquinone aglycones and anthraquinone glycosoides which are the active laxative form, while rhein is a major component. The degree of laxative potency is dependent on the content of anthraquinone glycosoides. The plant contains anthraquinone glycosoides as both aglycones and glycosoides has been used as an alternative

source of raw material for various herbal laxative drugs preparations (**Sakulpanich and Gritsanapan, 2009**).

The golden shower tree was known as aragvadha in Ayurvedic medicine, meaning "disease killer". *Cassia fistula* leaf extracts seem to be possible sources of anti-obesity and hypolipidemic compounds which could be developed as phytomedicines or drugs **(Christine *et al.*, 2011)**.Leaf and Root Extract of *Cassia fistula* may play a beneficial role in the management of bacterial infections **(Awal *et al.*, 2010).** *Cassia fistula* bark extracts are used to treat inflammation.

Plants which belong to Caesalpinaceae family are rich in flavonoids and bio flavonoids are known for their anti-inflammatory and antioxidant activities **(Ilavarasan et al., 2005).** The seeds of *Cassia fistula* are known to have defense proteins include a lipid transfer protein homologue and a protease inhibitory plant defensin **(Wijaya et al., 2000)**. *Cassia fistula* seeds have been used as herbal medicine and have pharmacological activity which includes anti-bacterial, anti-fungal and antioxidant properties. The preliminary phytochemical screening of the plant seed revealed the presence of anthraquinones, flavonoids, saponins, tannins and terpenoids. Seed extract of *Cassia fistula* confirmed the presence of roseanone with antiyeast activity **(Jothy *et al.*, 2011).**

Cassia fistula is mainly propagated by seeds. Seeds of *Cassia fistula* can survive for a very long period of time and their viability can be maintained for more than 3 years. The seeds are stored in room temperature with 13 ± 2% moisture content. Since the seeds have a very hard seed coat some pre-treatments are done to break the seed coat. Acid scarification and hot water soaking is mostly done. For acid scarification, sulphuric acid is used. The seedlings raised in containers will be ready for planting out after 2-3 years. Seedlings require large amount of water during the first stages of growth. Flowering and pod production occurs only after 8-9 years of planting.

Cassia fistula has been reported to help to revegetate overgrazed lands. This tree is tolerant of a wide range of soils and climate. *Cassia fistula* has reported to be invasive in Australia. It could have a negative impact on the environment.

Cassia fistula is an important source of naturally occurring bioactive compounds. Polyphenolics abundantly present in both in vivo and in vitro extracts may prove to be very important, non-toxic chemo preventive agents against various oxidative stresses. *Cassia fistula* callus cultures could represent an interesting supply of potential antioxidative and chemoprotective components like flavonoids and anthraquinones **(Bahorun et al., 2005).**

The study of the reproductive biology of *Cassia fistula* was carried out with the following objectives:

1. To study the phenological changes in ***Cassia fistula***.
2. To find out the pollen viability in ***Cassia fistula.***
3. To study the pollination and breeding behavior of ***Cassia fistula***.

CHAPTER II

CHAPTER- Ⅱ

REVIEW OF LITERATURE

Reproductive biology is the basis for species improvement and a thorough understanding for this is required for plant improvement, whether by conventional or biotechnological methods. Reproductive biology has its focus on phenology, pollination, pollen-pistil interaction and breeding systems. These are valuable for basic and applied research having implications to ecological and evolutionary studies and conservational biology. Information on pollination biology is not only required for comprehensive understanding of the efficiency of breeding system of the species and its evolutionary success but also for effective optimization of yield, conservation and rational genetic improvement **(Shivanna and Mohan,1993).** Very little information is available on the reproductive biology of tropical and sub-tropical trees such that references are meager and that too very generalized. Therefore, the various aspects of reproductive biology are reviewed under the following heads.

1) Phenology
2) Pollen studies
3) Breeding behavior

PHENOLOGY

Shukla *et al.*, (1982) studied the phenological observations were made on 122 tree species in a subtropical humid seasonal forest in north-eastern India. The forest had a high proportion of evergreen compared to deciduous species. Leaf-fall of most of the tree species coincided with the dry season. Flushing started towards the end of the dry season for a majority of the tree species, the degree and period of leaflessness varying with the species. Leaf production in the overstorey species extended over a longer period compared to the understorey species. For most of the species, flowering coincided with leaflessness. Proportionately more overstorey species flowered during the dry season and wet season flowering was more characteristic of understorey species. A majority of the species produced fleshy fruits during the wet season. Fruits, produced during the dry season, were mostly dry.

Srivastava (1983) studied the floral biology of *Butea monosperma* and revealed that current year's shoot bear flower and the flowering remain remained for 22-32 days. The average development process of the flower buds was observed within 20-25 days.

Jindal *et al.*, (1985) studied the phenology and breeding system of *Tecomella undulata* and found that the defoliation and flowering started from November and continued till March. Flowering was asynchronous within a wide range (59-103 days). Its peak started from the end of February to mid-March (9-19 days). The floral bud development started in November, December and January and fell before their opening. Only buds developed after February sets fruits.

Srivastava *et al.*, (1987) examined the floral biology of a Lac Host. *Bara salpan (Moghania chapper)*. The flowering period remained between 24 to 26 days from bud to full bloom. The average size of flower was found 10 to 12 mm and anther dehisced longitudinally between 6:30 to 7:30 am.

Borua (1988) analyzed the presence of heterostyly condition with three types of styles (12-15mm, 5mm and 1mm) length in *Solanum mammosum*. The blooming period took 102 to 126 days and the time of Anthesis and dehiscence of anthers was brought to light from 5:30 to 10:00 am. Flowers were found fully opened just before the anthesis.

Sudheendra kumar *et al.* (1993) investigated the phenology of teak, in 6 to 8 years old plantation. In general, flushing started in late March and was completed by April while most of the leaf fall occurred from December to February.

Dhillon *et al.*, (1994) observed the floral biology of *Acacia nilotica* species indica which flowered in June and remained at its peak upto September-October. Flower opening was noticed to take place during the night.

Reddy *et al.*, (1996) reported that *Duranta repens* flowers chiefly from July to December. The species showed staggered Anthesis and the flowers were small and hermaphrodite. Krishnan (2000) Studied the flowering phenology and floral success in monocarpic *Barlaria involucrate var. elata*. Peak flowering was observed in the month of December.

Chavan *et al.*,(1999) studied the flowering phenology, floral biology and phenotypic variability for floral traits in *Tamarindus indica* in Karnataka and found that then flowering occurred between the last week of March and lasted mid-June. The anther dehiscence took place between 09.00 and 11.00 hrs. Inflorescence length, floral buds/inflorescence and floral dry weight were observed to have high CV values.

Kim *et al.*, (2001) reported that phenology characteristics of tree species in subtropical forests species species exhibited peak of leaf drop in cool dry period (January-February) and leaf flushing in the beginning of warm dry period (March-April) and another in rainy season (August) of the year. The peak period of fruit maturation occurred during September-October. Leaf flush and flowering were simultaneous in both over and under storey tree species whereas the fruiting of under storey tree species in one month earlier than that of over storey tree species.

Williams *et al.*, (2005) observed high correlations between temperature and tree phenology in cold-temperate climates are unique in that low temperature constitutes the only climatic variable that determines the length of the growing season in all broad-leaved trees by inhibiting growth and causing leaf abscission. In the tropics, severe water stress affects trees the same way, but in many seasonally dry tropical forests the growing season is not significantly reduced by prolonged periods with low rainfall, because soil water storage buffers trees against seasonal drought (Borchert 1994).

Echereme *et al*., (2015) collected information about the different phenological phases of *Delonix regia*. The four phenophases, leafing, flowering, fruiting and ripening were observed and recorded once in every two weeks for a yearly cycle. Two-digit codes were used for inclusion of the principal growth stages and the secondary growth stages, and these defined the phenological phases observed. The results showed that changes in the prevailing seasons influenced the leafing and flowering phenology in *D. regia*. Flowering phenology started soon after the tree had resumed leafing with the early rains in the rainy season. Leafing, flowering and fruiting phenology started at different time periods and peaked synchronously. Also, the result showed that deciduousness in *D. regia* started with the onset of dry season in November and ended shortly before the rains in February.

Wei (2016) found that leafing, flowering and fruiting phenophases in various tree species at community level. At community level, leaf initiation begins with a major peak in December to January (winter) and a minor peak in April (summer). Leaf expansion begins in January (winter) to February (summer) and June (rainy). Leaf senescence is from August to October (rainy), January (winter) to March (summer) with major and minor peak. Initiation of flower bud occurs during the months of November and January and pollination in December (winter). Fruit bud initiates occurs during January (winter) and April (summer). Ripened fruit starts from January (winter) to April (summer) and in July (rainy). Fruit fall in the month of June (rainy) and March (summer) with a major and minor peak.

Marak and Wani,*(*2018) studied the vegetative and floral characters of *Gliricidia sepium* and observed that bud swell started from mid-January and continued up to second week of June. Leaf fall was observed to be from last week of December to last week of January. The flowering started from first week of March till first week of April with a peak period of flowering from second week of March to fourth week of March. Anthesis and anther dehiscence were also studied and observed to take place during morning hours after the onset of Sun. The maximum anthesis and anther dehiscence were observed between 6:30 to 8:30 a.m. and 10:30 to 12:30 a.m. respectively.

Paudel (2018) studied the seasonal variation in phenophases of *Mimosa pudica* in grazed pasture of Barandabhar corridor forest Chitwan,Nepal. He observed that sporadic seed germination in *Mimosa pudica* occurred in March and that most of the seeds germinated in the last week of June. Vegetative growth in *Mimosa Pudica* occurred between March and August and that flowering took place from August to April. Fruiting was observed to be from October to May and seed maturation was observed to be between Novembers to May with leaf fall occurring mainly between November – December.

POLLEN STUDIES

Nath *et al.,* (1959) revealed that in pomegranate 78 percent pollen germination took place in 5 percent solution and pollen viability ranged from 67.70 to 91.54 per cent.

Vasil (1960) found that Cucurbitaceae pollen were cultured in artificial nutrient media. Among the sugars, sucrose proved to be the best for germination of pollen. Although growth regulators, vitamins, antibiotics and some other chemical substances also improved germination of pollen and length of pollen tubes, the effect of boric acid (0.005—0.02%) and borax (0.01%) was most outstanding.

Nalawdi *et al.,* (1973) obtained 69 percent germination in cultivar 'Dholka' in 5 percent sucrose solution after 48 hours of incubation and reported 92 to 96 percent pollen viability during peak period of anthesis in 2 percent acetocarmine solutions.

Khurana *et al.,* (1979) examined high pollen viability in *Juglans regia* (96%), *Rhododendron arboretum* (73.90%), *Populus ciliate* (68.70%), *Pyrus pashia* (90.78%) and *Salix tetrasperma* (92.40%) and also found that for *Salix tetrasperma*, pollen germination was highest (24.40%) in 15 percent sugar solution whereas, for *Populous deltoides* (23.00%) and *Pyrus pashia* (17.00%) seeded highest in 10 percent sugar solution. The pollen of *Rhododendron arboretum* and *Populus ciliata* responded poorly to these media.

Egenti (1981) revealed that 14 percent sucrose solution was the best media for teak pollen grain germination. Pollen production varied in teak flower and pollen grain remained viable upto two days after anthesis.

Srivastava *et al.,* (1982) reported the 35.89 µm average size of fresh pollen grains of *Maughania macrophylla* and 95 percent pollen was found viable in 0.5 percent acetocarmine solution. The pollen germination was observed maximum in 45 percent sucrose solution and 1 percent agar-agar. Pollen stored at room temperature remained viable upto 72 hours and at Frigidaire condition it prolonged upto 140 hours.

Kuruvilla (1989) observed the breeding system of *Mudhuca indica* and reported that freshly dehisced pollen was sticky, became viable only after two days of drying and remained viable for about 5 to 6 days under normal condition.

Radicati *et al.*, (1990) carried out in vitro germinability and viability test on freshly collected or stored pollen collected or stored pollen (at 4 ºC or -20 ºC) in walnut. In vitro germinability was completely lost after one month of storage, but the pollen remained viable as confirmed by an in vitro germination test on stigma.

Bhattacharya *et al.*, (2000) studied the pollination biology in *Bombax ceiba Linn*. In vitro pollen germination study indicated that best germination (97%) along with 2940 µm tube development, takes place in 20% sucrose combined with 500 µg/ml H_3BO_3(Boric acid) solution. Among different salts of Ca, Mg and K, only Ca(NO3)2.4H2O (Calcium Nitrate Tertrahydrade) showed significant result with 54% germinating pollen along with 420 µm tube length in 50 µg/ml Ca(NO3)2.4H2O solution.

Kopp *et al.*, (2002) studied the pollen of Burdur dimriti, Sariemin, Tilki kuyrugu, Razaki, Buzgulu, Siyah buzgulu, and Siyah gemre table grape varieties *(Vitis vinifera L.*) grown in Isparta, Turkey. The pollens were tested in vitro for viability, germination capability, and production level. It was determined that active pollen levels varied between 23.8% and 80.8% in FDA (fluorescein diacetate), 31.5% and 68.8% in TTC (2, 3, 5-triphenyl tetrazolium chloride) tests. In respect to pollen germination rates, the best medium in hanging drop method and saturated petri method were 20% sucrose concentration and 1% agar +15% sucrose solution, respectively. The highest and the lowest pollen production levels per flower were determined 9000 in Siyah gemre and 2906 in Siyah dimrit grape varieties, respectively.

Lu *et al.*, (2007) the family Orobanchaceae was studied and illustrated with light microscopy (LM) and scanning electron microscopy (SEM). Five major pollen types were recognized on the basis of exine orna-mentation. Within these major types, minor types (subtypes) were distinguished based on exine sur-face pattern, size, shape, and form, colpi and colpus membrane. These types and subtypes are as follows: type I. retipilate: subtype Ia. Regular etipilate: (1) pollen size < 27 lm, (2) pollen size> 27 lm, subtype Ib. irregular retipilate; type II.verrucate: subtype IIa. macro-verrucate, subtypeIIb. Verrucate, subtype IIc. sparse verrucate; typeIII. retirugulate; type IV. granulate; type V. micro-reticulate.

Asmat (2011) studied the pollen morphology of Scrophulariaceae, a selected species in the Upper Dir district of Pakistan. A total of 5 genera and 9 species under this family were estimated for this research. Pollen grains were usually radially symmetrical, isopolar, oblate-spheroidal or prolate-spheroidal or sub-prolate, tricolporate and psilate, except Pedicularis oederi

which had bisyncolpate pollen. Pollen characters such as size, shape, colpi and exine thickness, and P/E ratio were found considerably important for systematic utilization. Pollen fertility estimation ranged from 70 to 98%, which showed that pollen flora of selected species was well established.

David, (2016) found that pollen viability was noticed to be maximum between 4 AM to 12 Noon. On the whole, the percentage of viability varied with the same sample in different staining techniques. In T. bellerica 72.72% viability was observed in TTC (2, 3, 5 Triphenyle Tetrazolium Chloride), 78.87% in Benzidine, 73.95% in Methylene blue and Fuchsine and 73.95% in Acetocarmine.

Marak and Wani,(2018) studied the pollen morphology and viability of *Gliricidia sepium* and found that the pollen colour was yellow and pollen grains were circular in shape with three germpores. The size of the pollen grain was 0.40µm and showed maximum percentage of pollen viability by acetocarmine test at room temperature was found to be maximum (87.50%) in the first day of pollen collection and decreased to 18.50 % after 15 days of storage at room temperature. The in-vitro pollen germination showed maximum germination (75.50 %) in 20 per cent sucrose concentration. Within 20 per cent sucrose concentration the storage temperature of -18 ± 1˚C and 4 ± 1˚C gave germination percent of 59.50 per cent and 53.00 per cent after 90 days of storage. The pollen stored in room temperature lost viability after 60 days of storage.

BREEDING BEHAVIOR

Khosla *et al.*, (1982) studied the breeding system of *Bombax ceiba* and reported that the species was both, cross and self compatible with the preponderance of the former. The studies further revealed 2.8 to 25.2 percent fruit set under different modes of pollination. Selfing by bagging resulted in the lowest fruit set (2.8%) while hand self pollination gave 25.2 percent fruit set thus, indicating self compatible nature of breeding system.

Sullivan (1983) examined pollination mechanism in two closely related species viz., *Acer pensulvanicum* and *A.spicatum*. Intra-specific crossing experiments on pollen stainability, ovule number and observations of the flowers in the field revealed that neither species bore functionally hermaphrodite flowers, but reproduced by xenogamous condition. Out crossing was an important means of reproduction in *A.spicatum.*

Ramirez *et al.*, (1984) examined the floral biology and breeding system of *Bauhinia benthamiana* and found that artificial pollination in flower with long and short pistil was genetically self incompatible and functionally andromonoecious. In other study of the floral and pollination biology of Parkia, Hopkins (1984) reported that out of 11 species, 9 were bat

pollinated with the most important pollinator being Phyllostomus discolor and two species partly or wholly entomophilous.

Bawa *et al.*, (1985) reported the floral biology of *Clintonia borealis* and found that pollination increased significantly by peak bloom and subsequently remained high. Since bee fly mostly between neighbouring stems, much of the pollen transferred might have lead to geitonomous nature. Selfed flowers were observed to set fewer and smaller seed than those of crossed ones. In *Clintonia boralis* seed number and seed size were limited by a balanced maternal resource availability and the amount of out crossing provided by the pollinators.

Jindal *et al.*, (1985) examined that in *Tecomella undulata* the fruit set varied from 0.64 percent selfing to 3.94 percent for cross pollination, indicating the presence of self compatability in the species.

Mcdade (1985) reported the breeding systems of Aphelandra. Five species experienced low levels of geitonogamous pollen transfer, produced few flowers daily which were pollinated by humming birds. Except *A.storkii,* these plants were fully compatible and seed from selfing appeared as viable as crossed ones *Aphelandra storkii* was partially self-incompatible and produced seed from self that germinated less successfully than crossed ones.

Reddy and Aluri (1997) studied reproductive biology of *Bruguiera gymorrhiza*, *Avicennia officinalis* and *Acanthus ilicifolius* and concluded that they did not reproduce by autogamy but xenogamy and geitonogamy. The flower was 100 percent and 60 percent successful by xenogamy and geitonogamy, respectively.

Ram (2001) studied the pollination biology and breeding system of *Acacia Senegal*. In his study he found that there were three types of flowers: autogamous, geitonogampus and xenogamous. The pollen tube entry was traced as 71.12% in autogamous, 82.16% in geitonogamous and 82.07% in xenogamous types of pollinated pistils. Sections of resin embedded pistils confirmed the entry of pollen tube into the embryo sac. Manual in vivo pollination studies showed that the species was self-incompatible. Self-incompatibility appeared to operate inside the embryo sac. Under natural conditions fruit set was observed to be as low as 0.36%. Insufficient pollination is the main cause of low fruit set. Manual xenogamous pollinations substantially improved fruit set to 30%.

Tandon *et al.*, (2003) reported that *Butea monosperma* showed a weak form of self-incompatibility. Fruit set following manual self-pollination (5.25%) was comparable with open-pollination (approx. 5%) but was significantly lower than manual cross-pollination (21.51%). This indicates that there is a high degree of geitonogamous pollination in this species, which may lead to a weakening of self-incompatibility as a means of reproductive assurance.

Smitha and Thondaiman (2016) observed the breeding behavior of *Saraca asoca* and recorded it to have poly-embryonic nature having 2–4 seedlings/seed. To record the percentage of polyembryony, the seeds were collected from 37 trees of Anupam mission and DMAPR campus (Boriavi and Lambhvel farms) and kept for germination. Among 454 seeds germinated, 23 were polyembryonic, which accounted to 5.07%. Further, it was also observed that the polyembryony phenomenon was tree-specific.

Deepthikumary *et al*.,(2019) studied the breeding system and seed biology of Humboldtia vahliana wight and found that the floral visitors include honey bees, stingless bees, butterflies and ants. Fruit set was observed to be highest in xenogamy flowed by open pollination and geitonogamy. The seeds were observed to be recalcitrant and seed germination was registered as 80 ±0.9% at the time of dehiscence..

CHAPTER III

CHAPTER-III

MATERIALS AND METHODS

The present study entitled "Monitoring of reproductive biology of *Cassia fistula*" was conducted in the campus of Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj. The details of the methods and materials used are given below.

1. LOCATION: The site on which the present research study was carried out is at the elevation of 98 m above sea level at 25^{o} 28' N latitude and 81^{o} 55' E longitude. All the required materials and facilities for the study were readily available in the College of Forestry.

2. CLIMATE AND WEATHER CONDITIONS: Prayagraj is located in the south eastern part of Uttar Pradesh and has tropical to subtropical climate with extreme of summer and winter. There are three seasonal variations in the area: Winter (December to January), Summer (April to June) and Rainy (July to September). The summer temperature peaks to about 48^{o} C and during the winter it drops to almost 5^{o} C.

3. FIELD SAMPLING AND DATA COLLECTION: A total of five trees were selected for observation. These trees were selected on the basis of their diameter measured at breast height using Vernier calipers. Ten branches from each tree, totaling fifty branches in all were selected in all directions and tagged with a transparent tag. The data about the reproductive biology on the basis of the selected branches was recorded on initiation and completion of phenological events of vegetative character and reproductive character. In the vegetative character leaf bud swell, leaf bud burst leafing and senescence were studied and in the reproductive character flower bud swell, flower bud burst, flowering, anthesis and seed dispersal were all studied and recorded. A record was also made for dehiscence of anthers, pollen studies, stigma receptivity breeding behavior and pollinator activity.

4. DATA ANALYSIS: A total of fifty branches were marked for data analysis.

5. VEGETATIVE CHARACTER:

a. **Leaf bud swell:** The dates of the first and last leaf bud swell in the tagged branches together with the number of days taken for a single leaf bud burst was recorded.

b. Leaf bud burst: The dates of the first and last leaf bud burst in the tagged branches together with the number of days taken for a single leaf bud burst were also recorded.

c. Leafing: The duration of leafing period in a total of fifty tagged branches was recorded. Also the number days taken for a single leaf bud to grow into a leaf from the day of initiation were recorded for all fifty braches.

d. Senescence: The leaf fall period in all of the fifty tagged branches was recorded.

6. REPRODUCTIVE CHARACTERS

a. Flower bud swell: The dates of the first and last flower bud swell were recorded. The number of days taken for a single flower bud to swell in all the fifty tagged branches was also recorded.

b. Flower bud burst: The dates of the first and last flower bud burst were recorded. The number of days required for a single flower bud to burst in all of the fifty tagged branches was also recorded.

c. Flower bud development: The morphological changes, in the shape of the flower buds from the time of their emergence to the time of anthesis were observed. Based on the observation the distinct characteristic features were grouped into 12 stages of bud development. For recording the data, five buds from five trees were tagged and observed to find out the number of days taken by floral buds to pass from one stage to the next one. Total number of days taken for a floral bud to develop into a full flower was also studied.

d. Anthesis: Ten flowers per tree for five trees at balloon stage were tagged the previous day and observed till they fully opened at an interval of every two hours from 6:30 onwards.

e. Stamen and pistil length: Length of stamen and pistil for fifty random flowers from five selected trees were taken with a measuring scale in cm.

f. Fruiting: The time and duration of fruiting was recorded in all of the fifty branches tagged in the five selected trees.

7. DEHISCENCE OF ANTHERS: Prior to anthesis, the mode of anther dehiscence were carefully observed and recorded for each flower tagged. For this, ten flowers were observed individually to determine the time of anther dehiscence in all of the five selected trees.

8. POLLEN STUDIES

a. Pollen collection:

- Flowers were collected early morning and the filaments and anthers were excised on the butter paper and kept for shade drying until dehiscence.
- After dehiscence the pollens were collected and divided into two parts: one used for viability test and in vitro germination test, while the other portion was stored in the desiccator with silica gel for 24 hours and later stored in vitals at room temperature (RT), 4 ± 1^0C and -18 ± 1^0C. Pollen mixtures were obtained from 10-20 flowers from each tree. Three replications were carried out for each condition.

b. Pollen grain characteristics: The collected pollen grains were observed under a microscope at 10× objective and the following parameters were recorded:

- Pollen color
- Shape of pollen grain

c. Pollen grain viability percentage:

- Pollens were dusted on the slide and one to two drops of aceto-carmine was added onto it.
- Pollens were mixed using mixed using sterile needle and observed under a microscope.
- Stained pollens were counted as viable and unstained pollens as non-viable.
- Pollen viability percentage was determined as follows:
  Pollen Viability % = (No. of stained pollens / No. of dusted pollen) × 100

d. Pollen germination: For carrying out the pollen germination test (in vitro)

For in-vitro pollen germination: A few drops of sucrose solution were dropped on the cavity slide, the pollen grains were then dusted onto the slides and evenly mixed in the solution. Total of three slides for each sucrose treatment were prepared. The prepared slides were stored in moist condition and observed under the microscope after 24 hours. Pollen grains were considered as germinated when the length of the pollen tube exceeds its diameter. The media to be used for in-vitro pollen germination constitute of 5, 10, 15, 20 & 25 percent sucrose solution. The in vitro germination percentage of the pollen was calculated using the formula:

Germination %: $\frac{No.of\ pollen\ grains\ showing\ tube\ length\ longer\ than\ pollen\ diameter}{Total\ no.of\ pollen} \times 100$

e. Treatments and replications: The following treatments each replicated three times were used for testing pollen viability and in-vitro germination:-

Treatments:-

- To – Acetocarmine test
- T1- 5% Sucrose solution
- T2- 10% Sucrose solution

- T3- 15% Sucrose solution
- T4- 20% Sucrose solution
- T5- 25% Sucrose solution

f. Pollen storage: To study the pollen viability, pollen were stored in individual vacuum vials. The vials were stored at room temperature and also in different refrigerated condition. The stored pollens were tested for viability and in-vitro germinability after every 7 days interval upto 60 days. The in-vitro germination tests of the stored pollen was conducted using the appropriate sucrose concentrations for different species. The sucrose concentration showing maximum germination in freshly collected pollens for a species was considered appropriate for that particular species and utilized for the complete duration of the storage period.

g. Floral Morphology: Following morphological characters were also recorded:

- Flowering period
- Flower colour
- Flower type
- Anthesis time
- Anthesis dehiscence time
- Mean number of anthers
- Mean number of stigma
- Pollinator type

9. POLLINATION BEHAVIOUR (Breeding behavior)

a. Autogamy: In this, self-pollinated flowers were bagged and then observed for fruit formation.

b. Allogamy: The flower buds were emasculated before anthesis and then the pollen grains collected from another mature flower were deposited on receptive stigma. The cross pollinated flowers were bagged and then extent of fruit set was observed.

c. Open-pollination: In this, flowers that were openly pollinated by vectors were left without bagging and observed for fruit set. The mature fruits and seed developed were counted and from this data the fecundity were calculated. The results are expressed as percentage of fruit and seed set.

10. **STATISTICAL ANALYSIS**

The data was analyzed statistically for the assessment of analysis of variance.

a. Critical difference (CD)

The critical difference (CD) was calculated as under:

CD= SE × t0.005 (error degree of freedom)

Where;

SE is the standard error of difference calculated as;

S.Em. = $\sqrt{\frac{2Xmsse}{r}}$

MSSE = Mean Sum of Square due to error

R = Number of replication

T0.05 = Tabulated value of t at 5 percent level of significance

Mean difference between any two families greater than calculated CD value was taken as significant difference.

b. ANNOVA TABLE (CRD)

For pollen viability and pollen germination

SOURCE OF VARIATION	D.f	SS	MSS	F(Cal)	F(tab) at 5%
Due to treatment	(t-1)	SST	MSST=$\frac{SST}{(t-1)}$	$\frac{MSST}{MSSE}$	
Due to error	t(r-1)	SSE	MSSE=$\frac{SSE}{t(r-1)}$		
Total	rt-1	TSS			

c. ANNOVA TABLE (RBD)

For vegetative and reproductive characters

SOURCE OF VARIATION	D.f	SS	MSS	F(Cal)	F(tab) at 5%
Due to replication	(r-1)	SSR	MSSR=$\frac{SSR}{(r-1)}$	$\frac{MSSR}{MSSE}$	
Due to treatment	(t-1)	SST	MSST=$\frac{SST}{(t-1)}$	$\frac{MSST}{MSSE}$	
Due to error	(r-1)(t-1)	SSE	MSSE=$\frac{SSE}{(r-1)(t-1)}$		
Total		TSS			

Where,

t= Number of treatments

r= Number of replications

SSR= Sum of squares due to replication

SST= Sum of squares due to treatments

SSE= Sum of squares due to error

TSS= Total sum of square

MSSR= Mean sum of square due to replication

MSST= Mean sum of square due to treatment

MSSE= Mean sum of square due to error

F (Cal) = Calculated value of F

F (tab) = Value of F from variance ratio table

CHAPTER IV

CHAPTER- IV

RESULTS

4.1 VEGETATIVE CHARACTERS

The study of all the vegetative characters such as leaf bud swell, leaf bud burst, duration of leafing, senescence is crucial for tree improvement programme. The results obtained from the study carried out for vegetative characters of *Cassia fistula* is presented as under:

4.1.1 Leaf Bud Swell

The data appended in Table 4.1 and Fig 4.1 revealed the total number of days taken for leaf bud swell was recorded maximum in T_3 (62.50 days) and minimum in T_2 (58.20 days)

4.1.2 Leaf Bud Burst

From Table 4.1 and Fig 4.2 revealed that total number of days taken for leaf bud burst was recorded maximum in T_3 (32.80 days) and minimum in T_5(30.90 days).

4.1.3 Leafing

Examination of the data in table 4.1 and Fig 4.3 showed that the total number of days for leafing period was higher in T_4 (103.20days) and minimum in T_1 (92.90 days).

4.1.4 Leaf fall

Perusal of Table 4.1 and Fig 4.4 revealed that the total number of days for leaf fall period was highest in T_4 (52.20 days) and minimum in T_1 (45.80 days)

Table 4.1 Total no of days taken for various phytophases in *Cassia fistula*

Trees no.	Leaf Bud Swell	Leaf Bud Burst	Leafing	Leaf fall
T_1	60.6	33.1	92.9	45.8
T_2	58.2	32	96	50.5
T_3	62.5	32.8	102.9	48.1
T_4	58.5	31.1	103.2	52.2
T_5	58.8	30.9	95.7	47.7
Mean	**59.72**	**31.98**	**98.14**	**48.86**
F- test	S	S	S	S
S. Ed. (±)	1.32	0.76	1.89	1.40
C. D. at 5%	2.69	1.55	3.85	2.86
C.V.	4.95	5.31	4.31	6.42

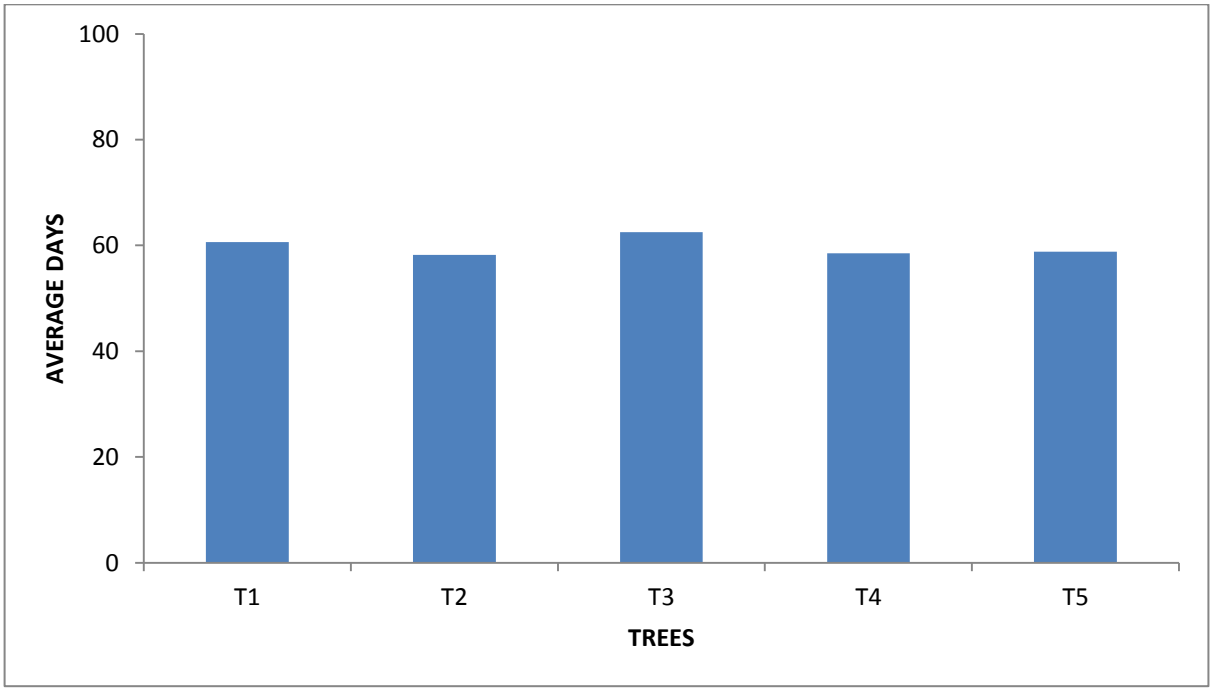

Fig. 4.1 Total no. of days taken for leaf bud swell in *Cassia fistula*

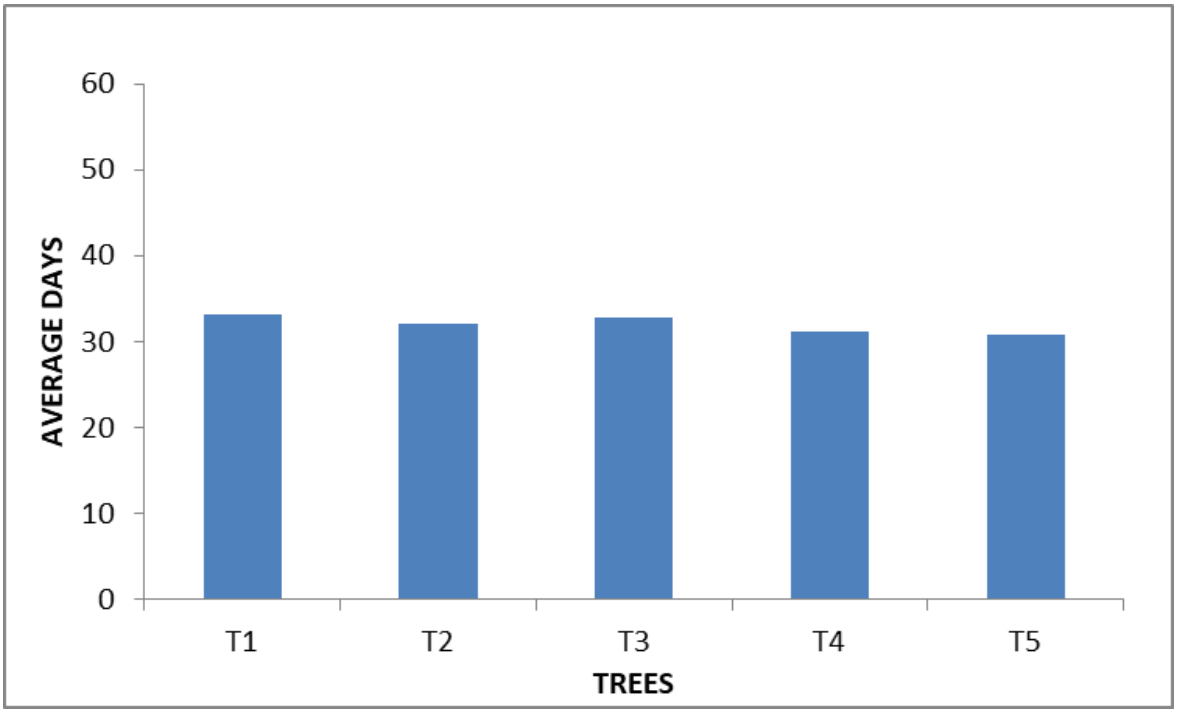

Fig. 4.2 Total no. of days taken for leaf bud burst in *Cassia fistula*

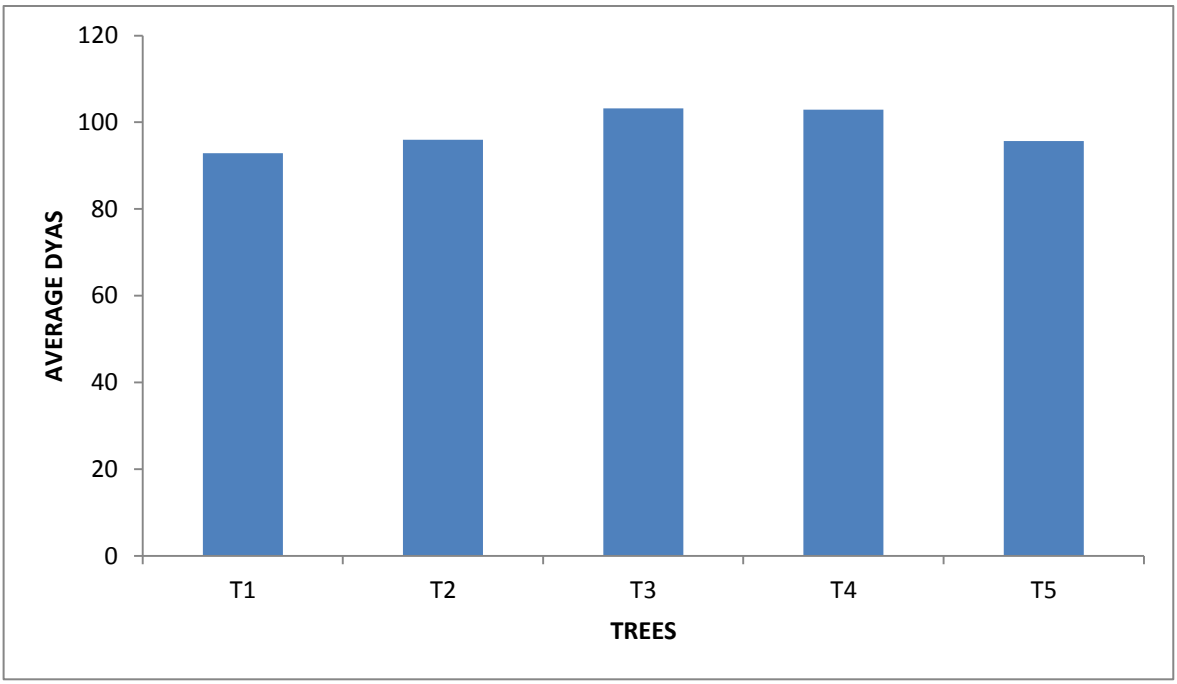

Fig. 4.3 Total no. of days for leafing in *Cassia fistula*

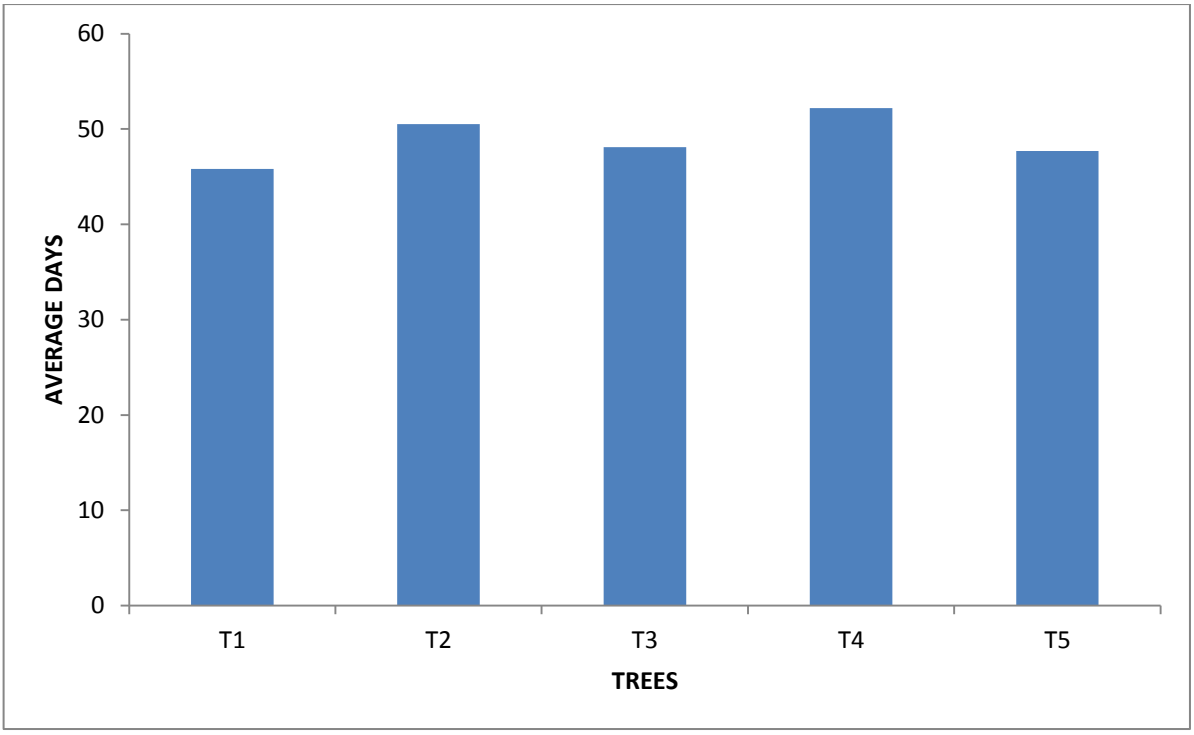

Fig. 4.4 Total no. of days taken for leaf fall in *Cassia fistula*

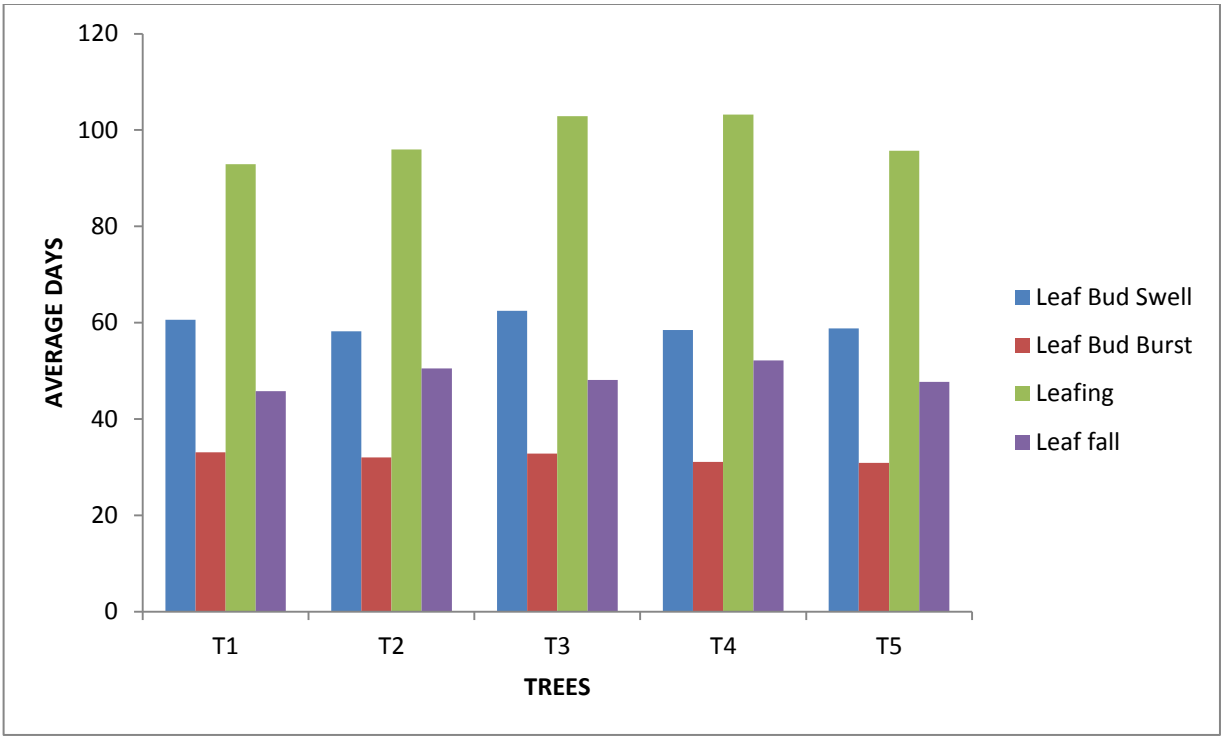

Fig. 4.5 Total no. of days taken for vegetative phytophases in *Cassia fistula*

Fig-I: Leaf bud swell in *C.fistula*

Fig-II: Leaf bud burst in *C.fistula*

Fig-III: Leaf bud enlargement in *C.fistula*

Fig-IV: Leafing in *C.fistula*

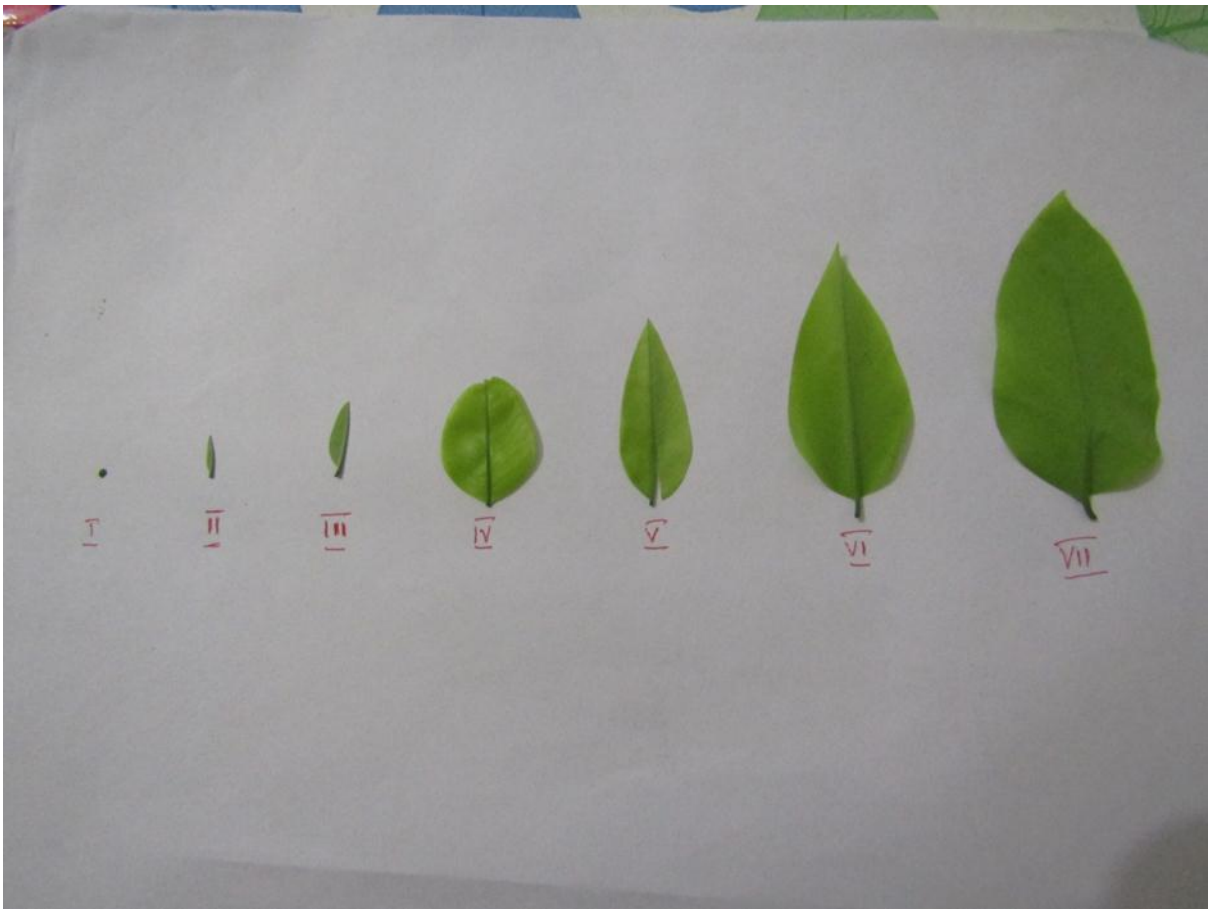

Fig-V: Different stages of leaf development in *Cassia fistula.*

Plate no.1- Different phenophases in *Cassia fistula*.

4.2 REPRODUCTIVE CHARACTERS

The different reproductive characters including flower bud swell, flower bud burst, flowering, fruiting. These characters must be examined deeply as they are necessary for attempting crosses between genetically different forms which in invariably involved in breeding programme.

4.2.1 Flower Bud Swell

From Table 4.2 and Fig. 4.6 it is clear that total duration of flower bud swell was recorded maximum in T_1 (37.60 days) and minimum in T_4 (34.40 days).

4.2.2 Flower Bud Burst

Data from Table 4.2 and Fig. 4.7 shows that the total duration of flower bud burst was maximum in T_2 (44.4 days) and minimum in T_4 (41.5 days).

4.2.3 Flowering

Examination of Table 4.2 and Fig. 4.8 revealed that the maximum duration of flowering was seen in T_5 (42.3 days) and minimum in T_4 (39.90 days)

4.2.4 Fruiting

Appraisal of Table 4.2 and Fig. 4.9 revealed that the duration of fruiting was longest in T_5 (45.5 days) and shortest in T_1 (42.5 days)

Table 4.2 Total no. of days taken for various reproductive phytophases in *Cassia fistula*

Trees	Flower bud swell	Flower bud burst	Flowering	Fruiting
T_1	37.60	41.9	40.6	42.5
T_2	37.30	44.4	42.1	44.1
T_3	36.70	43.7	42	43.4
T_4	34.40	41.5	39.9	44.2
T_5	36.10	43.2	42.3	45.5
Mean	**36.42**	**42.94**	**41.38**	**43.94**
F-test	**S**	**S**	**S**	**S**
S.Ed(±)	**0.62**	**1.04**	**0.79**	**0.89**
C.D at 5%	**1.26**	**2.11**	**1.60**	**1.82**
C.V	**3.79**	**5.40**	**4.26**	**4.54**

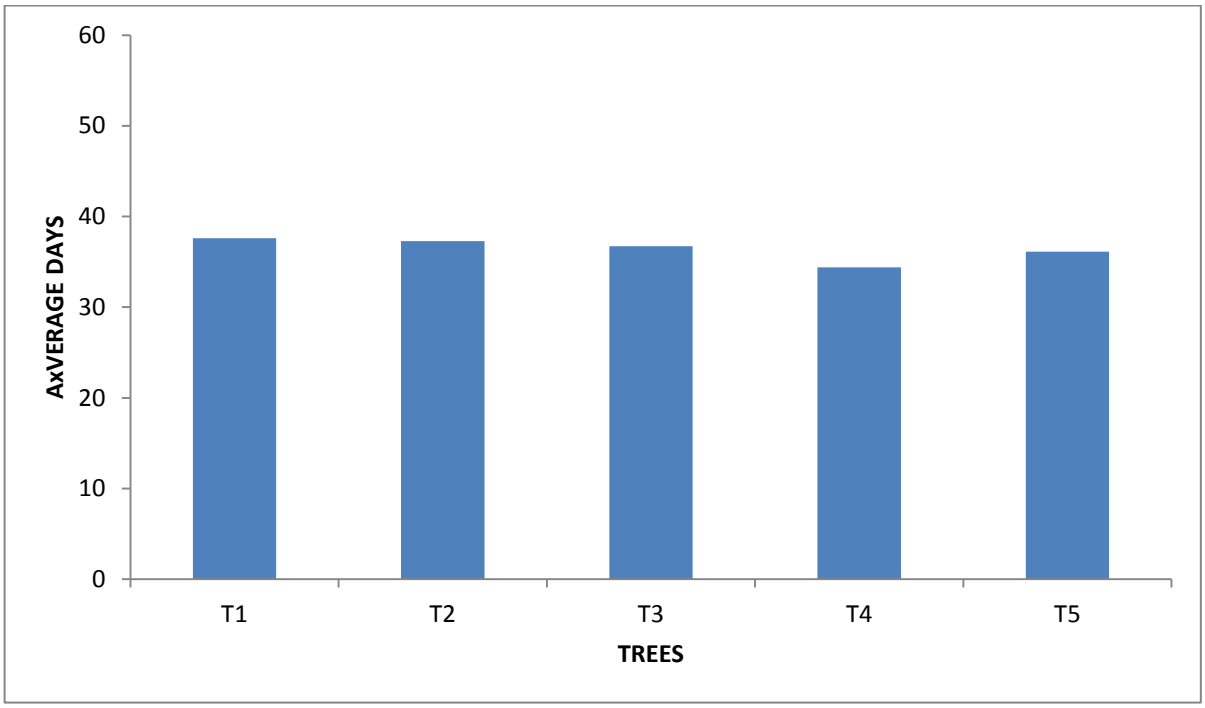

Fig.4.6 Total no. of days required for flower bud swell in *Cassia fistula*

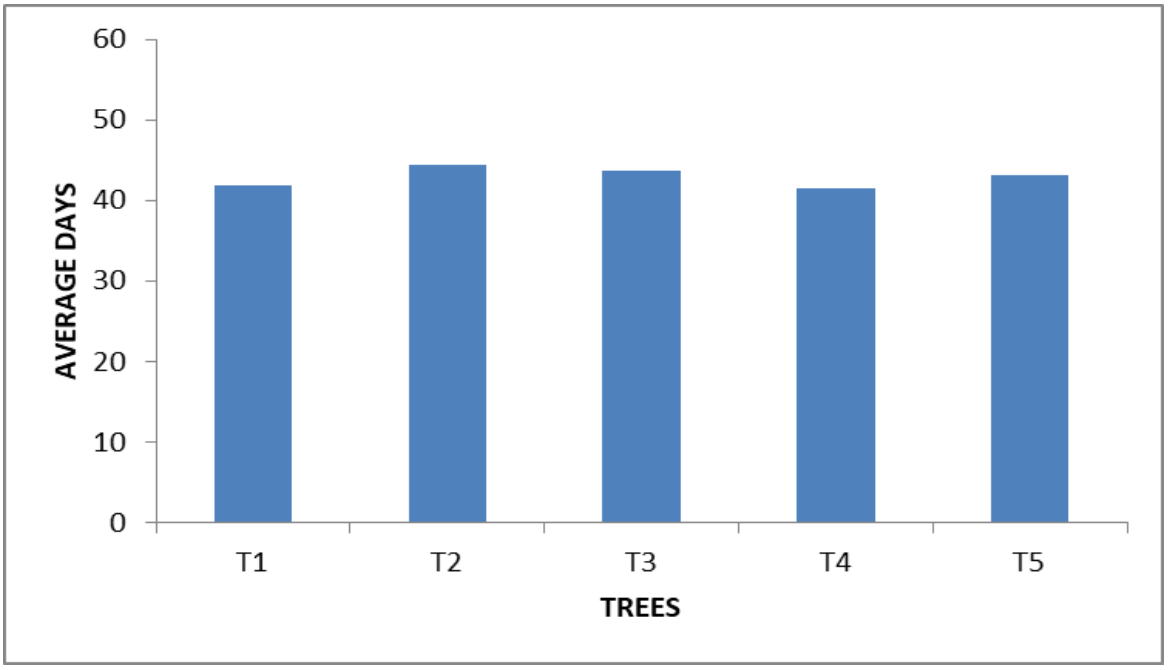

Fig.4.7 Total no. of days taken for flower bud burst in *Cassia fistula*

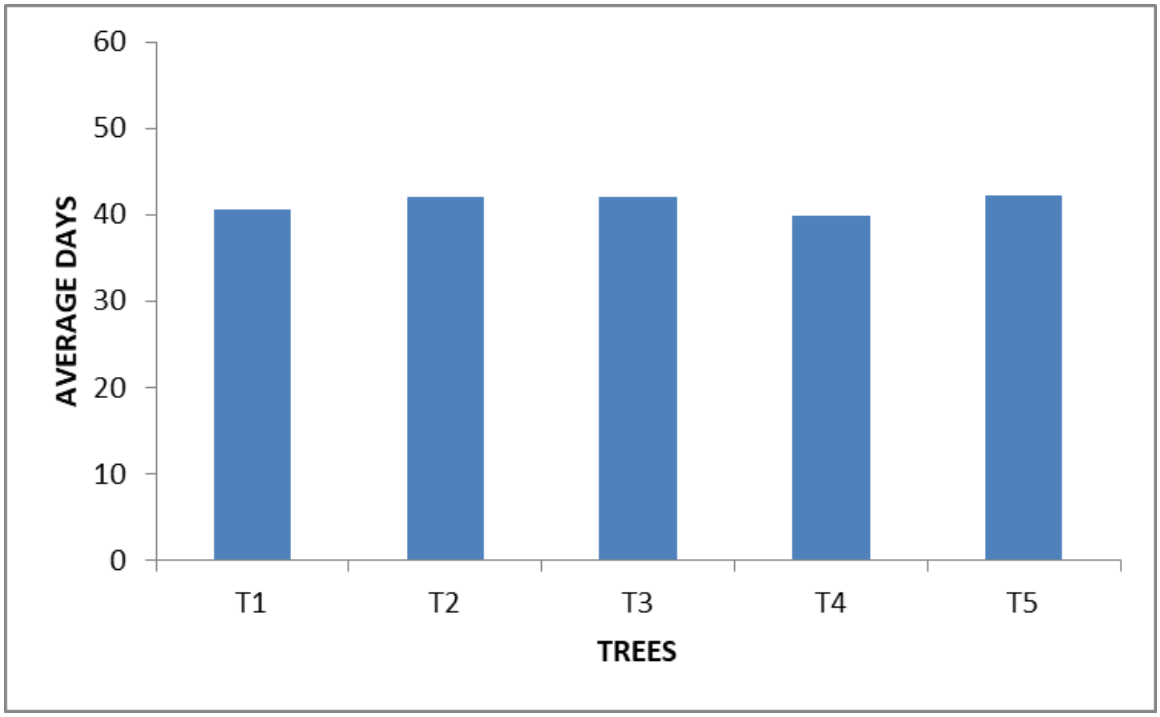

Fig.4.8 Total no. of days taken for flowering in *Cassia fistula*

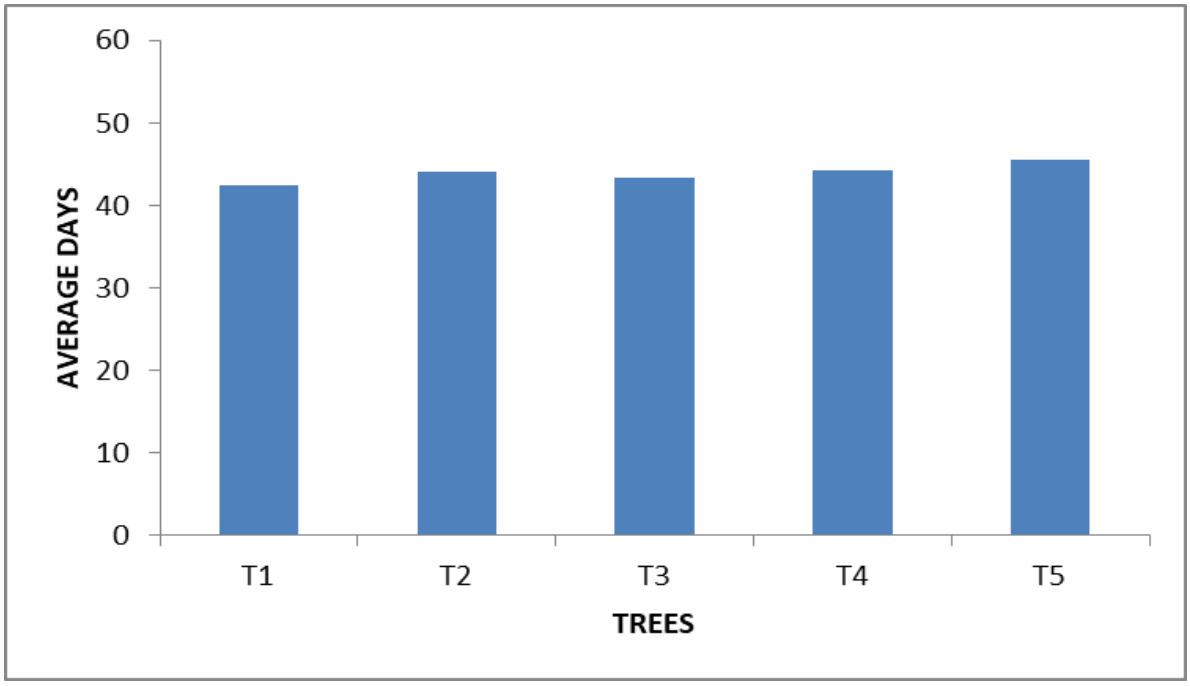

Fig.4.9 Total no. of days taken for fruiting in *Cassia fistula*

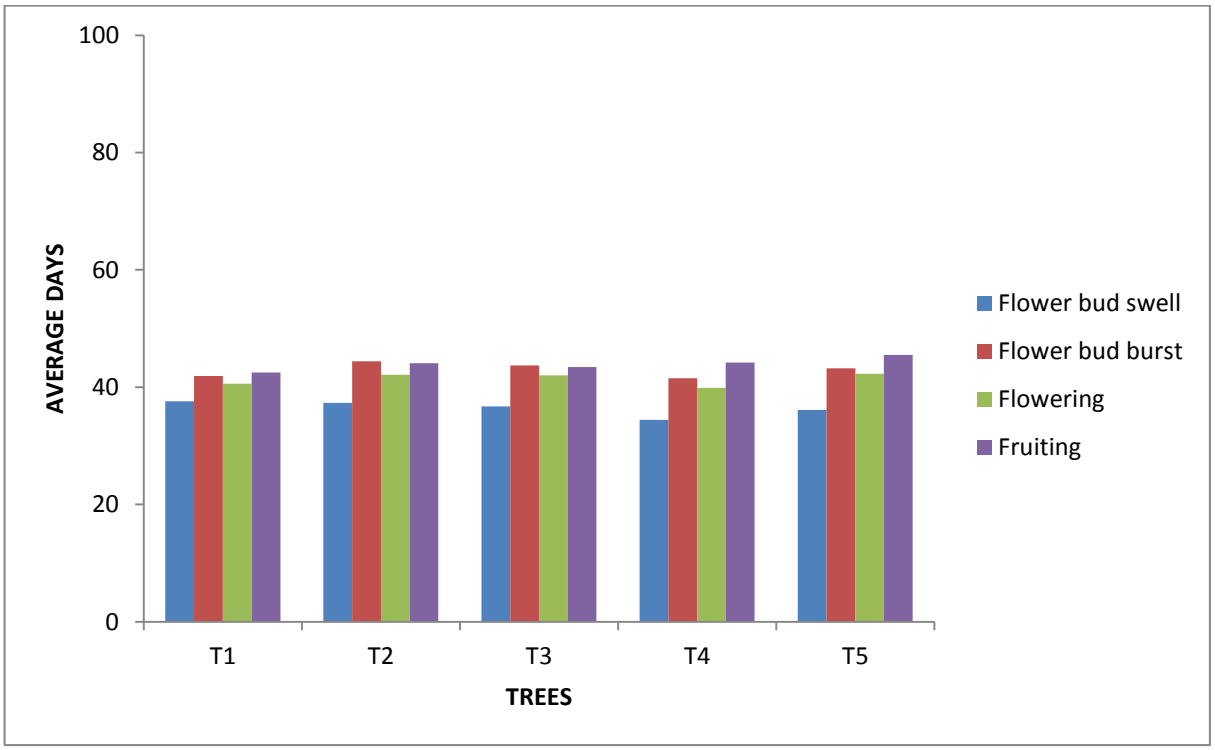

Fig.4.10 Total no. of days taken for reproductive phytophases in *Cassia fistula*

Fig- Ⅰ: Flower bud swell in *Cassia fistula*

Fig- ⅠⅠ: Flower bud burst in *Cassia fistula*

Plate no 2: Reproductive phenophases of *Cassia fistula*

4.2.5 Flower Bud Development

Appraisal of the data in Table 4.5 revealed that the maximum number of days to anthesis were recorded for T_2 (34.10) and the minimum number of days were recorded for T_5 (33.00) under twelve distinct stages of flower bud development number, in which stage (V I I-V I I I) took maximum number of days averaged at (days) and the minimum number of days averaged at (1.46) were taken by stage (X I-X I I) to anthesis.

Table 4.3: Mean value of different stages of reproductive bud development to anthesis in *Cassia fistula*.

Plus tree		**No. of days taken during different buid development stages**										**Anthesis time**	**Total no. of days**
		I-I I	**I I-I I I**	**I I I-IV**	**IV-V**	**V-V I**	**V I-V I I**	**V I I-V I I I**	**V I I I-IX-**	**IX-X**	**X-X I**	**X I-X I I**	
DAYS	T_1	2.01	2.51	3.11	3.28	4.41	4.64	5.24	3.34	2.41	1.78	1.11	33.40
	T_2	2.31	2.80	3.07	3.33	4.11	4.31	5.19	3.10	2.66	2.01	1.66	34.10
	T_3	2.41	2.41	2.81	3.51	3.81	4.41	5.41	3.21	2.21	2.31	1.31	33.80
	T_4	2.11	2.61	3.21	3.11	4.21	4.21	5.01	3.11	2.11	2.11	1.81	33.60
	T_5	2.01	2.61	2.71	3.21	3.81	4.31	5.31	2.81	2.41	2.41	1.41	33.00
Mean		**2.17**	**2.59**	**2.98**	**3.29**	**4.07**	**4.38**	**5.23**	**3.11**	**2.36**	**2.12**	**1.46**	**33.58**

4.2.6 Anthesis Timing

The data in the Table 4.6 and Fig. 4.12, it is clear that maximum anthesis (2.94) occurred between 8:30-10:30 a.m. hours. At 8:30 a.m. hours, almost all the flowers reached the second phase, i.e., 8:30-10:30 a.m. by which they are completely opened and by 14:30 p.m. the anthers became darker.

Table 4.4 Average value for anthesis timing in *Cassia fistula*

ANTHESIS TIMING						
TREES	**6:30 a.m-8:30 a.m**	**8:30 a.m-10:30 a.m**	**10:30 a.m-12:30 a.m**	**12:30 a.m-14:30 a.m**	**14:30 a.m-16:30 a.m**	**16:30 a.m-18:30 a.m**
T_1	1.21	1.91	1.51	0.66	1.00	0.56
T_2	0.91	1.86	1.11	0.43	0.70	0.33
T_3	1.01	3.61	1.21	0.56	0.80	0.46
T_4	0.67	4.11	1.51	0.34	0.50	0.33
T_5	1.29	3.21	0.39	0.71	0.90	0.23
Mean	**1.02**	**2.94**	**1.15**	**0.54**	**0.39**	**0.38**
SD±	**0.25**	**1.01**	**0.46**	**0.15**	**0.19**	**0.13**

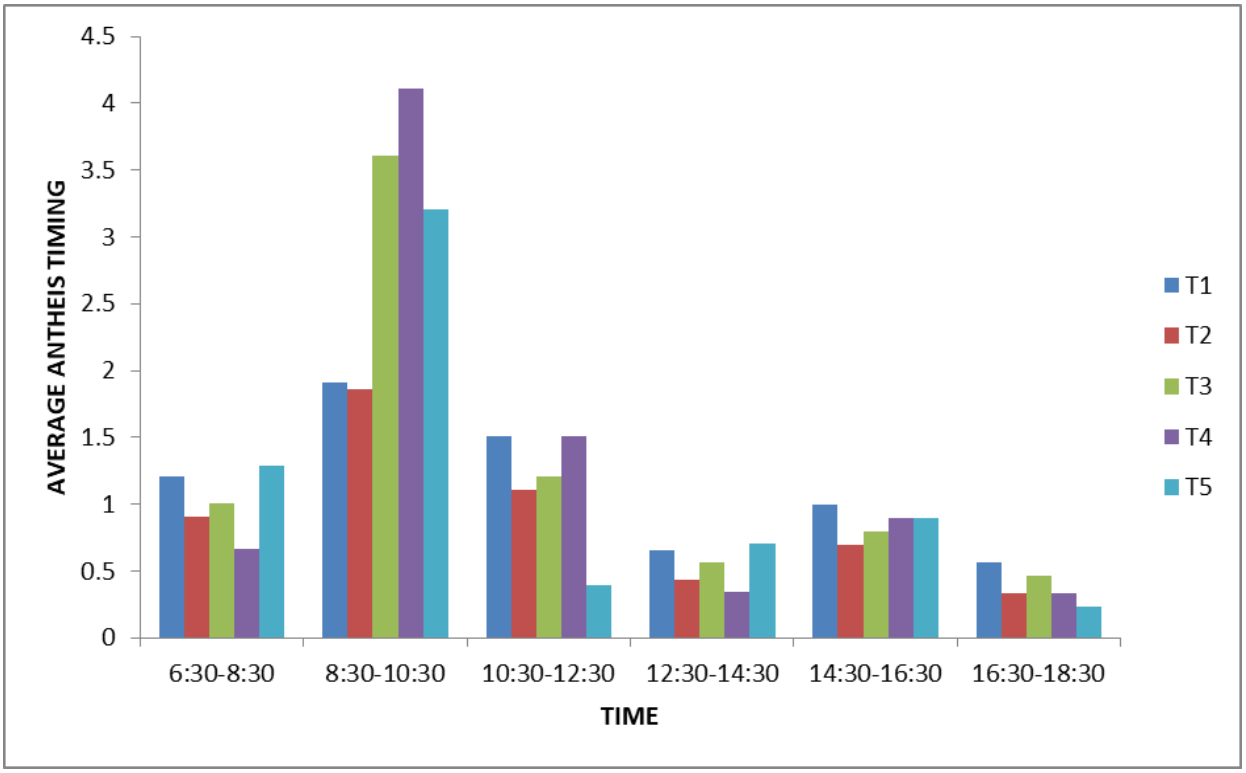

Fig. 4.11 Average value for Anthesis timing in *Cassia fistula*

4.2.7 Anther dehiscence

The data in Table 4.7 and Fig. 4.13, shows that maximum anther dehiscence is seen between 12:30-14:30 hours, followed by 14:30-16:30 hours.

Table 4.5 Average value for anther dehiscence in *Cassia fistula.*

ANTHER DEHISCENCE						
TREES	**6:30-8:30**	**8:30-10:30**	**10:30-12:30**	**12:30-14:30**	**14:30-16:30**	**16:30-18:30**
T_1	0.66	0.3	0.56	1.26	0.50	0.50
T_2	0.2	0.8	0.27	1.13	0.50	0.09
T_3	0.12	0.23	0.36	1.96	0.34	0.21
T_4	0.1	0.2	0.23	0.83	0.20	0.18
T_5	0.3	0.7	1.23	1.43	1.20	0.17
Mean	**1.38**	**2.23**	**2.65**	**6.61**	**2.74**	**1.15**
SD±	**0.28**	**0.45**	**0.53**	**1.32**	**0.55**	**0.23**

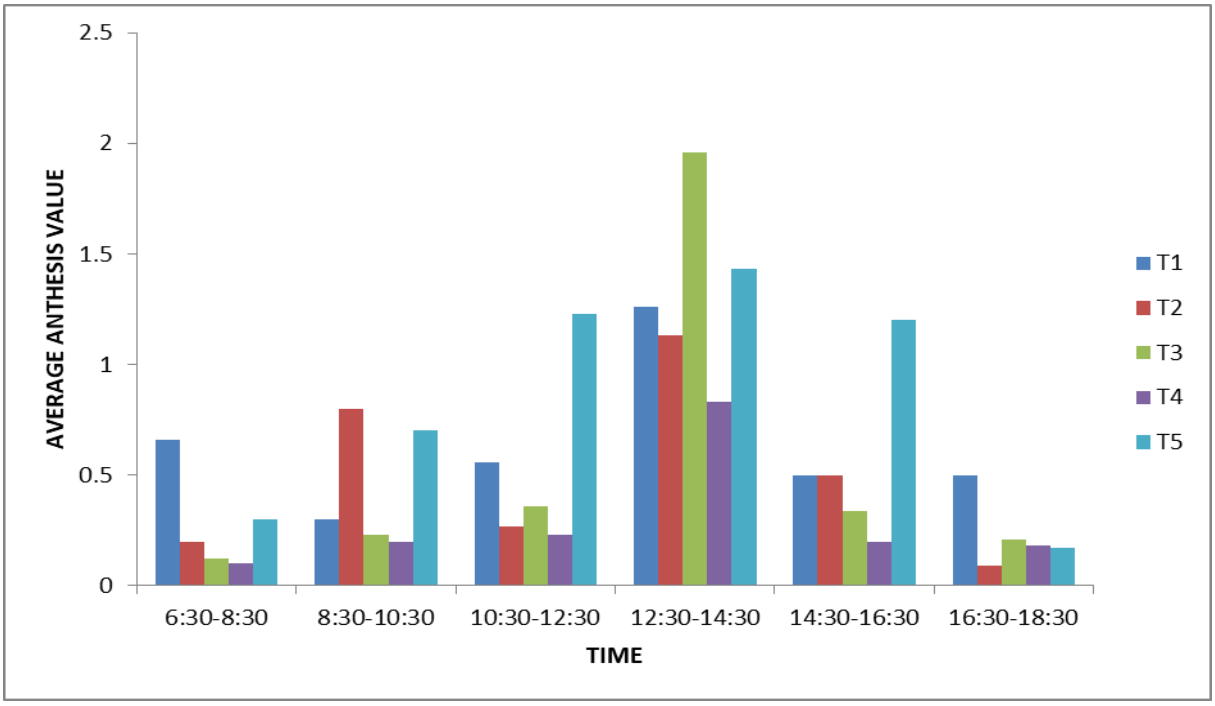

Fig. 4.12 Average value for anther dehiscence in *Cassia fistula*

Fig- Ⅰ: Balloon stage in *C.fistula*

Fig- ⅠⅠ: Opening of petals in *C.fistula*

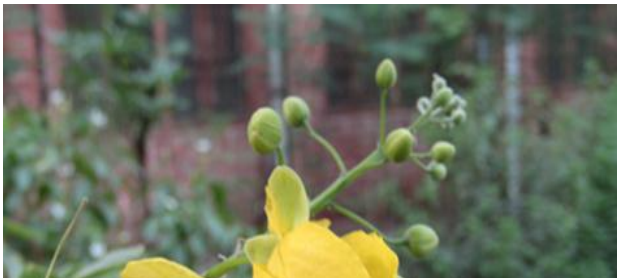

Fig-ⅠⅠⅠ: Anthesis in process in *C.fistula* **Fig-ⅠV: Fully opened flower of *C.fistula***

Plate no 3: Flower anthesis in *Cassia fistula*

4.3 POLLEN STUDY

Pollen is the male gametophyte ranging from 15-200 µm. Pollen viability is the ability of pollen to survive even in poor conditions. The germination capability of pollen is related to various factors such as, varieties, nutrition conditions and environmental factors. The pollen viability differs among different plant species. There are three categories in which different plant species are put under according to their pollen viability, viz: a) long lived pollen (six months to a year), (b) Pollen with medium life span (approximately 1-3 months) and (c) Short lived pollen (from few minutes to a couple of days). The pollen of *C.fistula* stays viable for a few months. The pollen viability can be evaluated by various methods depending on the species.

4.3.1 Pollen grain viability percentage at room temperature (25°C±5°C)

Data in Table 4.8 and Fig. 4.14 shows that the maximum mean viability percentage was seen in T_5 (99.00%) followed by T_2 (97.80%) with a mean of 97.14%. The lowest was observed in T_5 (46.70%) with a mean of 48.30%.

Table 4.6 Pollen grain viability of five trees in *Cassia fistula.*

Treatments	1st day	7th day	15th day
T_1	97.50	84.80	49.20
T_2	97.80	84.30	50.90
T_3	97.10	82.20	47.20
T_4	94.30	85.50	47.50
T_5	99.00	85.20	46.70
Mean	**97.14**	**84.40**	**48.30**
F-test	**S**	**S**	**S**
S.Ed. (±)	**1.489**	**1.118**	**1.238**
C.D at 5%	**3.000**	**2.252**	**2.493**
C.V.	**3.428**	**2.963**	**5.730**

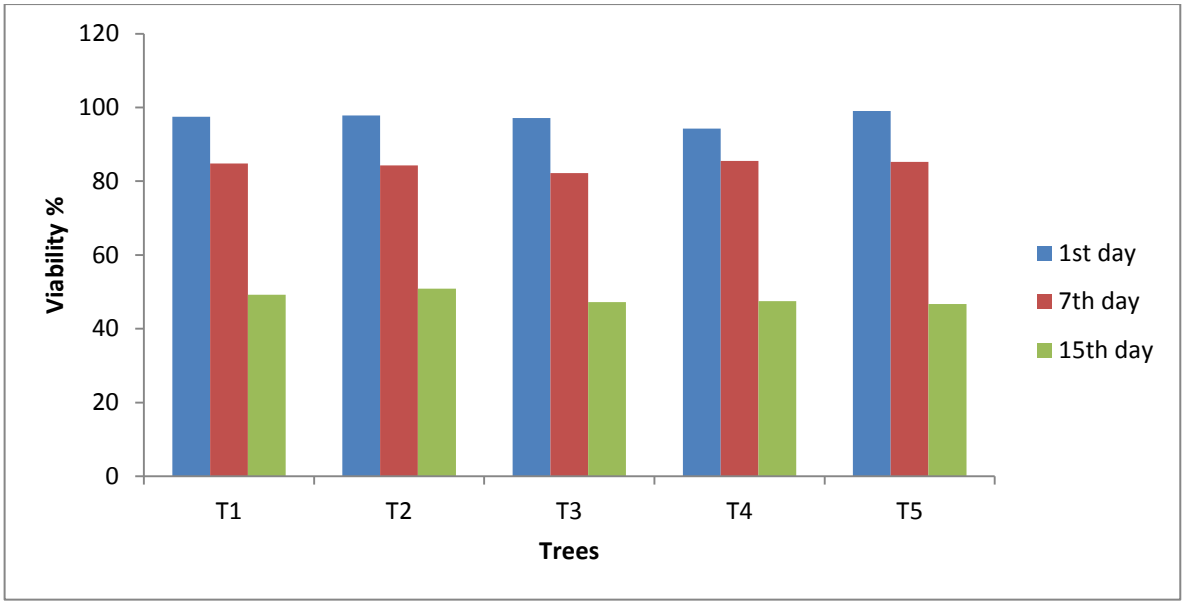

Fig. 4.13.Pollen grain viability percentage of *Cassia fistula* at room temperature (25°C±1°C)

4.3.2 Pollen grain germination percentage at room temperature (25^0C±5^0C)

The data presented in Table 4.9 and Fig. 4.15 reveal that maximum mean germination percentage (41.07%) which was recorded in 25% sucrose concentration followed by 15% sucrose conc. which gave a mean of 37.47 . The lowest mean germination (7.60%) was seen in 5% sucrose conc. With regard to trees the highest germination was observed in T_5 (42.67%) followed by T_2 (42.00 %).

Table 4.7: Pollen grain germination percentage at room temperature (25^0C±5^0C) for different sucrose concentration after pollen collection.

Treatments	5%	10%	15%	20%	25%
T_1	6.67	14.67	38.33	33.00	41.00
T_2	7.67	17.67	36.33	32.67	42.00
T_3	9.00	15.00	36.67	32.67	38.67
T_4	8.00	16.00	37.67	30.00	41.00
T_5	6.67	16.67	38.33	25.00	42.67
Mean	**7.60**	**16.00**	**37.47**	**30.67**	**41.07**
F-test	**S**	**S**	**S**	**S**	**S**
S.Ed	**0.730**	**0.894**	**0.699**	**2.385**	**1.135**
C.D. at 5%	**1.648**	**2.019**	**1.558**	**5.314**	**2.532**
C.V.	**11.769**	**6.847**	**2.286**	**9.526**	**3.386**

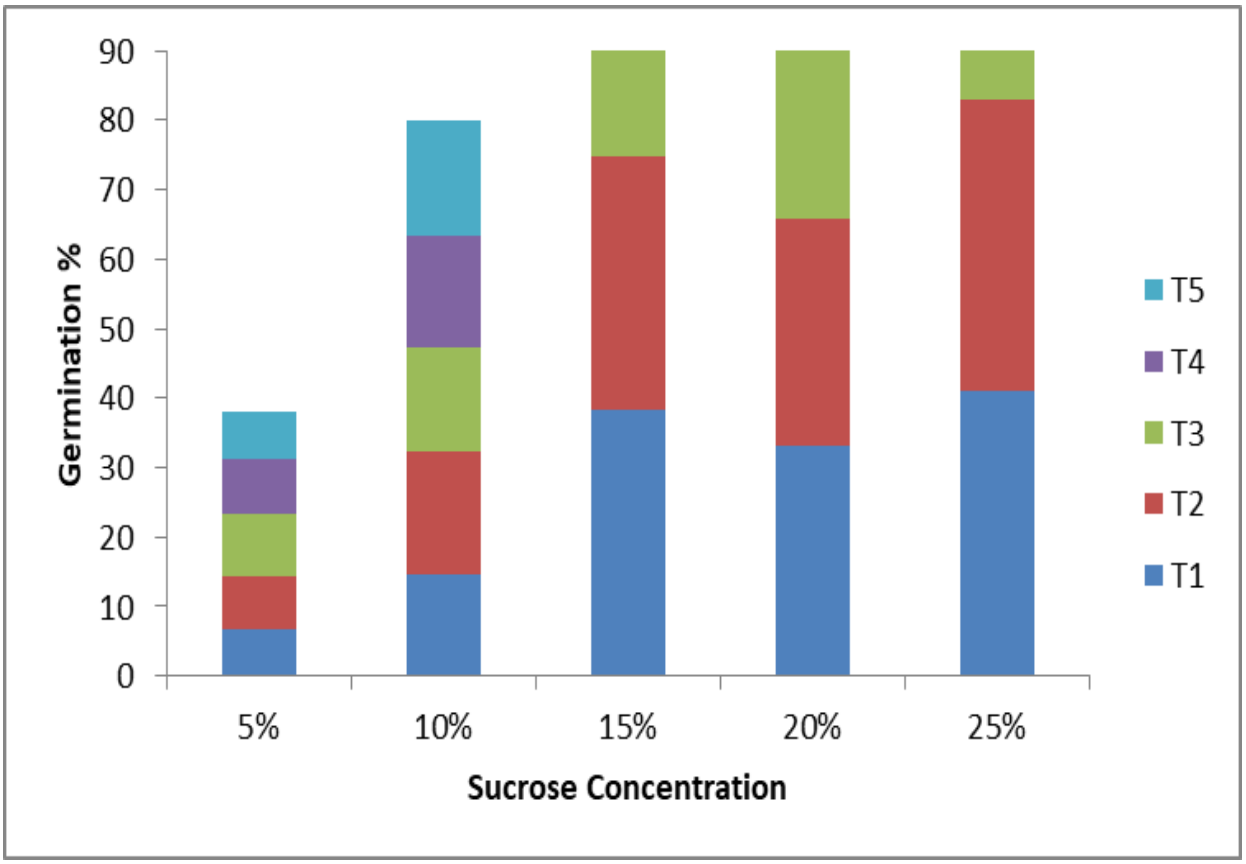

Fig.4.14 Pollen grain germination percentage at room temperature for different sucrose concentration.

4.3.3 Pollen grain germination percentage at various storage period and temperature in 25% sucrose concentration.

Data appended in Table 4.10 and Fig. 4.16 depicted that with the increase in number of days in room temperature the germination of pollen grains decreased. In temperature of -18±1°C the germination was 89.33% after seven days of storage which then decreased to 78% at 60 days.

Table 4.8: Pollen grain germination percentage at 25% sucrose conc. at 3 different storage temperatures.

Storage period	Pollen grain germination percentage at		
	Room temperature	**4±1°C**	**-18±1°C**
P_0 (0 days)	95.67	95.67	95.67
P_1(7 days)	78	87.33	89.33
P_2(15 days)	71	83.33	85.67
P_3(30 days)	60	80.33	82.33
P_4(45 days)	52.67	77.67	80.67
P_5(60 days)	40.33	72.33	78
Mean	**66.28**	**82.78**	**85.28**
F-test	**S**	**S**	**S**
S. Em	**0.653**	**0.527**	**1.027**
C.D. (P=0.05)	**2.011**	**1.624**	**3.166**

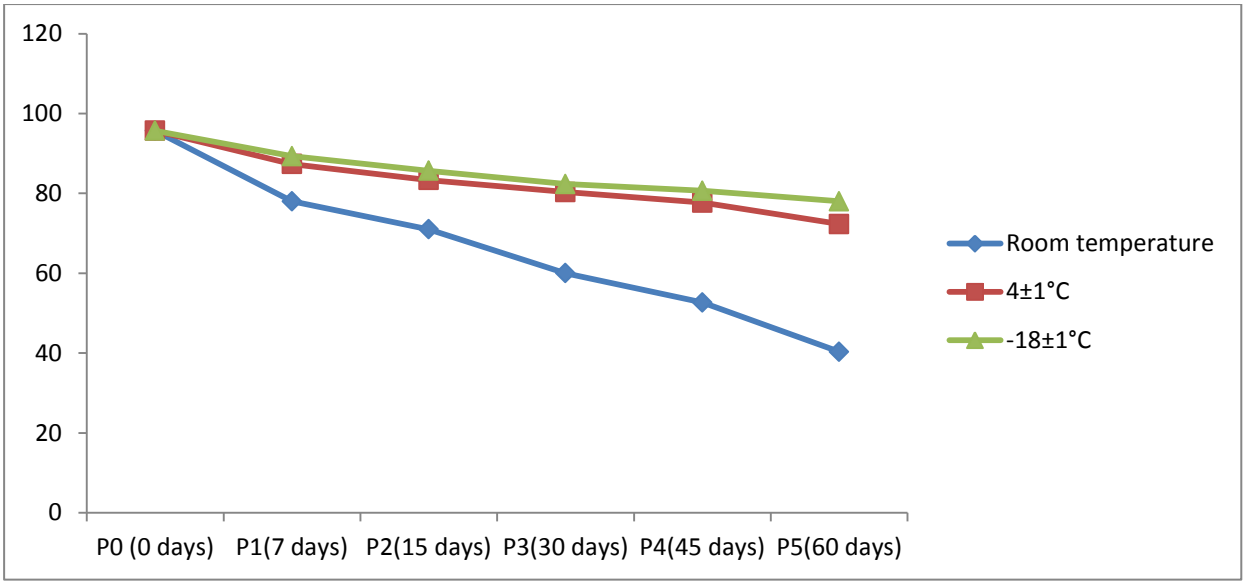

Fig.4.15 Pollen grain germination percentage at 25% sucrose conc. at 3 different temperatures.

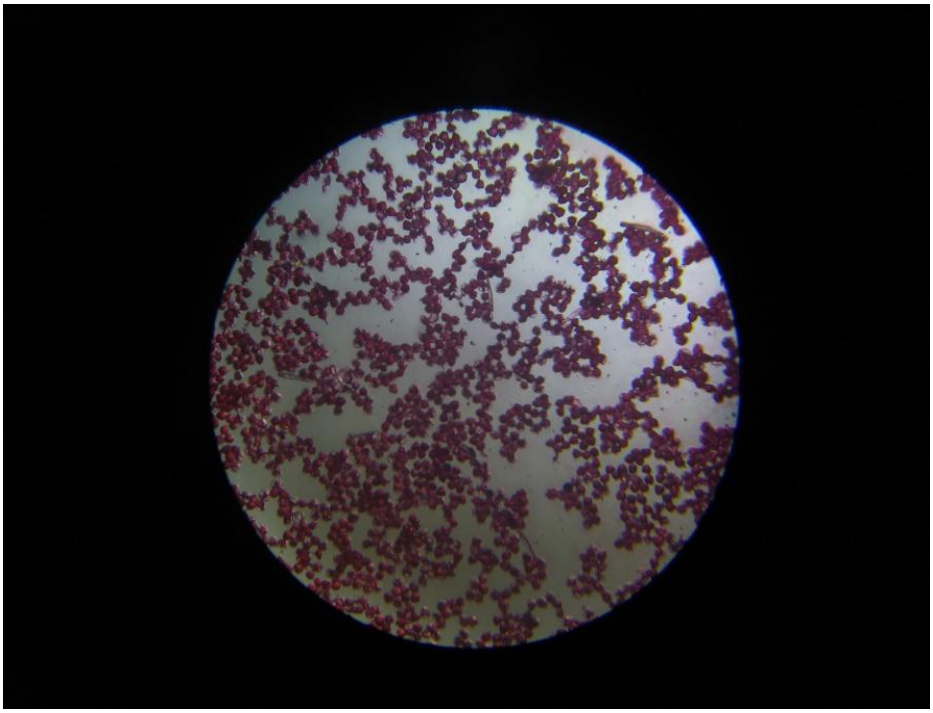

Fig- Ⅰ: Stained pollen grain in acetocarmine test of *C.fistula*

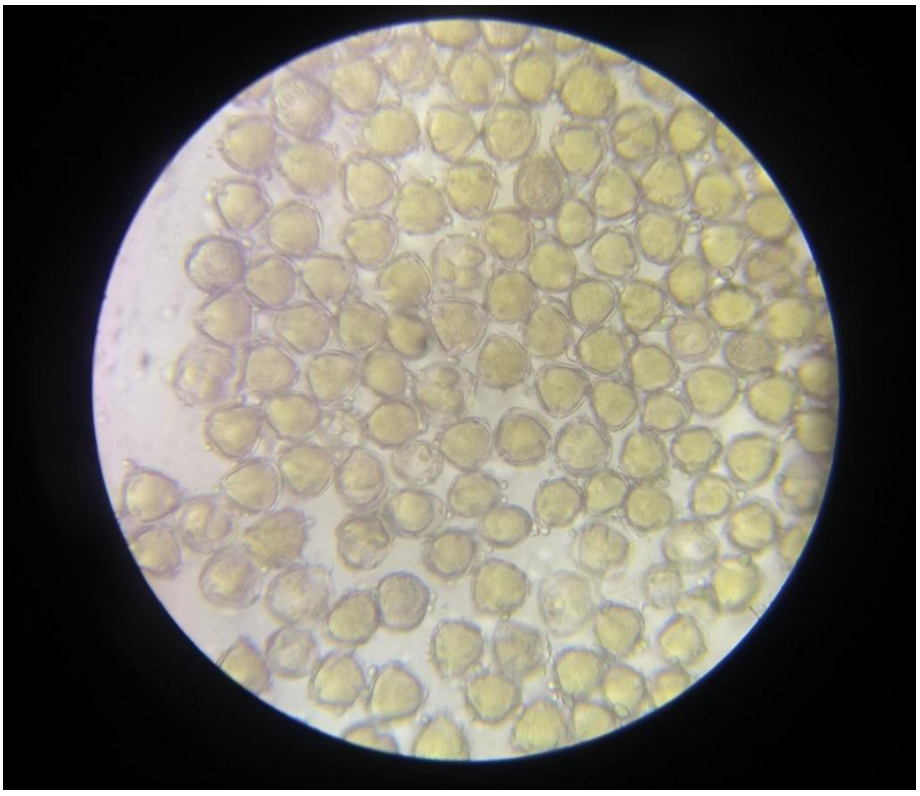

Fig- Ⅱ: Shape of pollen grain of *C.fistula.*

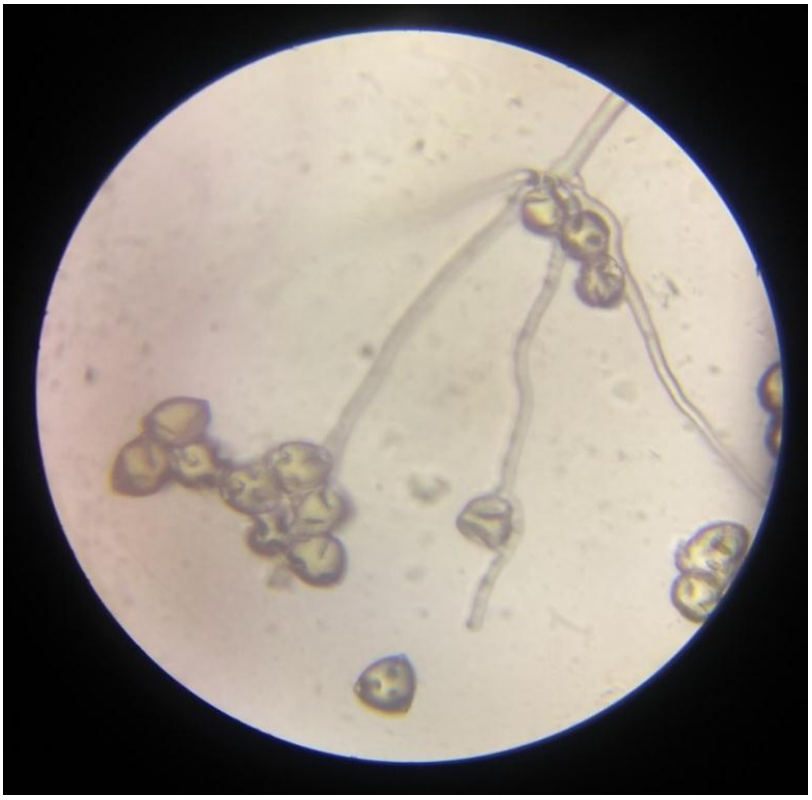

Fig-ⅠⅠⅠ: Pollen tube germination in *C.fistula.*

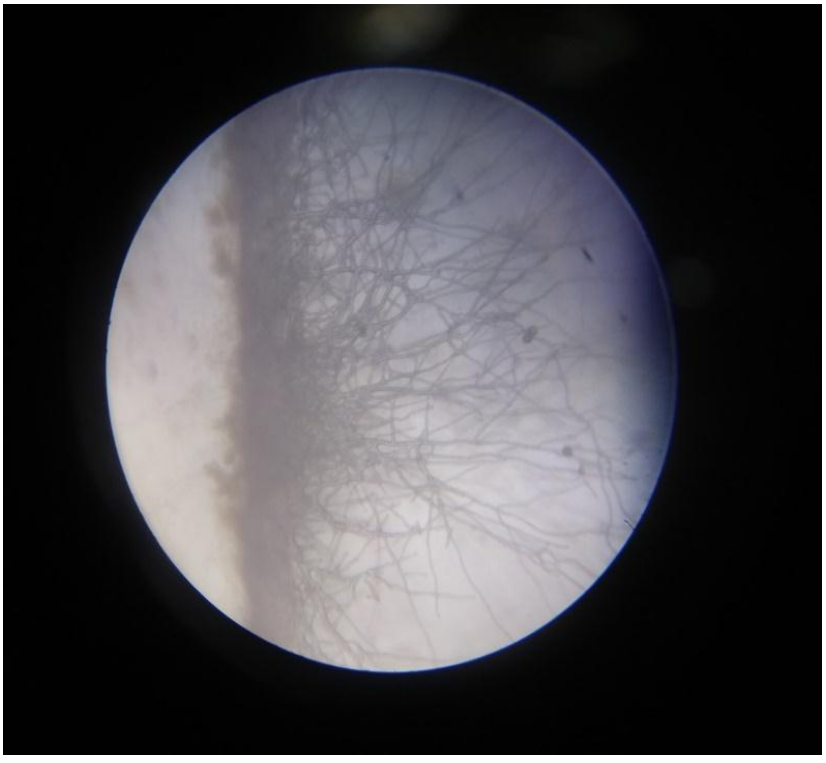

Fig-ⅠV: Pollen tube germination at 25% sucrose solution

Plate no.4- Pollen of *Cassia fistula*

4.4 FLORAL MORPHOLOGY

Table 4.9 Floral characteristics of *Cassia fistula*

Floral Character	Observation
Leaf bud swell	March-April
Leafing	April- May
Senescence	March-April
Flowering period	April-July
Fruiting period	August-October
Flower colour	Bright yellow
Inflorescence	Raceme
Flower type	Complete, hermaphrodite
Number of sepals	5
Number of petals	5
Fruit type	Indehiscent pod (drooping)
No. of seed in pod	25-100
Mean number of anthers	10
Mean number of stigma	1
Flower Anthesis time	8:30-10:30 hours
Flower dehiscence time	12:30-14:30 hours
Pollinator type	Bees, birds
Pollen colour	Pale yellow

4.4.1 Stamen and pistil length (cm)

The appraisal of data in Table 4.11 and Table 4.12 and Fig. 4.17 and Fig. 4.18 revealed that the maximum length of the stamen recorded was 2.10 cm and maximum length of pistil was recorded to be 2.80 cm.

Table 4.10 Stamen length of *Cassia fistula* (cm)

S.No	T1	T2	T3	T4	T5
1	2	1.9	1.4	1.8	1.7
2	1.9	1.9	2	1.9	1.7
3	1.8	1.5	1.9	1.9	1.4
4	1.6	1.4	1.6	1.6	1.4
5	2.9	1.6	1.9	1.7	1.9
6	1.9	1.4	2	1.8	1.7
7	2	1.9	1.9	1.8	1.9
8	1.7	1.5	1.9	1.9	1.7
9	1.9	1.5	1.6	1.8	2.1
10	2.1	1.9	1.8	1.8	2
Mean	**1.98**	**1.65**	**1.80**	**1.80**	**1.75**
Range	**1.6-2.1**	**1.4-1.9**	**1.4-2**	**1.6-1.9**	**1.4-2.1**

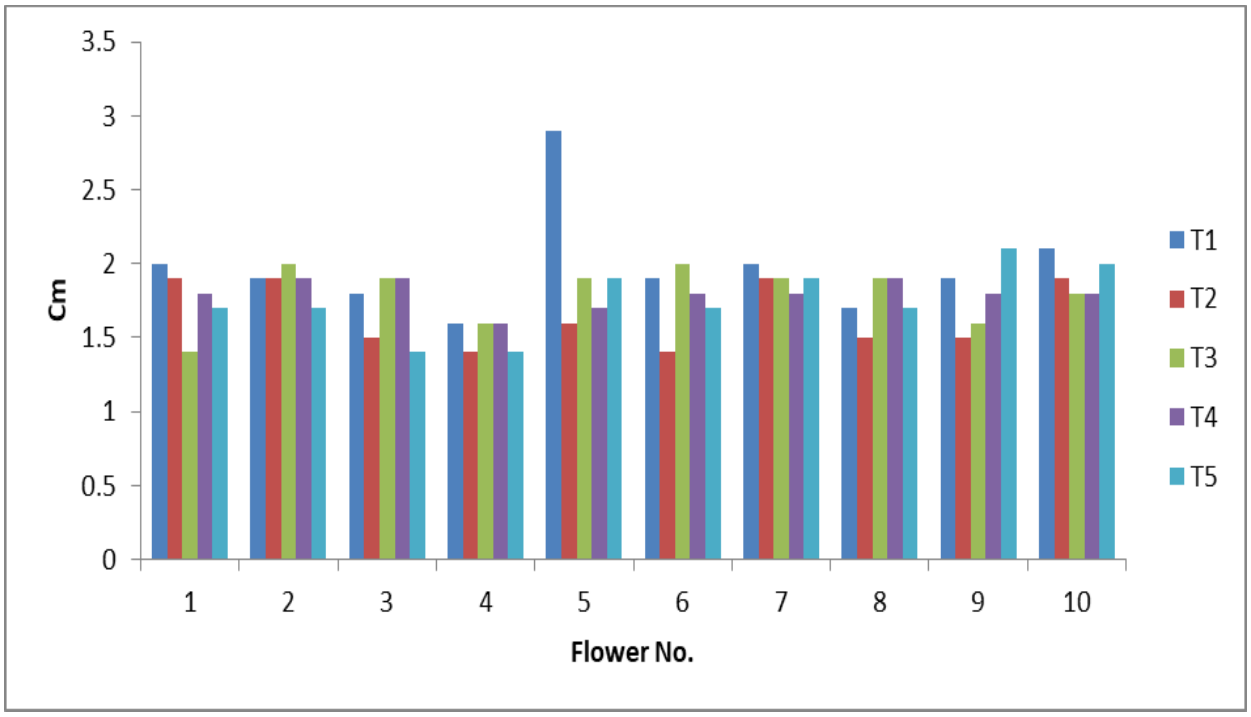

Fig. 4.16 Length of Stamen of *Cassia fistula* (cm)

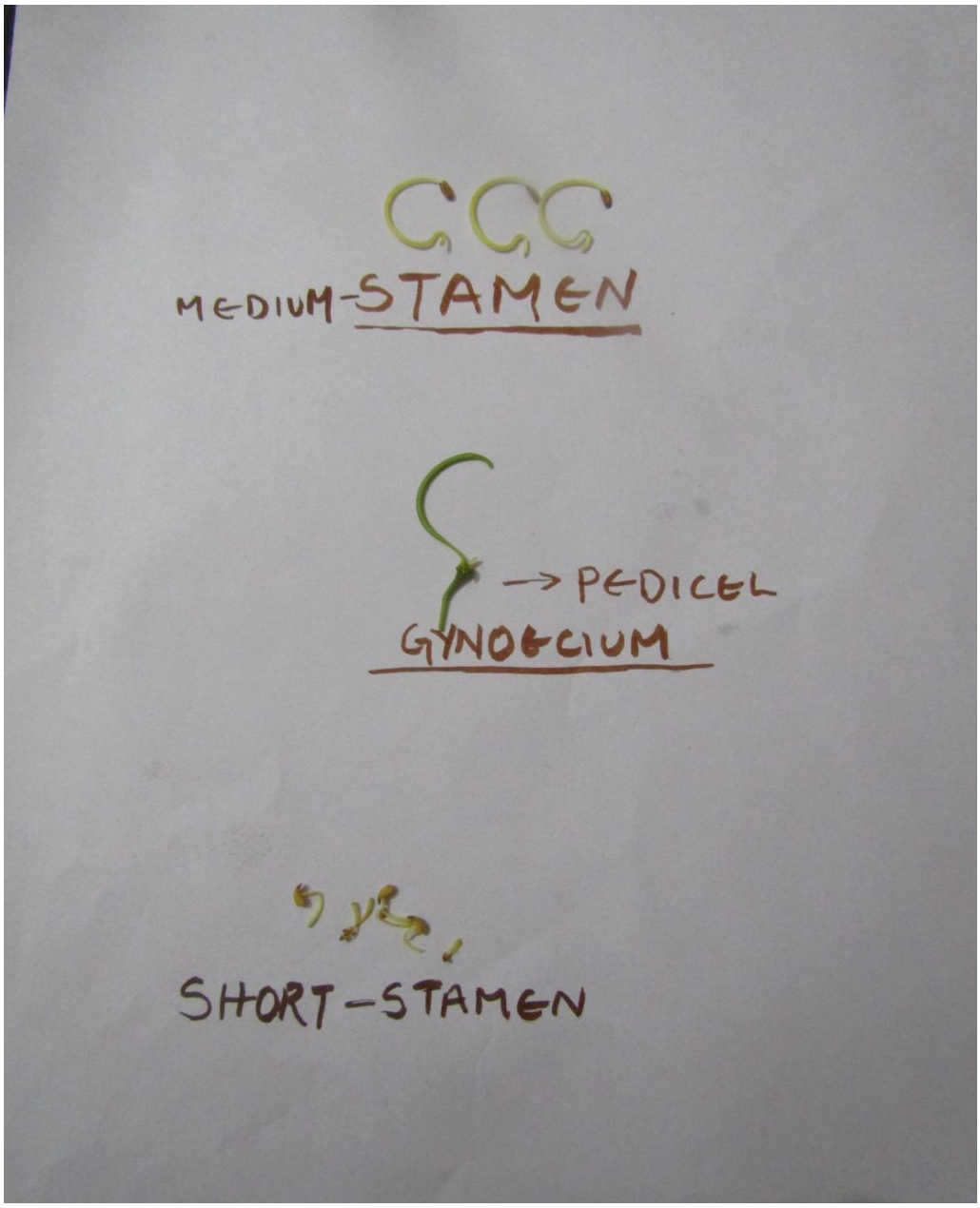

Fig- I: Reproductive parts of *Cassia fistula*

Table 4.11 Pistil length of *Cassia fistula* (cm)

S.No	T1	T2	T3	T4	T5
1	2.40	2.10	2.20	2.40	2.20
2	2.70	2.40	2.40	2.40	2.20
3	2.50	2.30	2.60	2.30	2.10
4	2.40	2.20	2.60	2.50	1.80
5	2.80	2.40	2.40	2.60	2.40
6	2.50	2.30	2.50	2.30	2.00
7	2.60	2.40	2.80	2.50	2.70
8	2.50	2.10	2.40	2.40	2.10
9	2.60	2.60	2.60	2.40	2.60
10	2.70	2.30	2.40	2.60	2.40
Mean	**2.57**	**2.31**	**2.49**	**2.44**	**2.25**
RANGE	**2.4-2.8**	**2.1-2.6**	**2.2-2.8**	**2.3-2.6**	**1.8-2.7**

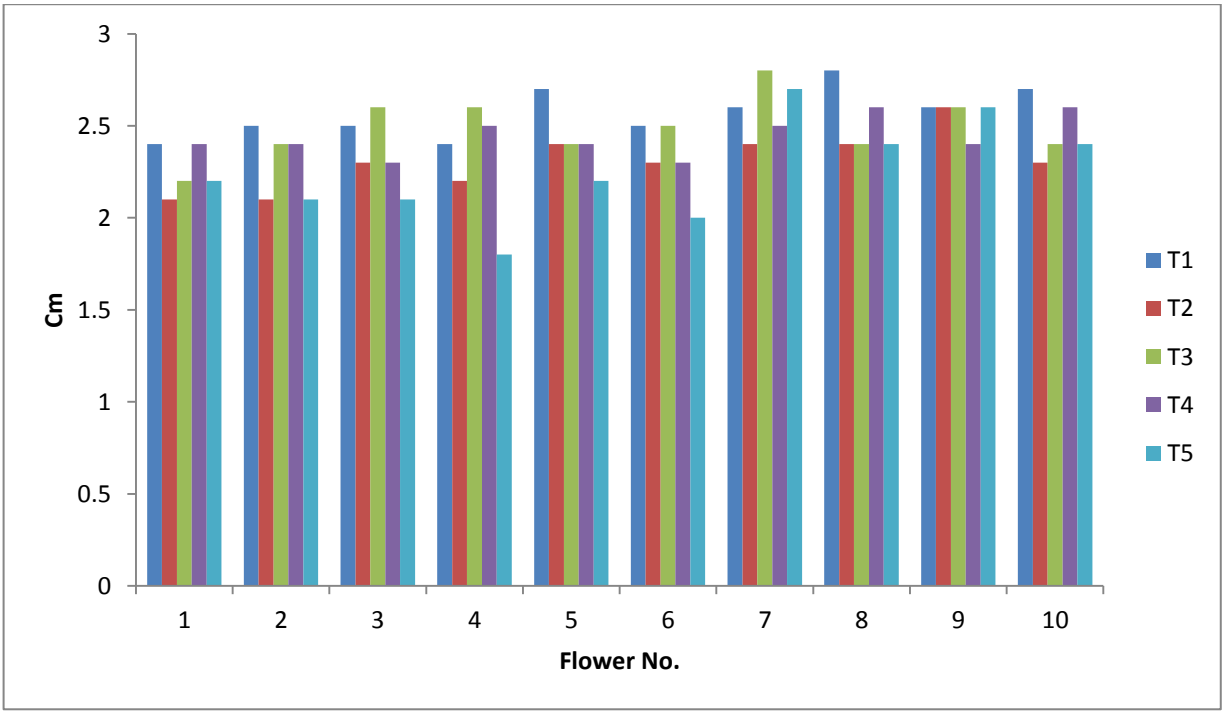

Fig. 4.17 Length of pistil of *Cassia fistula* (cm)

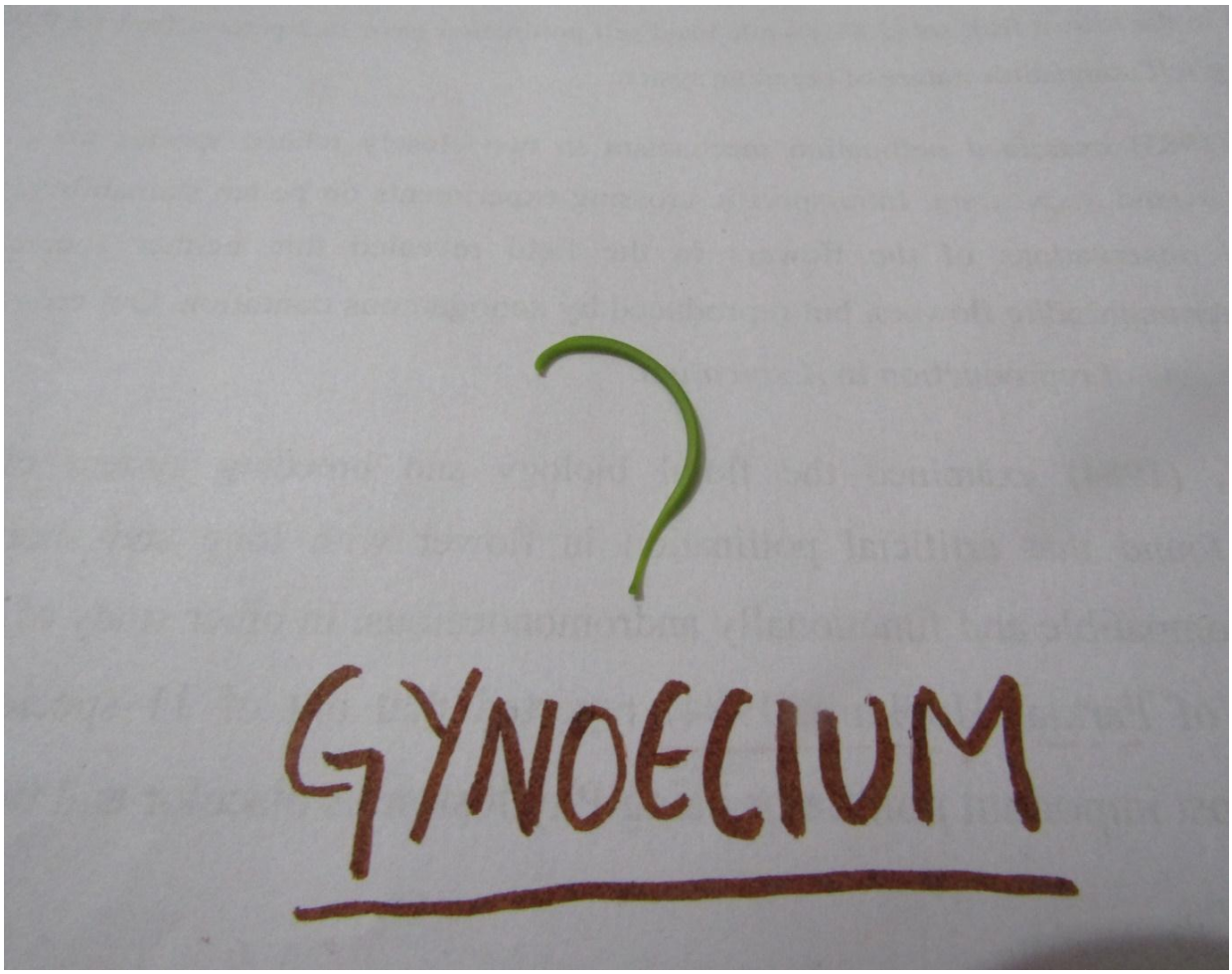

Fig- I: Female reproductive part of *Cassia fistula*

4.5 BREEDING BEHAVIOUR

The pollination studies was conducted to determine the mode of pollination in *Cassia fistula*. The fruit and seed setting from different modes of pollination were also observed.

4.5.1. Autogamy

Flower buds which were expected to open the next day were bagged with perforated butter paper bags before anthesis. Fruit set was recorded after 15-20 days, interval. The data on fruit set under selfing (by bagging) is presented in Table 4.14.

4.5.2 Allogamy

The cross pollinated flowers were bagged and data on the fruit set is also presented in Table 4.14 which showed that T_5 (13%).

4.5.3 Open pollination

The appraisal of data in Table 4.14 revealed the number of fruit set in open pollination

Table 4.12 Pollination studies in *Cassia fistula*

S.No	Type of pollination	Candidate plus tree	No. of flowers pollinated	No. of flowers set fruit	Fruit set %
1.	Autogamy	T_1	50	5	10
		T_2	50	7	14
		T_3	50	6	12
		T_4	50	4	8
		T_5	50	2	4
2.	Allogamy	T_1	50	6	12
		T_2	50	10	20
		T_3	50	9	18
		T_4	50	11	22
		T_5	50	13	26
3.	Open-pollination	T_1	50	3	6
		T_2	50	7	14
		T_3	50	6	12
		T_4	50	3	6
		T_5	50	8	16

Fig- Ⅰ: Bagging of *Cassia fistula* flowers.

Fig- ⅠⅠ: Fruiting in *Cassia fistula*

Plate no. 5 (A) Bagging of cross-pollinated flowers (B) Bagging of self-pollinated flowers

4.6 POLLINATOR ACTIVITIES

The flowering in *Cassia fistula* was observed from April-June. These pollinators or flower visitors are vital for the survival and evolution of plants. Giant Honey bees were found to be the most common pollinator and were active from morning 6:30 till late evening. From about 7:00 am the Indian palm squirrel was also a frequent visitor and also feeds on the flowers.

Table no. 4.13 List of the pollinators visiting *Cassia fistula* flowers.

Visitor with order/family	Common Name	Visiting Time
Hymenoptera • Apis dorsata • Ropalidia marginata	Giant Honey Bee Yellow Paper Wasp	Day Day
Passeriformes • Pycnonotus jocosus • Pycnonotus cafer • Argya striata	Red-whiskered bulbul Red-vented bulbul Jungle babbler	Day Day Day
Piciformes • Psilipogon zeylancius	Brown-headed barbet or large green barbet	Day
Rodentia • Funambulus palmarum	Indian palm squirrel	Day

Fig- Ⅰ: Red whiskered bulbul

Fig- ⅠⅠ: Red Vented Bulbul

Fig- ⅠⅠⅠ: Jungler Babbler

Fig- ⅠV: Green Barbet

Fig- V: Yellow Paper Wasp

Fig- V I: Giant Honey Bee

Fig- V I I: Indian Palm Squirrel

Plate no. 6- Pollinators in *Cassia fistula*

CHAPTER V

CHAPTER-V

DISCUSSION

The knowledge of the time of flowering and mode of pollination in forest tree species is crucial for a forest breeder. Thus, the detailed study of the different characters vegetative and reproductive, with anthesis timing, pollen germination, breeding programme, pollen study is of utmost importance.The term phenology was first introduced by the Belgian botanist **Charles Morren in 1853**, which is derived from the Greek words phaino meaning "to appear, to come into view" and logos mean "to study". So phenology is the study of timing of seasonal biological activities **(Brian P. Haggerty and Susan J. Mazer 2008)** or Phenology is literally "the science of appearance." **(Kasarkar R. and Kulkarni D. 2011**) or Phenology is the periodic phenomena of plants in relation to changes in season and climate **(Namita nath 2012).** Fabaceae is the second after Poaceae in turn of agricultural and economic point of view. Fabaceae is one of the most important family not only medicinal but also economical point of view. Legumes include a large number of plant species which are harvested as a food for human and animal consumption also harvested for oils, fiber, fuel, fertilizers, timber, medicinal, and chemicals **(Lewis et.al., 2005)**. Golden Shower Tree (*Cassia fistula L.*) is a member of the family Caesalpiniaceae known for its characteristic bunches of beautiful yellow flowers and grows throughout India. The tree is a native plant of India, naturalized in Africa, West Indies and South America. It has attained importance as an ornamental and avenue plant **(Arora, 1988).**

Vegetative characters

Cassia fistula belongs to the Leguminosae or Fabaceae family with compound,paripinnate leaves. The leaf bud swell started from last week of March. Total number of days taken for leaf bud swell and leaf bud burst were 62.5 and 33 days respectively. Leafing took mthe longest number of days i.e, 103.2 days. Leaf fall started around March and went on till April which was about 52.2 days. New leaflets were always seen during the whole course of study **(Kimkim and Yadava,2001).**

Reproductive characters

According to the results analyzed the flower is drooping raceme and bright yellow in colour and inflorescence was found to be raceme. The flower bud swell took around 37.6 days whereas the leaf bud burst took 44 days. Flowering and fruiting was highest in T_5 taking 45.3 and 45.5 days respectively. The flowering was at its peak in the month of May. Flowers have 5 sepals (usually about 6mm long), ovate or oblong, puberulent; petals 5, about 2 cm long or longer, ovate-orbicular, short-clawed, venose; stamens 3-morphic, the 3 lowermost almost 3 cm, the

anthers ovate-oblong, 4-5mm long , glabrous, dehiscent apically and basally; the 4 median stamens about 1 cm long, the anther ovate-oblong, sagittate, about 4 mm long, dehiscent from the basal lobes (and apical pores); 3 uppermost stamens shorter and smaller, somewhat unequal,the anthers similar to the median ones; ovary slender, slightly pubescent. Legume reportedly cylindric, about 50 cm long, indehiscent with horizontal seeds (**Flora of Panama, 2014)**

The fruiting took place in the first week of August after completion of flowering period. At the end of the flowering period, the flowers lose their bright yellow colour and turn pale yellow. The fruit is drooping pod of about 30-60 cm in length and 1.5-2.5 cm in breadth.

The flower bud development had 10 stages before anthesis which was similar to findings of **Nalawadi *et al***., who observed this in pomegranate.

Anthesis was observed to be at 8:30-10:30 hours. Similar findings were found in *Senna corymbosa* by **Cecilia laporta.** And anther dehiscence was observed to be at 12:30-14:30 hours which was similar to findings of **Hong Zhang *et al.,*** in peach.

POLLEN STUDIES

Pollen viability percentage at room temperature through acetocarmine test was found to be maximum on the first day with 99% which then decreased to 46.70% after 15 days. This is similar to **Kanthaswamy (2006)** who reported high viability on the first day of pollen collection and decrease in the following days.

The pollen germination percentage at room temperature for different sucrose concentration was also observed. Pollen grain germination was seen to be maximum in 25% sucrose followed by 15% sucrose solution.

POLLINATOR ACTIVITIES

Cassia fistula flowers are bright yellow in colour and hence attract many birds and insects especially bees. Honey bees were observed to be the most common visitor **(Katende 1995).** These results supports the findings of **Kimkim 2001**, in tree species in subtropical forests of Manipur.

BREEDING BEHAVIOUR

Cross-pollination showed maximum fruiting result. This species is considered geitonogamic or xenogamic. **Dundas et al.,(2007)** observed that *Trifolium glanduliferum* showed similar results of maximum fruit set under cross pollination.

CHAPTER V1

CHAPTER-V I

SUMMARY AND CONCLUSION

The present research entitled, "Monitoring of reproductive biology of *Cassia fistula*" was conducted at the Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj. For study of phenology, pollination and breeding behavior, five trees were selected and from each tree ten branches were chosen and tagged. The investigation was done in Randomized Block Design in field condition having five treatments and ten replications whereas Completely Randomized Block design was used for the laboratory work with five treatments and three replicates.

The observations of the investigation are summarized below:

- Vegetative characters of *Cassia fistula* showed that the maximum number of days taken for leaf bud swell and leaf bud burst was seen in T_3 whereas leaf fall too k the longest in T_4.
- Reproductive characters showed that the maximum number of days taken for flower bud swell was inT_1 and flower bud burst in T_2 with Flowering and fruiting showing maximum duration in T_5.
- Anthesis was observed to be peak at 8:30 a.m to 10:30 a.m with anther dehiscence taking place just after anthesis. Anther dehiscence was maximum at 12:30 -14:30 hours.
- Pollen grain viability percentage at room temperature (25°C±5°C) was observed to be maximum at T_5 on the first day with a mean of 99% which decreased to 46.7% after 15 days.
- In vitro pollen germination was recorded highest under room temperature (25°C±5°C) at 25% sucrose solution on the first day of pollen collection. The germination decreased to 40.33% after at 60 days. In 4±1°C and -18±1°C germination was 72.33% and 78% respectively.
- The study of the breeding behavior revealed that maximum fruit set was seen in cross pollination.
- The most common pollinator was Giant-honey bee which was observed to be active from 6: 30 a.m till late evening.

The study of the vegetative of *Cassia fistula* showed that leafing take the maximum number of days to complete. The leaf bud swell, leaf bud burst and leafing was longest in T_3 and leaf fall

had the longest duration in T_4. Reproductive characters viz. flowering and fruiting was maximum in T_5 and flower bud swell in T_1 and flower bud burst in T_2. Anthesis took place throughout the day with peak at 8:30 a.m and anther dehiscence taking place at 12:30-14:30 hours. The pollen grains were viable for more than a month showing 99% viability on the first day after collection. In-vitro pollen germination was observed to be highest at room temperature in 25% sucrose solution treatment. The study of the breeding behavior revealed that cross pollination showed the maximum fruits. The major pollinator in *Cassia fistula* was Giant Honey Bee.

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APPENDIX

APPENDIX

Leaf bud swell

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	141.680			
Treatment	4	131.480	32.870	3.758	0.01180
Error	36	314.920	8.748		
Total	49	588.080			

Leaf bud burst

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	18.580			
Treatment	4	38.680	9.670	3.356	0.01961
Error	36	103.720	2.881		
Total	49	160.980			

Leafing

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	142.020			
Treatment	4	862.520	215.630	12.064	0.00000
Error	36	643.480	17.874		
Total	49	1,648.020			

Leaf fall

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	86.020			
Treatment	4	251.320	62.830	6.377	0.00055
Error	36	354.680	9.852		
Total	49	692.020			

Flower bud swell

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	16.080			
Treatment	4	70.280	17.570	8.534	0.00006
Error	36	74.120	2.059		
Total	49	160.480			

Flower bud burst

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	81.620			
Treatment	4	59.320	14.830	2.754	0.04272
Error	36	193.880	5.386		
Total	49	334.820			

Flowering

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	20.580			
Treatment	4	45.480	11.370	3.664	0.01328
Error	36	111.720	3.103		
Total	49	177.780			

Fruiting

ANOVA

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	9	70.420			
Treatment	4	48.920	12.230	3.069	0.02837
Error	36	143.480	3.986		
Total	49	262.820			

Pollen grain viability percentage (1st day)

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	4	11.600	2.900	3.625	**S**	**NS**
Replication	2	1.200	0.600	0.750	**NS**	**NS**
Error	10	8.000	0.800			
	14					

***In vitro* germination of Cassia fistula pollen grain stored at room temperature (10% sucrose)**

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	4	18.000	4.500	3.750	**S**	**NS**
Replication	2	5.200	2.600	2.167	**NS**	**NS**
Error	10	12.000	1.200			
	14					

***In vitro* germination of Cassia fistula pollen grain stored at room temperature (15% sucrose)**

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	4	10.400	2.600	3.545	**S**	**NS**
Replication	2	0.133	0.067	0.091	**NS**	**NS**
Error	10	7.333	0.733			
	14					

***In vitro* germination of Cassia fistula pollen grain stored at room temperature (20% sucrose)**

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	4	138.000	34.500	4.043	**S**	**NS**
Replication	2	2.533	1.267	0.148	**NS**	**NS**
Error	10	85.333	8.533			
	14					

***In vitro* germination of Cassia fistula pollen grain stored at room temperature (25% sucrose)**

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	4	27.600	6.900	3.569	**S**	**NS**
Replication	2	14.933	7.467	3.862	**NS**	**NS**
Error	10	19.333	1.933			
	14					

***In vitro* germination of Cassia fistula pollen stored in room temperature**

ANOVA:

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	7	25530.958	3647.280	1535.697	**S**	**S**
Replication	2	5.583	2.792	1.175	**NS**	**NS**
Error	16	38.000	2.375			
	23					

***n vitro* germination of Cassia fistula stored in 4±1°C**

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	7	4088.625	584.089	318.594	**S**	**S**
Replication	2	3.083	1.542	0.841	**NS**	**NS**
Error	16	29.333	1.833			
	23					

***In vitro* germination of Cassia fistula stored in -18±1°C**

ANOVA

Source	D.F.	SS	MSS	Cal. F	TAB. F(5%)	TAB. F(1%)
Treatment	7	2531.292	361.613	149.633	**S**	**S**
Replication	2	18.583	9.292	3.845	**S**	**NS**
Error	16	38.667	2.417			
	23					