Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region

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Thesis submitted to the Odisha University of Agriculture and Technology in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Fruit Science and Horticulture Technology)

By

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Place: Bhubaneswar

Date: 10/9/2020

CERTIFICATE- I

This is to certify that the thesis entitled "**Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region**" submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (FRUIT SCIENCE AND HORTICULTURE TECHNOLOGY)** to the Odisha University of Agriculture and Technology, Bhubaneswar, is a faithful record of *bona fide* and original research work carried out by **ABHISHEK PADHY, Adm No. 18122I04** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma or published in any other form.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

Kishore 10/9/20

(**Dr. Kundan Kishore**)

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CERTIFICATE- II

This is to certify that the thesis entitled "**Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region**" submitted by **ABHISHEK PADHY** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** in the discipline of **FRUIT SCIENCE AND HORTICULTURE TECHNOLOGY** has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with the External Examiner.

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Abhishek Padhy.

Bhubaneswar **Abhishek Padhy**

Date: Adm. No. 18122I04

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ABBREVIATONS

% : Per cent

ANOVA : Analysis of variance

CD : Critical difference

cv (s) : Cultivar(s)

Deptt. : Department

et al. : Co-workers

i.e. : That is

cm : Centimeter

IIHR : Indian Institute of Horticultural Research

ICAR: Indian Council of Agricultural Research

CHES: Central Horticultural Experiment Station

ICAR: Indian Council of Agricultural Research

viz. : Namely

ABSTRACT

The present investigations entitled "**Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region**" was conducted at CHES (IIHR-ICAR), Bhubaneswar in 2019. Studies were conducted during April, May, June and July in two varieties namely Arka Neelachal Vikram and Red Sitaphal. Flowering behaviour was studied on the basis of variation in flowering intensity during April to July and maximum flower production was observed in May followed by April in both the varieties. Pollen viability was assessed by acetocarmine, lugol's solution, safranin and aniline blue stain and among them lugol's solution was found to be better due to high pollen staining capacity. The efficacy of pollen staining dyes was correlated with pollen germinability. Higher pollen viability % implied higher germination % and subsequent higher fruit set per cent in July. Maximum pollen viability was observed in July with 91.01% and 85.32% in Red Sitaphal and Arka Neelachal Vikram, respectively. Pollen germination medium was also optimized with sucrose (10%), boron (400ppm) and calcium (200 ppm). Maximum pollen germination was recorded in July with 64.10% in Red Sitaphal and 54.52% in Arka Neelachal Vikram, whereas it was minimum in April. Pollen production capacity (pollen /flower) was highest in the month of July in Arka Neelachal Vikram (41093.54) and Red Sitaphal (43628.33). Mean stigma receptivity was also highest in the month of July which was 44.50% in Arka Neelachal Vikram and 47.50% in Red Sitaphal. All of the above parameters tested were found to be positively correlated with fruit set. Maximum mean fruit set was obtained in Red Sitaphal in July (8.17%) followed by Arka Neelachal Vikram in July (7.89%). Relative humidity and average rainfall were found to be positively correlated with pollen production, pollen viability, pollen germination, stigma receptivity and fruit set, whereas temperature was negatively correlated.

The present investigation gave an indication that weather parameters play vital role in pollen production, pollen viability, germinability, stigma receptivity and in fruit set of sugar apple (*A.squamosa*) under eastern coastal Odisha condition. Mild temperature with high humidity was conducive for better fruit set in *Annona squamosa*.

CHAPTER-I

INTRODUCTION

INTRODUCTION

Annona is an important genus of tropical fruit belonging to the family Annonaceae. The name "*Annona"* has been derived from the Latin word "anon" meaning "annual harvest" (Lizana and Reginata, 1990). It is a delicious and important minor fruit crop of tropical region of the world. There are approximately 119 species (Guerts F.,1981) under the genus *Annona,* of which 109 species were native to Tropical America and rest 10 to Tropical Africa. Out of those 7 species namely *A. squamosa* L. (sugar apple), *A. atemoya (A. squamosa x A. cherimola), A. reticulata* L. (custard apple), *A. cherimola* Mill. (cherimoya), *A. muricata* L. (soursop), *A. diversifolia* (ilama) and *A. glabra* L. (pond apple) possess high commercial importance. Nakasone and Paull, 1998, stated that chromosome numbers of *Annona* are 2n = 2x = 14 and 16, except for *A. glabra*, which is a tetraploid species (2n = 4x = 28).

Pinto *et al.* 2005, reported that most of the *Annona* species originated in tropical America, while origin of wild soursop (*A. muricata*) was speculated to be in Africa. The Spaniards probably carried seeds from the New World to the Philippines and the Portuguese were assumed to introduce the sugar apple to south India before 1590 (Morton, 1987). Nowadays, it is mainly cultivated in tropical South America, West Indies, Africa, Australia, China, India, Mexico, Southern United States, Phillipines, and Thailand. In India, *A. squamosa* is the most important commercial species of genus *Annona*. However, *A. reticulata* and *A. cherimola* is also cultivated in north India and south India, respectively. In India, the family is represented by 26 genera and about 200 species found mostly in the peninsular region of India. India is considered as secondary center of origin for *A. squamosa* (Anuragi *et al.*, 2016).

Annona squamosa is commonly known as sugar apple and custard apple. In Hindi it is known as Sharifa, in Telugu Sitaphal and Sitaphalam in Tamil (Pandey and Barve, 2011). Sugar apple is cultivated in about 46,000 ha in India mainly in the states of Andhra Pradesh, Maharashtra, Assam, Tamil Nadu and grows wild in Deccan plateau and some parts of central India. (Horticulture statistics at a glance, 2018, MoAF&W,GOI). Custard apple is a good source of

vitamins, dietary fibre, carbohydrate and minerals. It is considered as a good energy source with a value of 104 kcal and is high in protein about 1.89g (Srivastava *et al.*, 2017). Phytochemical investigations on *A. squamosa* revealed its several pharmacological activities such as antibacterial (leaf extract), antidiabetic (root extracts), antioxidant (polar extract), antitumor (seed extract), antimalarial activity and antihelminthic (Chakraverty and Bhattacharya, 2016).

The sugar apple tree is deciduous, hardy tree reaching 3–8m in tall having lateral branches. Lenticels are present on stems with older shoots being smooth and young stems are pubescent. Leaves are dark green colour dorsally and bluish-green ventrally with petioles 0.7-1.5 cm in length. The leaves present oblong lanceolate or lanceolate, 6–17 cm long and 3–5 cm wide with an obtuse or acuminate apex and are alternately arranged on short petioles. Flowers were greenish, fleshy, fragrant, drooping, positionally axillary or terminal and are borne in paired, single or in multi-flowered fascicles either on newer or older branches and can even be seen on the main trunk, i.e., cauliflorous condition) (Kishore *et al.,* 2012). Flowers are present more on leafy shoots than on the older wood and tend to open as shoot elongates. The fruit is round, heart-shaped, 5.5-10 cm in diameter, 6-10 cm in length and weighs 120 to 330 g. The fruit has tuberculate surface and pulp is white in colour with sweet-sour flavour. It contains many black seeds, each 1.5 to 2.0 cm in length and 0.6 to 0.8 cm in width. Seeds are oblong, smooth, shiny, blackish or dark brown are scattered throughout the flesh and are poisonous if chewed. (Pinto *et al.*, 2005; Chen *et al.*, 2011).

Flowering in custard apple begins in March and continues till September. George and Nissen (1988) reported that flowering and fruit set percent were affected by both vegetative flushing of the tree, microclimate and prevalent weather conditions of the place. He further added that even though significant numbers of flowers are produced in a sugar apple plant to obtain a good crop but the main cause is low fruit set percent which result in fewer yields. They only found one to eight percent fruit set in 'African Pride' cultivar of *Annona atemoya* under natural conditions. Kishore et al. (2012) reported that there were two major flowering waves April and September under Indian tropical humid eastern coastal region. Flowering coinciding with summer months were more prone to low fruit set due to poor pollination which has been attributed to both the external and internal

factors, such as very high and low humidity prevailing at the time of flowering, soil moisture stress, competition between vegetative and floral growth, hypogyny, dichogamy, poor pollen germination and death of insect pollinators. High temperature, low humidity and dry wind desiccates pollen and stigma, flower dries up prematurely causing no effective pollination and eventually no fruit. Hence a thorough investigation was made to find out the productive and unproductive phases in *Annona squamosa*, its flowering behaviour, reproductive biology and how weather parameters are correlated with reproductive biology. The knowledge of flowering behaviour and reproductive biology is used in in artificial pollination and in breeding experiments; is also important in the understanding of sterility problems, fruit hybridization & breeding programs, and evolutionary ecology.

In our experiment for reproductive biology, pollen viability tests has been performed with several pollen viability testing chemicals of which the most reliable testing chemical was chosen for further investigation. Stigma receptivity of any stigma is evaluated by performing a stigma receptivity test that shows catalytic activity of the stigmatic surface with the use of hydrogen peroxide (H_2O_2) under Dafni methodology (1998). For evaluating best germination medium for *Annona squamosa*, boric acid, calcium chloride, potassium nitrate and sugar were evaluated to optimize the pollen germination medium. Flowering behaviour, reproductive biology and fruit set of *Annona* in relation to weather parameters has been poorly studied and work on this aspect is very scanty. Therefore, present investigation on "Studies on flowering behavior, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region" was planned and executed at Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar, Odisha during the year 2019-20 with the following objectives-

c) Objective of study

1. To study flowering behaviour of sugar apple.

2. Optimization of pollen viability and pollen germination medium.

3. To study pollen biology, stigma receptivity and fruit set in sugar apple.

4. To correlate weather parameters with reproductive biology.

CHAPTER-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Custard apple (*Annona squamosa* L.) is grown different parts of India except in temperate regions. It continues to gain importance as a valuable commercial crop in watershed areas, drought prone areas and arid regions. It can be grown under wide range of soils and diverse agro-climatic conditions. Because of its unique taste, luscious flavor and medicinal properties cultivation of this crop is increasing during the last decade. With the increasing demand for this crop, more and more quality planting materials of elite varieties are required by *Annona* farmers. Crop improvement by adopting suitable breeding methods can help achieve that necessary objective. But for breeding to be successful it is necessary to be thoroughly acquainted with the floral biology of this crop.

Annona squamosa was investigated for fruit set in Odisha where it is ranged between 0.75 to 3.33 percent (Sahoo *et al.*, 2000). In order to understand the causes of poor fruit set this research paper has been formulated. Poor pollen viability due to erratic climatic condition is a major setback in getting higher fruit set and thereby productivity which eventually restrain the expansion of *Annona* cultivation. Therefore, we can focus our approach in increasing pollen viability and resulting fruit set per cent that will help maximise the fruit yield and overcome this problem in Sitaphal. Flowering behaviour, reproductive biology and fruit set of *Annona* in relation to weather parameter has been poorly studied and there are very few works available on this aspect**.** After investigating several other researches and literature on custard apple, the relevant parts have been carefully reviewed and presented below in the following subheadings**.**

2.1. Flowering Behaviour

Flower buds appear along with vegetative flushes on the current shoots. Percentage of flower buds appearing on older woods is negligible. The flower buds were extra-axillary, opposite to the leaves in all *Annona* species as observed by Thakur and Singh (1965). Similar finding has also been reported in *Annona squamosa* by Nalwadi *et al.* (1975).

Whereas Kishore *et al.* (2012) reported that flowering in *A. squamosa* occurred in different flushes but the major bloom periods were April and

September under tropical humid eastern coastal regions of India. Flowers were either axillary or terminal in position. Flowers were borne singly, paired or in multiflowered fascicles either on old branches, newer branches or on the main trunk (cauliflorous). He further added that the most important functional character of pendulous flower of *A. squamosa* was floral chamber formed by three pale coloured perianths. These perianths open marginally during pistillate phase (female) and it expands during staminate (male) phase. Dehiscence (longitudinal splitting) of anthers takes place in the morning hours during male phase releasing medium size spherical pollens along with suture. The bisexual flowers exhibit protogynous dichogamy and are entomophilous, hand pollination is better in achieving higher fruit set than allowing natural insect pollination. It is seen that stigma receptivity starts at anthesis.

Shroeder (1951) and Richardson and Anderson (1996) reported that since flowers of *Annona* have many pistils (to form aggregate fruit), so these pistils are required to be fertilized with viable pollen under conducive environment to increase fruit quantitative and qualitative attributes. Cautin and Agusti (2005) opined that flowers of *Annona* species are hermaphroditic and there are six different stages in flowering phase : (1) flower opening as pre-female stage; (2) flowers partially open as female stage; (3) about 30% of flowers partial open, female stage; (4) full flowering that is at least 50% of flowers open completely as male stage; (5) flowers fading; and (6) end of flowering.

Hayes (1957) reported that the season and duration of flowering varies with different species of *Annona*. Flowering commenced from last week of March in *A. cherimola, A. glabra, A. atemoya* and *A. squamosa* whereas in case of *Annona reticulata* it was last week of April. *Annona squamosa* (green and red types) and *Annona glabra* reached at its peak flowering during second week of April. Whereas in *Annona atemoya* and *A. cherimola* its peak flowering was reached during first week of May and in *A. reticulata* during the first week of June. These observations were recorded under Delhi condition by Thakur and Singh (1965).The flowering was ceased in last week of August in *Annona squamosa* (green and red type), *Annona atemoya and Annona cherimola*. However, flowering was ceased in the first week of June and middle of November in case of *Annona glabra* and *Annona reticulata* respectively as reported by Thakur and Singh (1965). But

according to Nalwadi *et al.,* (1975) *A. squamosa* (green type) flowers from mid-April to mid-July with peak during May under Dharwar (Karnataka) condition

Kumar and Singh (1977) reported that in *Annona squamosa* var. Sahebganj flowering period is from March to August with peak flowering during April to May under Sabour condition. It was seen that both *A. reticulata* and *A. squamosa* flowered and new leaf flushing occurred simultaneously. Whereas in *A. muricata* flowering and flushing of leaves occurred throughout the year while in *A. senegalensis* and *A. reticulata* only flowering occurs throughout the year (Azeez and Folorunso, 2014).

Azeez and Folorunso (2014) stated that the peak flowering period for most of the species (*Annona senegalensis, Annona muricata, Annona squamosa, Cleistopholis patens, Monodora tenuifolia* and *A. reticulata)* was between March to April while in *Xylopia aethiopica* maximum flowering was observed in July. They observed that the leaf flushing and flowering occurred at the same time in *A. reticulata* and *A. squamosa* though the rate of flower and leaf production was not high.

2.2. Reproductive Development

Thakur and Singh (1965) reported that different species of *Annona* took about 27 to 35 days for complete bud development (i.e., from bud development to anthesis) and pass through. But Nalwadi *et al.,* (1975) recorded an average of 27 to 34 days (30.8 days) are required by *Annona squamosa* flowers from visual initiation of flower bud to complete blooming. Whereas Farooqi (1970) reported a total period of 45 days is required from flower bud initiation to anthesis. But Kshirsagar *et al.* (1976) recorded 31 days for complete bud development in *Annona atemoya*.

According to Thakur and Singh (1965), the anthesis in different species of *Annona* continues throughout the day with their peak period in the morning and evening time, specifically between 5.30 to 8.30 am in *Annona squamosa* (green and red type) and between 2.30 to 5.30 pm in *A. glabra* and *A. reticulata* .But in *A. cherimola,* two peak period of anthesis were observed i.e., between 5.30 to 8.30am 2.30 to 5.30 pm. Similarly in *A. atemoya* two peak period were observed (i.e.,

between 11.30 to 2.30 pm and 2.30 to 5.30 pm, under Delhi condition. In Dharwar (Karnataka) condition, the anthesis in *A. squamosa* commenced early in the morning at 6 am and continued till 6 pm with maximum at 6 am and minimum at 6 pm. Beyond 6 pm there was no anthesis as reported by Nalwadi *et al.,* 1975.

Kumar and Singh (1977) reported that maximum anthesis was observed between 17.30 hrs and 5.30 hrs in var. Sahebganj of *Annona squamosa* under Sabour condition. Kshirsagar *et al.* (1976) reported that maximum anthesis of 62 to 82% buds occurred between 5 to 8 am in *Annona atemoya*. Ahmed *et al*., (1935) reported that in *Annona* matured anthers are positively hygroscopic hence higher humidity favours anther dehiscence. *Annona cherimola* and *Annona squamosa* shed pollen in the afternoon hours from 3.30 to 6 pm (Wester, 1910). On the other hand, Venkatratnam (1959) reported that in *Annona squamosa* dehiscence took place between 4 am to 8 am. While in *Annona cherimola* and *Annona reticulata* it was from 4 am to 8 am and, in *Annona glabra* from 12 midnight to 4 am under Hyderabad condition.

Thakur *et al*. (1965) reported that in *Annona atemoya*, *A. cherimola, A. reticulata,* and *A.squamosa* (red and green type), anther dehiscence took place throughout the day but maximum was between 11.30 to 2.30 pm in all the species. In *Annona glabra,* anther dehiscence was between 4 am to 5am and at no other time of the day under Delhi condition.

Nalwadi *et al.,* (1975) reported that anther dehiscence took place between 12 midnight and 4am with peak anthesis at 2 am in *Annona squamosa* under Dharwar condition (Maharashtra). Kshirsagar *et al.,* (1976) reported that maximum anther dehiscence took place between 12 noon to 2 pm in *A. atemoya.* Kumar & Singh, (1977) reported that maximum anther dehiscence between 11.30 hrs to 14.30 hrs in *A. squamosa* under Sabour condition.

2.3. Pollen Biology

The pollen in all *Annona* species is compound. A compound pollen grain has four individual pollens closely packed within a thin covering. These pollen grains possess a thick exine without germpore. The average size of the individual pollen grain of the diploid species was found to be ranging between 36.5 µ to 45.8

µ (i.e., in *A. squamosa, A. cherimola, A. reticulata and A. atemoya*) whereas it was 94.8µ in *A. glabra*, a tetraploid species. The larger pollen size of the latter is owing to its higher ploidy status as reported by Thakur and Singh, (1965).

Nalwadi *et al.,* (1977) reported that shape of the dry pollen of *A. squamosa* was round to oval with an average length and breadth of 54.3 µ and 45.0µ respectively. Pollen viability is the ability of a pollen grain to germinate and develop as a pollen tube (Gerard, 1932).The growth of the pollen tube can be taken as the measure of pollen viability since the non-viable pollen could not make the growth of a pollen tube. We cannot guarantee a good fruit set unless the pollen used is viable with a high germination percentage.

The need for assessing viability of pollen used in artificial pollination and in breeding experiments is also important in the understanding of sterility problems and hybridisation programs, fruit breeding programs and evolutionary ecology (Rodríguez-Riaño & Dafni, Amots. (1988). Shivanna and Rangaswamy, 1992 opined that flower phonological changes due environmental impact and subsequent genotypic differences contribute to the variable pollen viability rate. Thakur and Singh (1965) observed that the percentage of fertile pollen as per acetocarmine test was found to range from 55.9% in *A. cherimola.* Nalwadi *et al.,* (1975) reported that 89.4% pollen were fertile and 10.6 % were sterile by acetocarmine test is *A. squamosa*.

Farooqi *et al.,* (1970) also observed 86.4% fertile and 13.6 % sterile pollen *Annona reticulata*. Loguercio (2002) reported that pollen bursting occurs due to increment in osmotic pressure and0low cell0wall resistance which causes rapid diffusion of water into the pollen grains and imbibition damage after significant loss of ions and soluble substances from cytoplasm. This improper rehydration causes the dehydration and disorganization of pollen membrane & metabolism which eventually lowers the pollen viability % (Wolters-Arts, 2002).

Kishore et al., (2012) in his studies investigated that *A. squamosa* produces numerous pollen grains and ovules and has moderate high pollen/ovule ratio. This moderately high pollen/ovule ratio is due to poor pollen delivery mechanism due to small stigmatic surface area and pollen wastage due to inefficient pollen carrier insects. (Kishore *et al.,* 2012).

2.4. Pollen germination

In vitro pollen germination in an improved culture medium is a technique that helps enhance the conditions of the style-stigma thus inducing germination and increase pollen tube growth. A specific protocol of culture medium is required to obtain adequate pollen germination for every species. After several researches on this it has been found that the culture medium should contain carbohydrates, germination-stimulating substances, such as calcium nitrate, magnesium sulphate & boric acid (Imani *et al., 2011*).

Bita and Gerats (2013) found that heat stress alters the carbohydrate and protein levels in pollen as well as the pent-up toxic by-products such as reactive oxygen species which result in sterility of pollen. This disturbs vegetative and reproductive development of plant and negatively affects the fruit set % and fruit yield. According to Acar and Kakani (2010) pollen grains which show pollen tube length greater than or equal to the diameter of the pollen grain were considered germinated.

In Japanese pear 'Kousui' pollen germination was strongly inhibited by fructose in agar medium while sucrose stimulated pollen germination per cent and pollen tube growth of pear. With this Okusaka and Hiratsuka (2009) suggested that fructose might have impeded a physiological trigger of germination and once this trigger is hindered, many physiological pathways including sugar biosynthesis are blocked, resulting in germination failure.

In vitro germination percentages of *Annona cherimola* ranged between 19% (Bettiol Neto *et al.*,2009) to 37% (Rosell *et al.*, 1999) when fresh pollen grains were used. Nietsche *et al.,* (2009) observed that the average germination was 50.2% with no significant differences in sugar apple pollen germination were observed when pollens were collected between 12:00 am and 7:00 am. Bettiol Neto *et al.,* (2009) explained that there are significant differences in germination percentages of sugar apple pollen collected during dry & humid seasons about 25.0% and 37.6%, respectively.

Rosell *et al.,* (1999) reported that the optimum temperature for *in vitro* pollen germination of cherimoya was 20℃–25°C, and pollen required a medium with provision of Ca, Boron, as well as Sucrose at 5–10%. Mendes et al., (2012) in

his investigation on sugar apple's pollen viability found that in seeded and seedless accessions of sugar apple the viability percentages ranged from 38.5 and 52.5%, respectively.

The average pollen germination rate of atemoya cultivar 'Gefner' and sugar apple cultivar 'Brazilian Seedless' was 52.5% and 5.9% respectively. Nietsche *et al.*, 2009 reported average pollen germination in sugar apple accession ranged from 46.75% to 53.62% whilst on the contrary Bettiol Neto *et al.*, (2009) observed germination rates of 19.70% and 26.24% respectively for pollen grains from 'Gefner' and sugar apple flowers.

Saavedra (1977) presented that *Annona* pollen had a good proportion of tetrads and dyads intermixed among individual viable, and non-viable pollen grains and he found that the tetrads were unable to germinate on stigmatic surface. He concluded that the inter-specific constitution was the main cause for high concentration of tetrads in atemoya.

Judd (2007) found that pollen viability is mostly affected by temperature and moisture variations and can be assessed by presence of cytoplasm, enzyme activity and testing germination. Mendes *et al.*, (2012) observed that optimum range of pollen germination and also to obtain good fruit set in the orchard is 20-25°C. Pollen requires pre-hydration treatment before *in vitro* germination along with suitable germination medium having calcium, boron and sucrose at 5 to 10%. Temperature is an inevitable factor that represents the environmental conditions along with other factors which significantly affect pollen grain germination (Pio, 2003). According to Worsley, (1959) *Pinus poderosa* start of germination in eight days at 3ºC, while at 15º C in two days and at 30ºC germination commenced in six hours. However, Dorman (1976) observed that the temperature range of 25°C-26ºC was more pronounced for pollen germination. Boden (1958) opined that the temperature range of 25ºC-30ºC was suitable for pollen germination of *Eucalyptus* species.

Silva (1996) found that for best temperature for passion fruit pollen germination was at 28 ºC. Ruggiero *et al.*, (1975) propounded that when the yellow passion fruit pollen grains were kept at 24 and 25ºC in the artificial medium for 30 minutes, there was initiation of germination observed and no pollen

tube formation was seen after 6 hours. Vasilakakis and Porling (1985) stated that below 15°C pollen germination was reduced but pollen tube growth increased in the temperature range of 5 to 25°C when assessed in a pear tree (*Pyrus communis*). The optimum temperature for pollen grain germination of cherimoya is 20-25°C (Rosell *et al.,* 1999) whereas for kiwi fruit the ideal temperature was 27°C (Boden, 1958). Tunistra and Wendel found that optimum temperature range for pollen germination in sorghum was 20-40°C but germination reduced below optimum at 10°C.

Prasad *et al.,* (2011) reported that pollens are collected at a suitable maturation stage in order to maintain the viability and capacity to germinate when the hybridization is performed. In several species, it is found that the highest and lowest % of pollen germination occurs during anthesis and post-anthesis respectively. This has been further substantiated with a fact that samples collected before the natural flower opening has immature pollens which eventually gives low germination percentage.

Kumari et al., (2009) observed that the pollen collection time affects the germination percentage. The best results were obtained when germination was executed at anthesis time, and minimal germination when observed 20 hr after anthesis. Luza and Polito, (1985) during their study on English walnuts observed that pollen grains collected at 1-2 days before anthesis showed 0.60% germination but gave 45.2% pollen germination in flowers collected after anthesis.

2.5. Stigma receptivity

Stigma receptivity refers to the ability of the stigma to support germination of viable, compatible pollen. According to Sanzol and Herrero, (2001), effective pollination period and fruit set is affected by three reproductive processes: stigma receptivity, pollen tube kinetics and ovule longevity. It is an important factor limiting the EPP and fruit set in kiwifruit, apricot, pear and cherry. A short life span of ovules is limiting to EPP in sweet and sour cherries and apricot (Yi *et al.,* 2006).

Thakur and Singh, (1965) observed that the stigmas were receptive a day before the anthesis. The receptivity was highest at the time of anthesis and then decreased abruptly. By at the time of anther dehiscence, the stigmas in all diploid

species turn almost non-receptive. However in *A. glabra* the receptivity was retained for a longer time and 37 to 50 percent of the stigmas were receptive at anther dehiscence stage. Farooqi *et al.,* (1970) reported that in *Annona reticulata,* the stigmas were receptive a day prior to anthesis with most receptive condition on the day of anthesis and later it declined to non-receptive condition after five days of anthesis.

Nalwadi *et al.,* (1975) observed that in *A. squamosa* the stigma was found to be receptive from a day prior to opening of flower to two days after opening of the flower. The percentage of receptive stigmas was 30% one day prior to opening of flower. From third day onwards after opening of flower the stigma was non-receptive in *Annona squamosa.*

Kishore et al., (2012) reported that stigma receptivity when evaluated on the basis of fruit set % showed significant temporal difference which starts at anthesis and remain receptive for 24 h. Using hydrogen peroxide, stigma receptivity can be assessed; if peroxidase enzyme is present on stigmatic surface it releases oxygen bubbles on reacting peroxide. More the receptivity of stigma more are the bubbles formed (Galen *et al.,* 1985). Jawale *et al.* (1990) observed stigma receptivity in garden rose and found that on the first day of flower opening stigma receptivity was very low but on the 4^{th} and 6^{th} day after flower opening it was found to be maximum. Jawale *et al.,* (1999) found that stigma receptivity in sunflower lasts for 22-72 hours.

Singh and Srivastava, (2000) reported that the receptivity of stigma was 19.99% one day before anthesis, 34.99% on the day of anthesis and 12.49% one day after anthesis when assessed in quince fruit. In onion, stigma remain receptive for six days after anthesis (Harikarunakar and Haripriya, 2000) whereas in pomegranate, stigma receptivity varied from 48-144 hours after anthesis.

2.6. Fruit set

Fruit set percent is an important component that determines profitability of fruit crop. The fruit set in fruit crops are mostly limited by three reproductive processes: stigma receptivity, pollen tube kinetics and ovule longevity (Sanzol and

Herrero, 2001). Various physiological, biochemical, genetic and weather factors does influence fruit set percent considerably.

Thakur and Singh (1965) reported that hand pollination resulted in higher fruit set % than natural pollination which ranged between 44.4 % to 60 % among different species, whereas fruit set was below 6 percent in open pollination and self-pollination. Lack of suitable pollinating agents, protogyny, hypogyny, short receptivity, delayed dehiscence after anthesis and low pollen germination appeared to be the causes of low fruit set under natural conditions.

Bautista (1975) in his study of 6 improved clones of *Annona cherimola* stated that higher percentage of fruit set was achieved by self-pollination and artificial crossing than by open pollination. Kshirsagar (1976) reported that hand pollination in atemoya resulted in 70 percent fruit set. Kumar and Singh (1977) observed that low fruit set of about 8 percent occurred naturally in var. sahebganj of *Annona squamosa.*

Cherimoya flowers were pollinated in the evening hours immediately after pollen collection was found to be more effective than pollinating newly opened overnight stored flowers whereas stigma was receptive equally over the two days. This suggests that pollen viability is the greatest setback to successful cherimoya pollination in a humid climate (Richardson and Anderson, 1996).

Peculiarities in sugar apple's floral anatomy such as perfect flowers and protogynous dichogamy (Kumar *et al.*, 1977) together with the expansion of the *Annona* crop to areas where natural pollinators are not found result in a need for artificial pollination in commercial fruit production (Kishore *et al.*, 2012).

Pollinating the flowers between 09:00 and 12:00 h on the day of anthesis resulted in high stigma receptivity and high percentage of fruit set. Kishore et al., (2012) observed that floral visitors mainly visited the floral chamber of day old flower which contained dehisced anther, stigma secretions and fruity odors. Floral chamber had no nectar production (Kishore *et al.*, 2012). Gazit *et al.*,(1982) stated that *Annona squamosa* flowers are hermaphrodite and protogynous. The female reproductive structure matures first and stigma remains receptive for 24 hours. It is seen that *Annona* species can bear flowers on both current and old season's

growth and rarely any reproductive growth is seen on older woods. Flowering period of custard apple is very long starting from March-April and continue up to July-August. April-May is considered as the peak flowering period but negligible fruit setting occurs during the entire spring and summer season. Plant start bearing fruit only in the rainy season.

2.7. Influence of weather parameters on flowering and fruit set

Thakur and Singh (1965) observed that the fruit set % was low in custard apple due to poor pollination which is mainly due to both the extrinsic and intrinsic factors such as soil moisture stress, very high and low humidity occurring at flowering time, competition between floral & vegetative growth, dichogamy, hypogyny, poor pollen germination% and the death of insect pollinators. Pollen grains were sterile and shrivelled during May-June when relative humidity was very low and temperature was very high.

Kumar *et al.,* (1977) reported that maximum flowering in custard apple was observed in summer season in Northern India when temperature is as high as 40°C whereas humidity is very low combined with desiccating winds & dry soil condition. Under such circumstances pollen production and fruit set does not occur. They observed that maximum pollen germination was observed under 20% sucrose-agar concentration. Loss of stigma receptivity was seen from the time of dehiscence and completely lost after 6 hours which resulted in poor fruit set %.

Hopping (1983) insinuated that low relative humidity and high temperature deteriorate pollen viability. Therefore, fruit set occurs in rainy season on reversal of environmental conditions. Early fruit set in *Annona squamosa* occurs in South India where temperature is high and relative humidity is lower than in North India.

George and Nissen, (1987) propounded that the temperature above 32°C was more conducive to vegetative flushing that increased competition between vegetative growth & development of fruitlets which resulted in reduced fruit set % in custard apple. Flowering and fruit set % were affected by environmental conditions during vegetative and flowering phases of the tree. It was observed that high temperature of 28°C and soil moisture stress of -2.0 MPa reduced flowering and fruit set %. They found that under high VPD condition if we hand pollinate the

flowers fruit set % significantly increases (>20%). Several cultural practices can be adopted to maximize the productivity of custard apple such as overhead misting, wind breaks, and efficient irrigation scheduling. He insinuated that protogynous dichogamy in atemoya is the limiting factor for fruit set %.

Sanewski, (1988) propounded that favourable temperature for flowering (October to February) are 25°C and 28°C during flowering (October to February) , these temperatures are also favourable for good fruit set. Shivanna and Cresti (1989) have studied on the effects of high temperature and humidity on pollen membrane constitution and integrity and also pollen vigour in *Nicotiana tabacum*. Different methods have been applied to assess pollen germination *in vitro* by Shivanna and Heslop-Harrison,(1981) and also *in vivo.*

George *et al.*, (1995) reported that suitable conditions for high fruit set are high relative humidity, moderate temperatures, lack of tree water stress and there should not be strong wind or continuously heavy rain. If relative humidity dips below 70 per cent, fruit set and fruit shape are critically affected. Higuchi *et al.*, (1998) revealed that high temperature lowers fruit set percentage in custard apple. However he further obtained highest percentage fruit set in the 'cool ± cool' flower pollination treatment (10 out of 10) and the lowest (3 out of 11) was observed in the 'warm ± warm' pollination treatment in custard apple. Warm temperature adversely affected pollen germination percentage and it came into light that pollen germination period is less heat sensitive than pollen development period.

Rosell *et al.*, (1999) propounded that temperature has a significant effect on pollen germination of cherimoya (*Annona cherimola* Mill.) the optimum temperature range between 20°C to 30°C. They also insinuated that these temperature agree with Sanewski (1985), who this temperature range to be the most adequate for fruit development in the orchard. Temperatures below 15°C and over 30°C reduced pollen germination and abnormal pollen tubes developed. Bita and Gerats, (2013) insinuated that at the metabolic level, heat stress is known to alter the protein and carbohydrate level in pollen and the build-up of toxic by-products such as reactive oxygen species (ROS), which ultimately lead to sterility

of pollens and affect vegetative and reproductive development of plants, with negative afffect on fruit set and yield.

It has been observed that high temperature (Hedhly, 2011), low nutrient availability (Poulton *et al.*, 2002), water stress (Saini, 1977) during the development of reproductive organs decreases viability of pollens. As tolerance to such abiotic factors varies with species so slight increase in temperature during growth and development of reproductive tissues may accelerate or delay flowering (Matsuda and Higuchi, 2012). Herrero, (2013) insinuated that the abiotic factors may also affect synchrony in the development of female and male reproductive systems and cause defect in the gametophytes (Lora *et al.*, 2014). These ultimately affect fruit set percent of that plant. According to Pereira et al., (2014) high temperature may affect the morphology, metabolism and chemical composition of pollen grains that affect pollen productivity and performance which ultimately influences seed production and fruiting.

CHAPTER-III

MATERIALS AND METHODS

MATERIALS AND METHODS

For crop improvement of *Annona squamosa*, it is essential to know about its floral biology and to investigate the relation of weather parameters with low fruit set. Accordingly this study was undertaken to know the flowering behaviour, flower length, pollen production, pollen viability, pollen germination, stigma receptivity, fruit set and then it was correlated with weather parameters.

3.1 Experimental site:

The present investigation entitled "Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region" was carried out at Central Horticultural Experiment Station, (ICAR-IIHR), Bhubaneswar during 2019. The location lies at 20°15 N latitude, 85°15 E longitude at 42 m above mean sea level.

3.2 Climate and weather conditions:

Geographically, the experimental site in Bhubaneswar falls under the 18th agro-climatic region of the country i.e., eastern coastal plain. Bhubaneswar has a tropical climate with hot summer and brief mild winter. It is positioned at about 60 kms away from Bay of Bengal. The average rainfall of Bhubaneswar is 1679.7 mm, of which approximately 85% is received during July to October. The Rainfall code of the place is D_1E_3 ($B_1A_2B_1$) $C_1D_1E_2$. The average temperature varies from 27.65 to 31.95°C and relative humidity varies between 73% and 91% from March to October (Table 3.2).

3.3 Experimental details

Name of the crop	: Sugar apple (*Annona squamosa* L.)
Year of study	: 2019
Age of Plant	: 5 years
Experimental Design	: Factorial randomized block design
No. of variety	: 2 (Arka Neelachal Vikram and Red Sitaphal)
Months	: 4 (April, May, June, July)

Number of treatments : 8

Number of replications : 4

Number of plants/replication : 2

Treatment details:

Table 3.1 Treatment Table

Variety	Month of study	Treatment	Combinations
Arka Neelachal Vikram V1	April (M1)	T1	V1M1
	May (M2)	T2	V1M2
	June (M3)	T3	V1M3
	July (M4)	T4	V1M4
Red Sitaphal V2	April (M1)	T5	V2M1
	May (M2)	T6	V2M2
	June (M3)	T7	V2M3
	July (M4)	T8	V2M4

3.4 Materials used in the study

1. Glassware- Different glassware, i.e., beaker, glass bottles with conical flasks, glass slides, cover slips, etc. and plastic wares such as micropipette, polyvials, scale for measuring flower length, etc.
2. Chemicals- Chemicals used were glycerine, distilled water, acetocarmine solution, lugol's solution, safranin solution, aniline blue solution, sucrose, agar, boric acid, calcium chloride of analytical reagent grade.
3. Cleaning of glasswares- Glasswares were cleaned with detergent and thoroughly washed with running tap water and once with distilled water.
4. Microscope- Leica compound microscope was used pollen viability and pollen production studies whereas for pollen germination, visualizing flower parts and stigma receptivity was seen through stereo-zoom Leica (30X) microscope

Table 3.2 Meteorological observations

Month	Max temp. (°C)	Min temp. (°C)	Average temp. (°C)	Relative humidity %	Rainfall (mm)
March	35.61	22.97	29.29	75.00	2.00
April	37.97	24.97	31.47	73.00	24.60
May	37.03	26.87	31.95	77.00	55.00
June	35.17	25.43	30.30	82.00	160.00
July	32.71	25.68	29.19	88.00	348.00
August	32.00	25.48	28.74	90.00	299.00
September	31.80	24.90	28.35	91.00	467.00
October	32.06	23.23	27.65	87.00	324.10

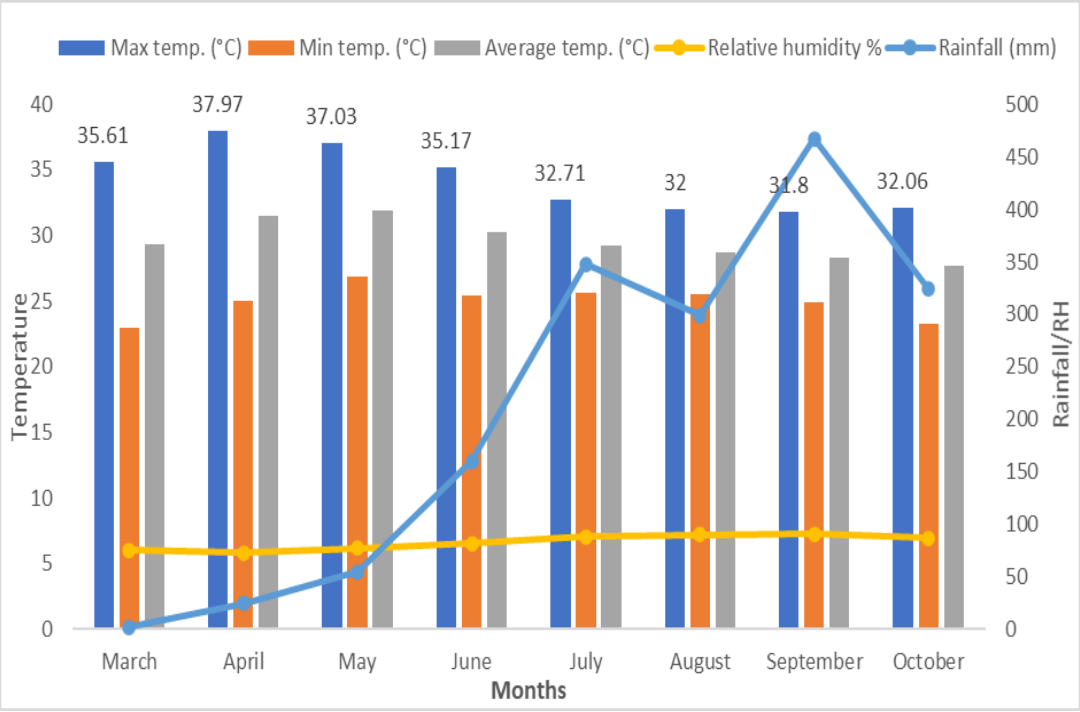


Figure 3.1 Metereological observations recorded in 2019

5. Digital weighing balance- The chemicals are weighed using a digital weighing balance.
6. Hemocytometer- It is used for counting number of pollen produced and also to count stained pollen.
7. Aluminum tags- tags were used to tag the branches that were going to be analysed for flower production and subsequent fruit set.
8. Marker pens- for labeling.

3.5 Methods followed in the study-

3.5.1 Selection of trees

Healthy, vigorous, disease free trees with uniform growth under similar management condition by adopting the package of practices as recommended by the CHES were utilized for this study. Out of which four trees from Arka Neelachal Vikram and Red Sitaphal were selected for the study. Each tree served as one replication.

3.5.2 Selection of branches

Five laterals from each tree were randomly selected from the peripheral region of the tree. The new shoots appearing on these- lateral branches were labeled for recording observations on pollen viability, pollen germination, pollen production, stigma receptivity and fruit set.

3.5.3 Recording of the observations

The observations were recorded from April 2019 to July 2019.

3.5.4 Flower bud development

Observations were recorded monthly on buds in the selected branches to know various stages pf development of flower bud. In each stage the length of the flower bud was measured with a centimeter scale. The measurement of length of bud was made from base of calyx to the apex then the average values of length of flower buds were recorded. Detailed flower parts were observed under Leica stereo zoom microscope at 10X magnification.

3.6 Pollen production

Pollen were counted from 10 flowers taken from both the varieties with eight treatments (each treatment taken as one month) and four replications each. Pollen were taken in a microcentrifuge tube mixed with glycerine and Lugol's solution in the ratio 1:2:1 and then shaken well. This pollen solution was loaded in hemocytometer glass slide using capillary action then put coverslip over it. This was observed under optical compound microscope (Leica). Pollen produced on the anthers of flower was quantified by hemocytometric method as described by Kearns and Inouye (1993). The number of pollen in each line traverse of hemocyometer was counted using an optical compound microscope (Leica with objective magnifications 10×; eyepiece magnification 10X). If the number of counted pollen (in any one of nine squares) is more than 100 dilution is required for which dilution factor is taken into consideration. The grid lines of the counting graticule fitted the field of the microscope and allowed the majority of the total pollen load to be counted and finally the pollen production per flower was calculated using the formula:

$$N = \frac{a \times v \times 10^4}{n}$$

where N is the number of pollen/flower; a is the mean number of pollen counted/corner square; v is the volume of suspension made with pollen; and n is the number of anther/flower.

3.7 Stigma receptivity

Stigma receptivity was evaluated in emasculated flowers (N=55) using 2% hydrogen peroxide test (Dafni and Motte, 1998). The flowers buds at the time of anthesis were collected from two listed cultivars and pistils were emasculated from flowers at 8:00 am. The collected pistils were immersed for 2-3 min at 28°C and a pistil was regarded as receptive when more than 75% of the stigmatic area were covered with bubbles and percentage of bubbles formed were recorded. Each treatment was carried out with 55 pistils in four replicates. Stigma receptivity was evaluated by percentage of bubble formation which was based on visual observation in Leica stereo-zoom microscope. Stigma receptivity was calculated by the ratio of number of stigma receptive to the total number of stigma observed

(N=55) in each experimental month. The stigma receptivity was known by observing the number of oxygen bubbles formed from floral stigmatic surface. More receptive stigma corresponds to more number of bubbles formed which depends on peroxidase reaction activity. To obtain reliable results, damaged stigmas or those with pollen on the surface were not used, avoiding obtaining false positive results. The receptivity results were submitted to ANOVA 5% probability with the help of the OPSTAT statistical analysis tool (O.P. Sheoran, HAU).

$$\text{Stigma receptivity \%} = \frac{\text{Number of receptive stigma per flower}}{\text{Total number of stigma under observation}} \times 100$$

3.8 Pollen viability

Pollen staining was optimized by staining with four different stains: 2 % Acetocarmine, Aniline Blue, Lugol's solution and Safranin. Five flowers were collected between 7 and 8 A.M. Anthers were carefully removed from flowers in a petridish and pollen were collected from them. Four microscopic slides taken for each staining material, one drop of staining material and one drop of glycerine was put on each microscopic slide. Then using aneedle transfer pollen from petridish to microscopic slide. A coverslip was placed over the stain without leaving bubbles and left for 5-10 minutes for proper staing of pollen grains. Slides were observed under optical compound microscope (Leica) under eyepiece 10X and objective 15X magnification).

- 2% Acetocarmine stained viable pollen red while sterile (mostly shrivelled) pollen remain colourless. Acetocarmine stains chromatic material in nucleus and cytoplasm in live pollen (R. G. Stanley and H. F. Linskens,1974).

- Lugol's solution (IKI_2) stained viable pollen dark brown to black whereas non-viable pollen remain colourless. Lugol's solution stains starch present in pollen (Machado,1987).

- Aniline Blue stained viable pollen deep blue color, while dead pollen remains unstained. Aniline Blue detects callose in pollen grain walls and pollen tubes. (Hauser and Morrison, 1964).

- Safranin stains chromosomes, nuclei, and lignified, suberized or cutinized cell walls. The viable pollen stained brilliant red, while dead pollen remains unstained. (Ruzin SE ,1999)

$$\text{Pollen viability (\%)} = \frac{\text{Total number of stained (viable) pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

Similarly pollen viability percent were counted in every month of the study period (April, May, June, July) for both the varieties (eight treatments) and for each treatment, four replications were made to reduce error in finding pollen viability percentage.

3.9 Pollen germination

3.9.1 Germination medium standardization and pollen germination in different months

Pollen germination *in vitro* is a reliable method to test the pollen viability. To standardize pollen germination medium for *Annona squamosa* L. initially a set of media were used. The complete medium for both the varieties was then standardized by altering the concentration of sucrose, boric acid and/or calcium chloride one by one to obtain maximum pollen germination and good pollen tube growth. Prepare 1% agar solution by dissolving 1 g of agar powder in 100ml warm distilled water heated on a hot plate. Allow the solution to boil until the agar is completely dissolved. Pour 2 ml of calcium chloride (200ppm) and 4 ml of boric acid (400ppm) as per required concentration for treatments taken, and then allow them to cool to 50°C and pour into labeled petridishes. Cover the dishes with their moist paper covered lids then place them in a dark and humid place. Similarly make replications for each treatment taken. After 6hours, visualize them in compound microscope from lower magnification to higher magnification. Note down the number of pollen observed to the number of pollen germinated obtained from the magnified field of study. Take similar observations in different months of the study period and note down the results.

A pollen grain was considered germinated when equal to or greater than the grain diameter (Kakani *et al.*, 2005). Germination percentage (%) was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field of view. Pollens were counted as germinated if they had pollen tube greater than half of the diameter of pollen grains.

$$\text{Pollen Germination (\%)} = \frac{\text{Total number of pollen grains germinated}}{\text{Total number of pollen grains observed}} \times 100$$

Similarly pollen germination percent were counted in every month of the study period (April, May, June, July) for both the varieties (eight treatments) and for each treatment, four replications were made to reduce error in finding pollen viability percentage.

3.10 Fruit set percentage

After some weeks of petal fall, fruit set was recorded in each mode of pollination. The fruit set percentage was evaluated out by counting total number of fruits developed and dividing it with total number of flowers developed and then covert it to percentage.

$$\text{Fruit set (\%)} = \frac{\text{Number of fruits developed}}{\text{Total number of flowers observed}} \times 100$$

3.11 Statistical Analysis

Data on various parameters were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to test statistical differences between means ($P < 0.05$) using OPSTAT statistical package. Correlations among various parameters were also worked out.

CHAPTER-IV

RESULTS

RESULTS

The experimental results obtained in the present investigation entitled "Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region" are presented and discussed under suitable heads. Observations recorded from the study of flowering behaviour, pollen biology, stigma receptivity and the role of weather parameters in influencing the fruit set in *Annona squamosa* L. have been presented by graphs and diagrams. The data of the final observation of the various characters were subjected to statistical analysis and have been given under suitable headings. During the course of investigations, the detail findings of the experiment are presented in this chapter. The data are presented in tabular form with a mean and critical difference (CD) at 5 % level of significance.

4.1 Flowering behaviour

The data on mean number of flowers produced in different months of the flowering season are presented in table 4.1. The data revealed that Red Sitaphal variety produced highest number of flowers per tree than Arka Neelachal Vikram in every month of the study period. The mean number of flowers produced in different months of the flowering season varied from 230.25 in April to 145.75 in July in Arka Neelachal Vikram whereas in Red Sitaphal variety it ranged from 258.25 in April to 171.25 in July. Flowering was maximum in May followed by April in both the cultivars 'Arka Neelachal Vikram' and in 'Red Sitaphal'. It was observed that the number of flowers increased from July (145.75), June (167.00), April (230.25), May (283.25) in Arka Neelachal Vikram variety. Similarly in Red Sitaphal spike in production of flowers was witnessed from July (171.25), June (192.75), April (258.25) and May (311.25). Flower length in *Annona squamosa* was also influenced by various treatments and it showed a positive correlation between flower production per tree and flower length. Flower length showed slight increase from 2.12cm in April to 2.65cm in July in Arka Neelachal Vikram whereas in Red Sitaphal it ranged from 2.00cm in April to 2.55cm in July (figure 4.2).

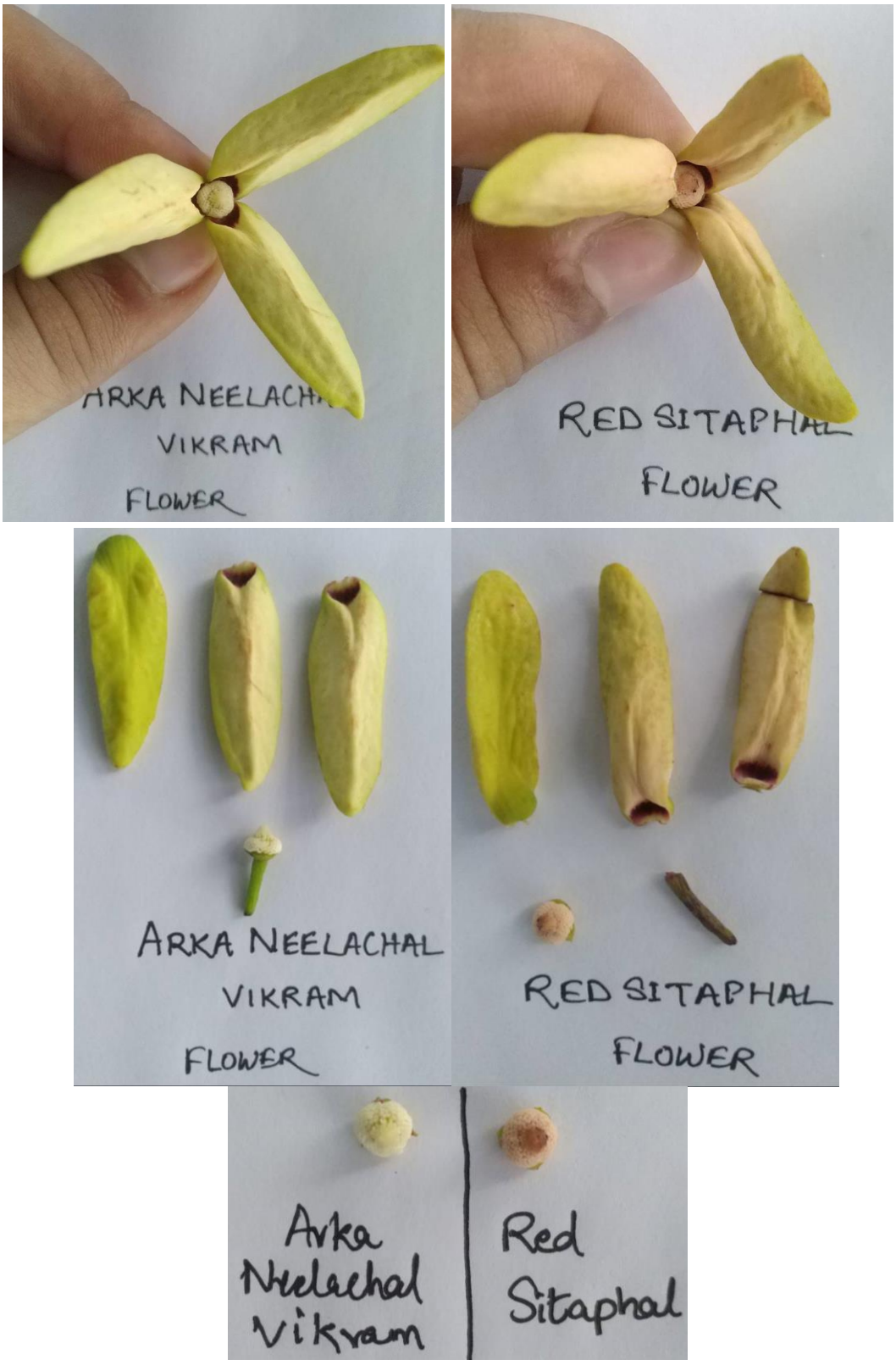


Figure. 4.1. Flower morphology of Arka Neelachal Vikram and Red Sitaphal

Table 4.1 Flowering behaviour in sugar apple under different treatments

Treatments	Mean number of flowers produced
T1	230.25
T2	283.25
T3	167.00
T4	145.75
T5	258.25
T6	311.25
T7	192.75
T8	171.25
CD (*0.05*)	16.74
SE(m)±	5.65
CV (%)	5.14

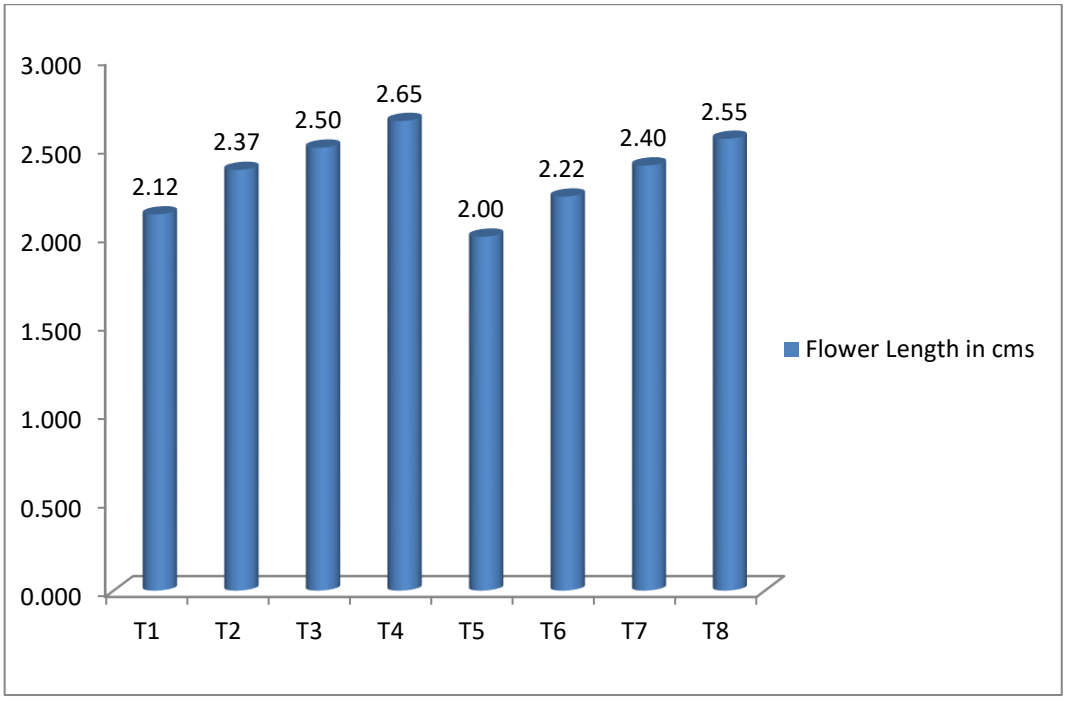


Figure 4.2 Flower length of sugar apple as influenced various treatments

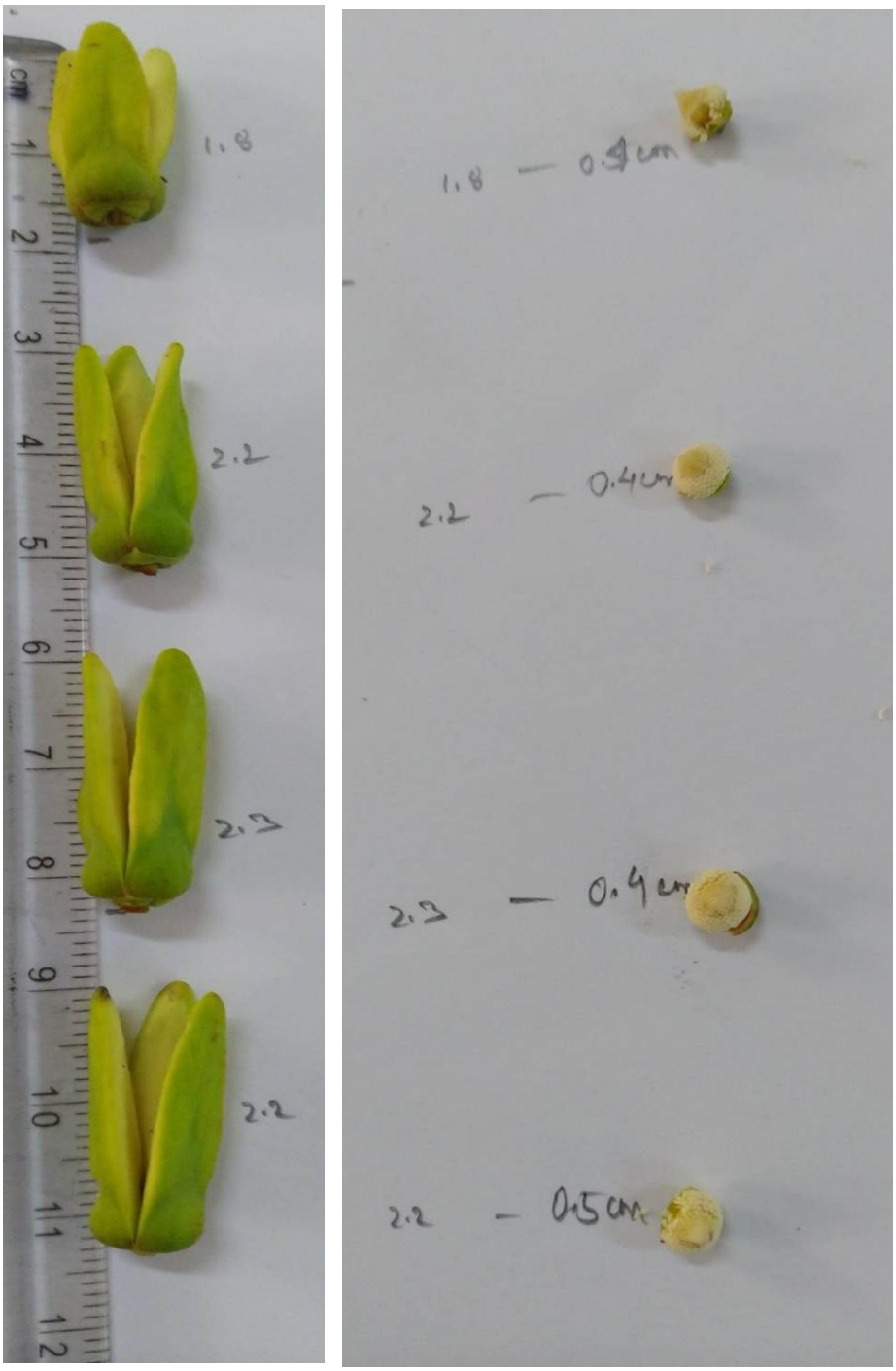


Figure 4.3 Flower length and stigma length of Arka Neelachal Vikram and Red Sitaphal

4.2 Pollen production

It is evident from the table 4.2 and figure 4.4 that pollen production was highest in Red Sitaphal than in Arka Neelachal Vikram in every month of the study period, but the peak production was observed in the month of July which varied between 41093.54 pollen per flower in Arka Neelachal Vikram to 43628.33 pollen per flower in Red Sitaphal. Mean pollen production of Arka Neelachal Vikram was in the range of 27769.34 to 41093.54 pollen per flower whereas for Red Sitaphal it varied from 31075.21 to 43628.33 pollen per flower during the study period. Mean pollen count was lowest in April which varied between 27769.34 pollen per flower in Arka Neelachal Vikram to 31075.21 pollen per flower in Red Sitaphal. In May, mean pollen count was 33909.56 per flower in Arka Neelachal Vikram to 35375.63 pollen per flower in Red Sitaphal whereas in June, mean pollen count was 36430.82 pollen per flower in Arka Neelachal Vikram to 38756.84 pollen per flower in Red Sitaphal.

Table 4.2. Mean number of pollen per flower in sugar apple under different treatments

Treatments	Mean number of anthers per flower	Mean number of pollen per flower
T1	185.50	27769.34
T2	194.40	33909.56
T3	210.80	36430.82
T4	213.90	41093.54
T5	205.00	31075.21
T6	213.90	35375.63
T7	217.50	38756.84
T8	221.50	43628.33
CD (*0.05*)	4.56	8.91
SE(m) ±	2.12	4.11
CV (%)	3.63	21.35

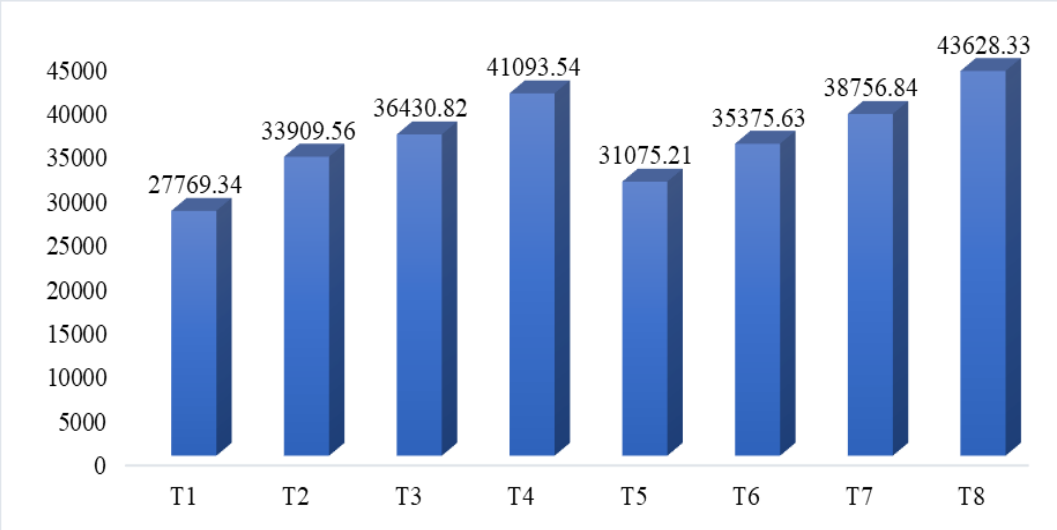


Figure 4.4 Pollen count per flower of sugar apple as influenced by various treatments

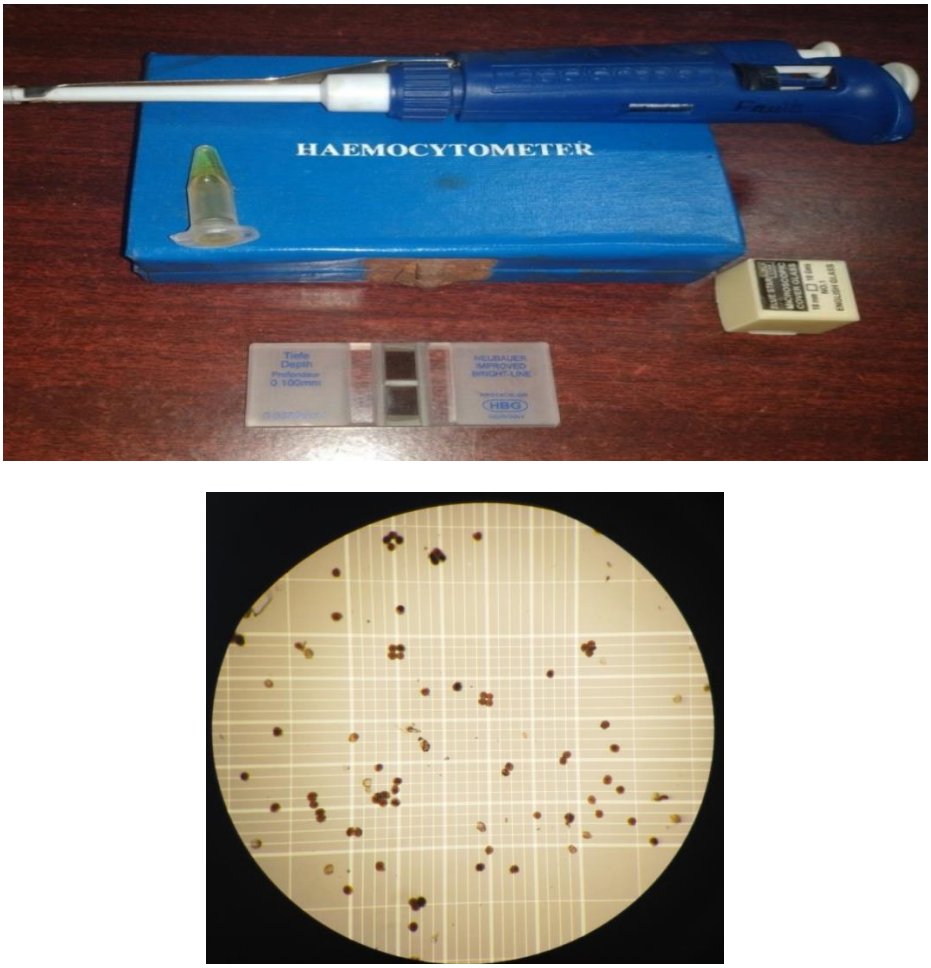


Figure 4.5 Haemocytometer and pollen count as observed under compound microscope

4.3 Pollen viability

Pollen staining material was optimized and the data on pollen viability of sugar apple as influenced by various staining materials were presented in figure 4.6, table 4.3 and figure 4.7. Lugol's solution was found to be the best staining material for both the varieties followed by Safranin, Aniline Blue and lastly Acetocarmine. Lugol's solution (IKI_2) stained viable pollen black and showed maximum mean pollen viability in both the varieties that is Arka Neelachal Vikram (74.41%) and Red Sitaphal (81.73%) whereas Safranin stained viable pollen brilliant red in both the varieties of *Annona squamosa* L. that is Arka Neelachal Vikram (69.60%) and in Red Sitaphal (76.82%). This shows that Red Sitaphal had more viable pollens than Arka Neelachal Vikram. Aniline Blue stained viable pollens deep blue and the mean pollen viability percent was 64.40% for Arka Neelachal Vikram and 71.58% for Red Sitaphal. Acetocarmine stained viable pollens red and showed minimum mean pollen viability in both the varieties that is Arka Neelachal Vikram (58.83%) and Red Sitaphal (66.85%). Since Lugol's iodine solution stained maximum pollens and showed highest mean pollen viability %, Lugol's solution was optimized to be the best staining material for sugar apple. Lugol's iodine solution was further used to test pollen viability in different months of the study period.

Lugol's iodine solution was further used to test pollen viability in different months of the study period. Pollen viability of sugar apple as influenced by various treatments was statistically analyzed and is presented in table 4.4 and figure 4.8. It was observed that Red Sitaphal variety outperformed Arka Neelachal Vikram and showed highest viability percentage in the month of July which varied from 91.01% in Red Sitaphal and 85.33 % in Arka Neelachal Vikram. Mean pollen viability was lowest in April which varied between 52.55% in Arka Neelachal Vikram to 56.743% in Red Sitaphal. In May, mean pollen viability was 63.53% in Arka Neelachal Vikram to 65.773% in Red Sitaphal whereas in June, mean pollen viability was 71.26% in Arka Neelachal Vikram to 76.16% in Red Sitaphal.

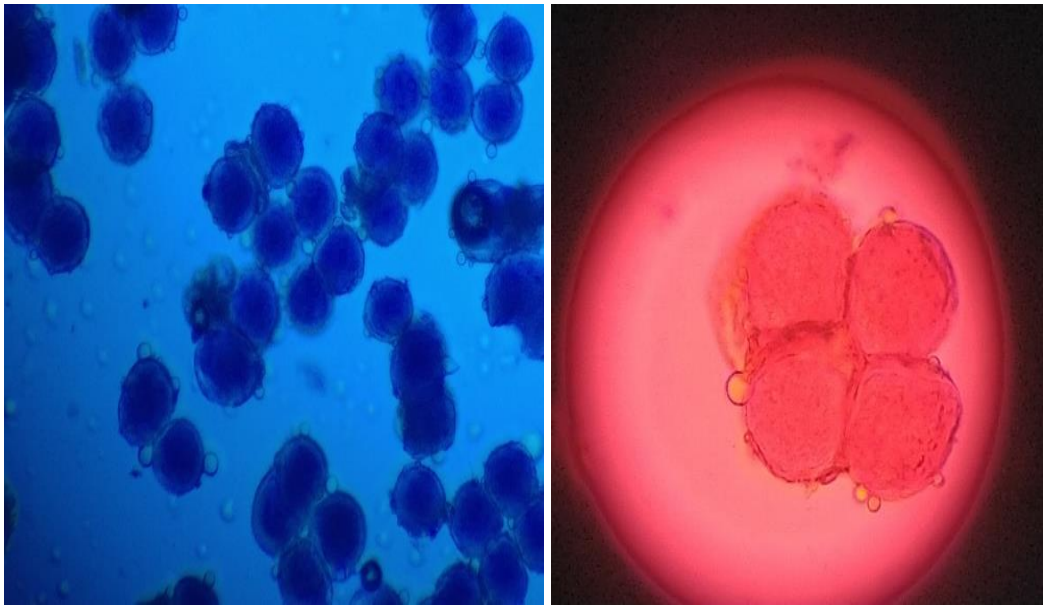
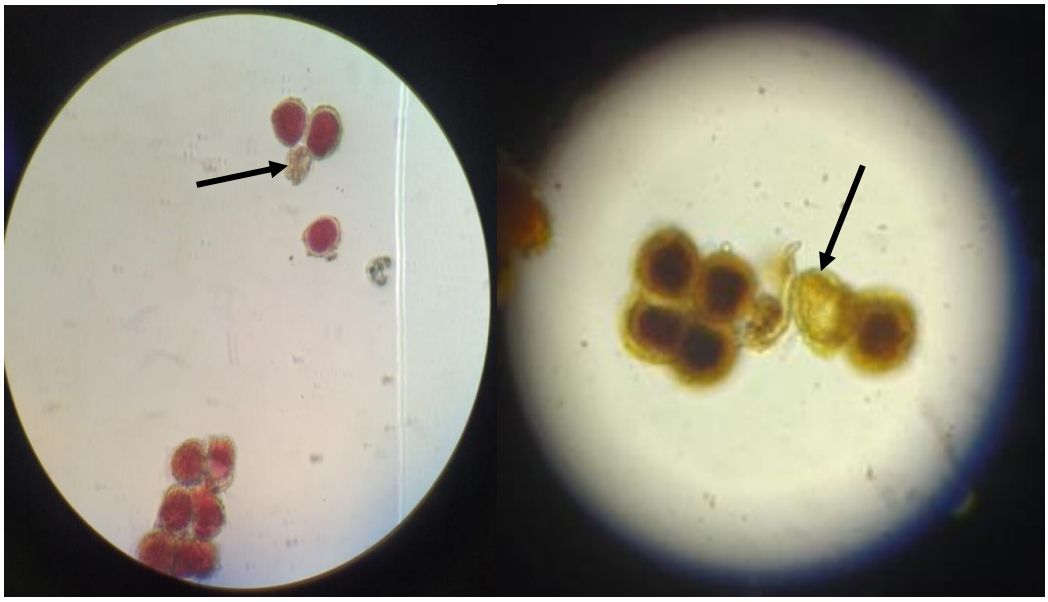


Figure 4.6 1. Aniline Blue stained pollen, 2. Safranin stained pollen,



3. Lugol's iodine solution stained pollen, 4. Acetocarmine stained pollen. Here unstained or colourless pollen (arrow marked) are non-viable.

Table 4.3 Optimization of pollen staining material in sugar apple

Variety	Staining material	Mean pollen viability %
Arka Neelachal Vikram	Lugol's solution	74.41
	Safranin	69.60
	Aniline Blue	64.40
	Acetocarmine	58.84
Red Sitaphal	Lugol's solution	81.73
	Safranin	76.82
	Aniline Blue	71.58
	Acetocarmine	66.85
C.D. (0.05)		4.08
S.E.(m) ±		1.38
C.V. (%)		3.90

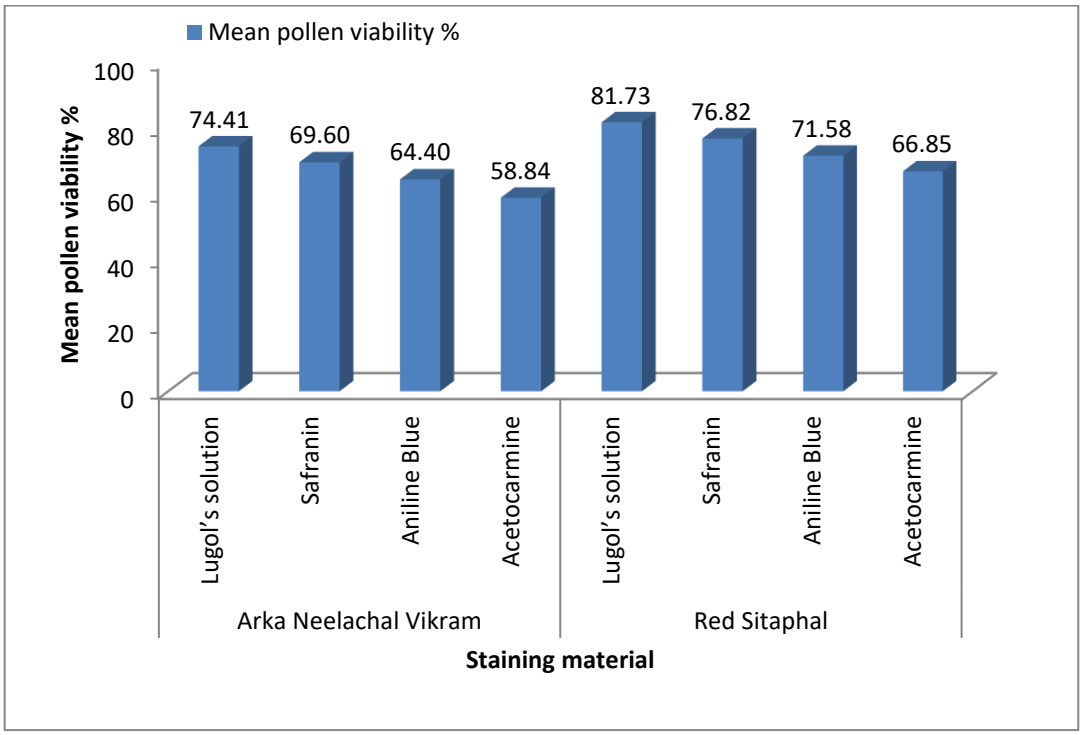


Figure 4.7 Optimization of pollen staining material in sugar apple

Table 4.4 Pollen viability in sugar apple under different treatments

Treatment	Mean number pollen observed	Mean number of pollen stained	Pollen viability %
T1	61.75	32.50	52.55
T2	71.75	45.50	63.53
T3	64.75	46.25	71.26
T4	73.75	63.00	85.33
T5	72.50	41.25	56.74
T6	67.50	44.75	65.77
T7	72.00	54.75	76.16
T8	92.25	84.00	91.01
C.D. (0.05)	15.60	11.97	4.06
S.E.(m) ±	5.27	4.04	1.37
C.V. (%)	14.62	15.70	3.90

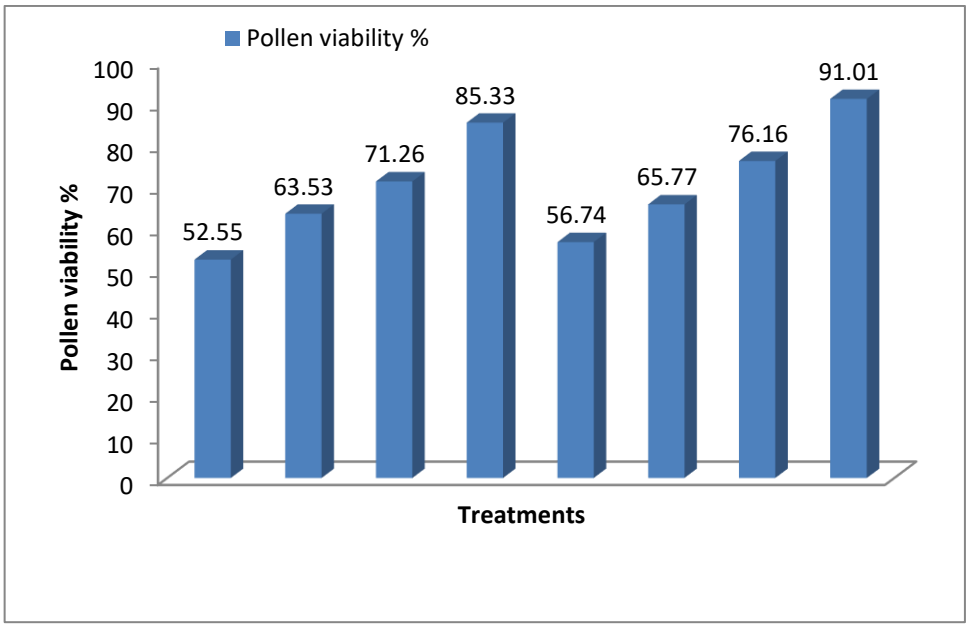


Figure 4.8 Pollen viability of sugar apple as influenced by various treatments

4.4 Pollen germination

Pollen germination medium was optimized for *Annona squamosa* L. and the results obtained were graphically presented in figure 4.9. It was found that 10% sucrose gave higher mean pollen germination (7.79% in Arka Neelachal Vikram and 9.25 in Red Sitaphal) than 15% sucrose (2.41% in Arka Neelachal Vikram and 2.54% in Red Sitaphal) in April. But when we took into consideration different mineral composition of different strengths, it was observed that 10% sucrose + boric acid 400 ppm + calcium chloride 200ppm gave maximum mean pollen germination (38.72%) followed by 10 % sucrose+ boric acid (14.65%), 15% sucrose + boric acid 400ppm + calcium chloride 200ppm (11.31%), 10% sucrose(8.52%) , 15% sucrose + boric acid 400ppm (3.71%) and 15% sucrose (2.47%). Thus, 10% sucrose+ 400ppm boric acid + 200 ppm calcium chloride were selected as best pollen germination medium for further germination studies during the study period.

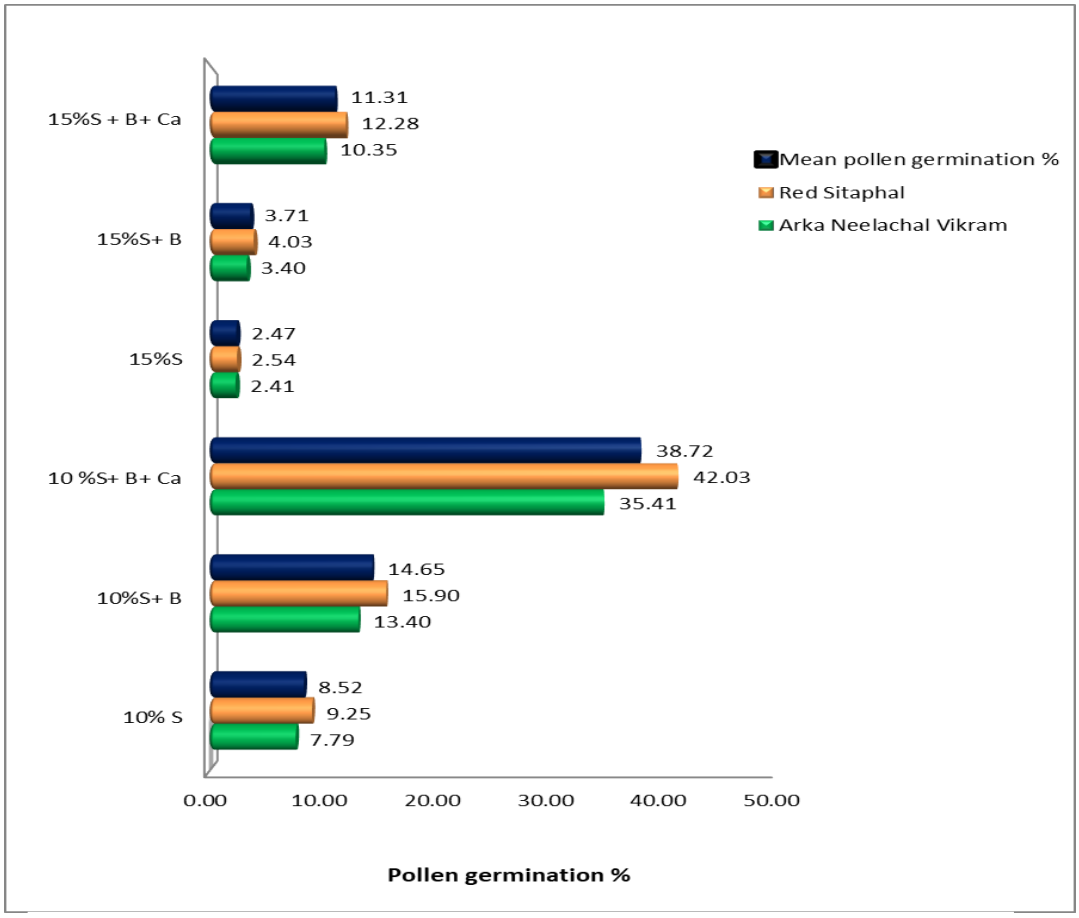


Fig. 4.9 Optimization of germination medium in sugar apple

The influence of various treatments on germination of *Annona* pollen was assessed and the results were presented in table 4.5 and figure 4.10. It was observed that the mean pollen germination was maximum in Red Sitaphal (42.13% to 64.10%) than Arka Neelachal Vikram (35.30 to 54.51%). Peak pollen germination period was observed in the month of July in both the varieties where it varied from 54.51% in Arka Neelachal Vikram to 64.10% in Red Sitaphal followed by June (46.69% in Arka Neelachal Vikram to 56.98% in Red Sitaphal), May (41.13% in Arka Neelachal Vikram to 49.12% in Red Siatphal). April showed the minimum pollen germination in both the varieties where it ranged between 35.30% in Arka Neelachal Vikram to 42.13% in Red Sitaphal.

Table 4.5 Pollen germination in sugar apple under different treatments

Treatment	Mean number pollen observed	Mean number of pollen germinated	Mean Pollen germination %
T1	208.25	73.75	35.31
T2	229.25	94.25	41.13
T3	225.00	105.25	46.69
T4	236.50	129.00	54.52
T5	204.00	85.75	42.13
T6	214.00	105.00	49.12
T7	219.50	125.00	56. 98
T8	246.00	157.50	64.10
C.D. (0.05)	19.93	11.59	1.90
S.E.(m) ±	6.73	3.91	0.64
C.V. (%)	6.04	7.15	2.63

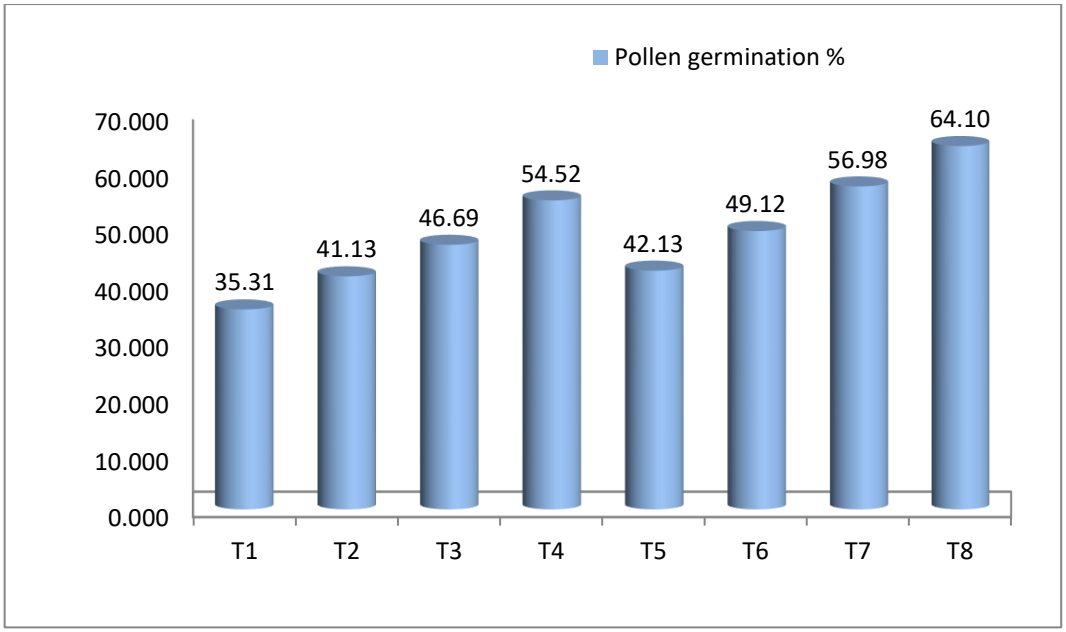


Figure 4.10 Pollen germination in sugar apple as influenced by various treatments

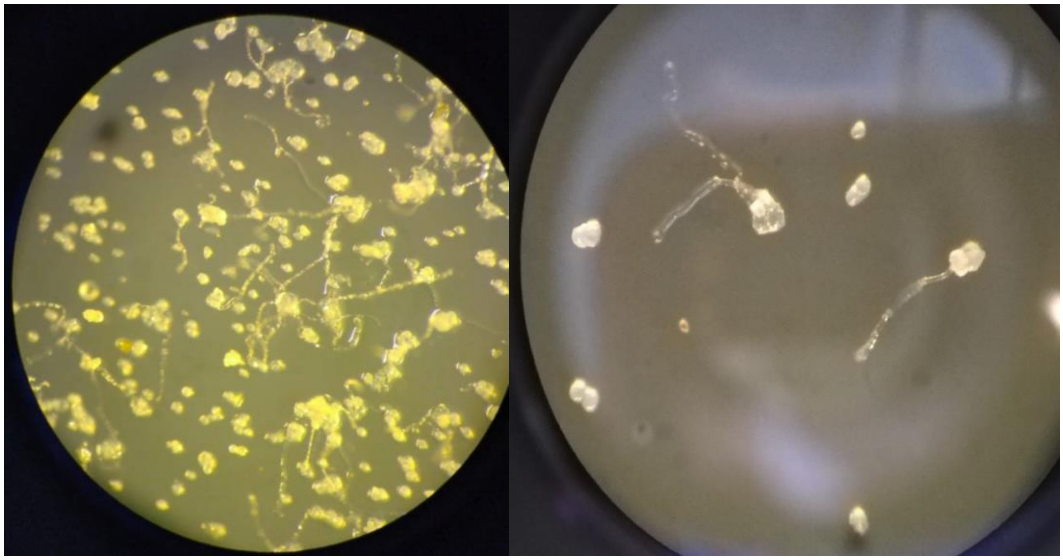


Figure 4.11 Pollen germination with Sucrose + Boron + Calcium

4.5 Stigma receptivity

The data recorded on stigma receptivity as ascertained by visual observations are presented in table 4.6 and figure 4.12. Here the data indicated that mean stigma receptivity percentage was highest in treatment 4 (44.50%) and treatment 8 (47.50%) in Arka Neelachal Vikram and Red Sitaphal respectively. In April, mean stigma receptivity was recorded lowest in the study period which varied between 31.75% in Arka Neelachal Vikram and 35.50% in Red Sitaphal. In May and June, mean stigma receptivity ranged from 35.50% to 45.75% in Arka Neelachal Vikram and it was 39.75% to 44.50% in Red Sitaphal.

Table 4.6 Stigma receptivity in sugar apple under different treatments

Treatment	Mean stigma receptivity %
T1	31.75
T2	35.50
T3	41.75
T4	44.50
T5	35.50
T6	39.75
T7	44.50
T8	47.50
C.D. (0.05)	2.17
S.E.(m) ±	0.73
C.V. (%)	3.66

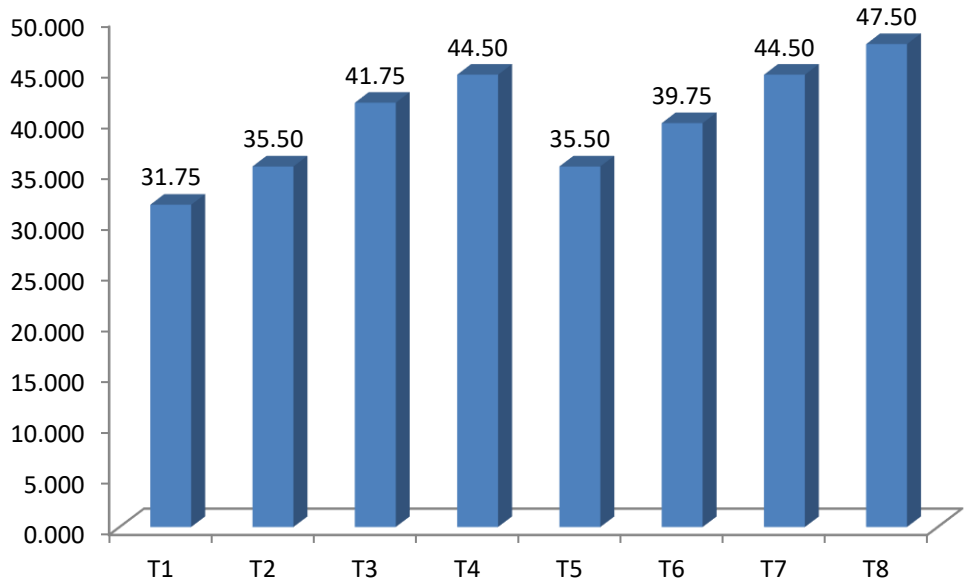


Fig.4.12 Stigma receptivity % of sugar apple as influenced by various treatments

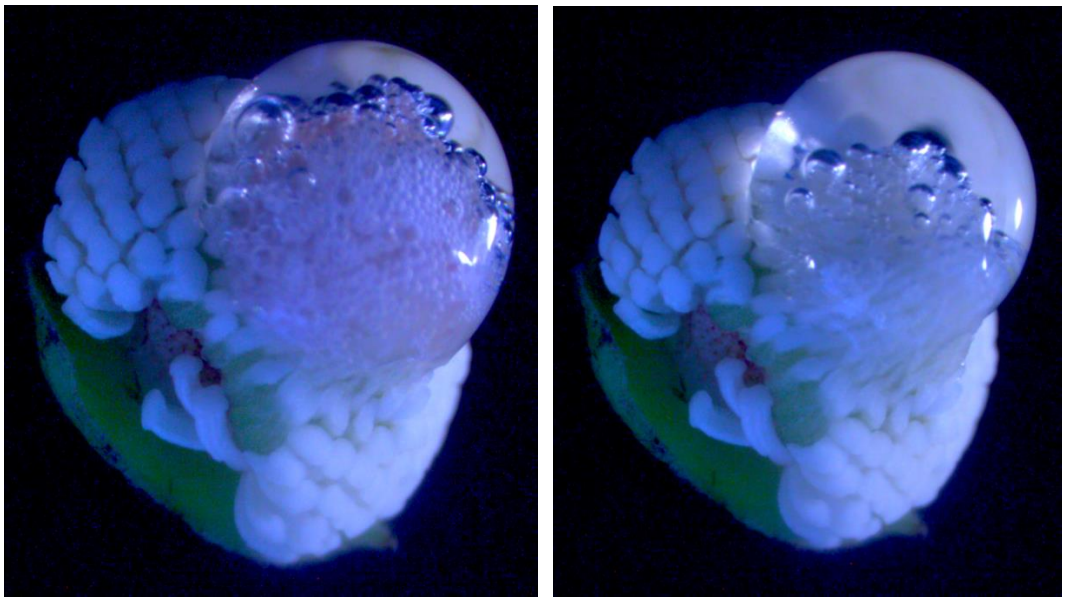


Figure 4.13 Stigma receptivity detected using H_2O_2 in Arka Neelachal Vikram

Figure 4.14 Initial fruit set in Arka Neelachal Vikram and receptive stigma shown in emasculated flower (arrow)

4.6 Fruit set

Data personified in table 4.7 and figure 4.15 showed that fruit set percentage was highest in Red Sitaphal than Arka Neelachal Vikram and maximum fruit set was obtained in the month of July in both the varieties. Mean fruit set percentage (%) in *Annona squamosa* L. ranged from 3.25% in April to 7.89% in July in Arka Neelachal Vikram whereas in Red Sitaphal it varied from 3.48% in April to 8.17% in July. In May, mean fruit set percentage (%) varied between 3.61% in Arka Neelachal Vikram and 3.85% in Red Sitaphal. Mean fruit set percentage in June was in the range of 6.28% in Arka Neelachal Vikram to 6.48% in Red Sitaphal.

Table 4.7 Flowering behavior and fruit set in sugar apple under different treatments

Treatment	Mean number of flowers/ plant	Mean number of fruit set / plant	Mean fruit set %
T1	230.25	7.50	3.25
T2	283.25	10.25	3.61
T3	167.00	10.50	6.28
T4	145.75	11.50	7.89
T5	258.25	9.00	3.48
T6	311.25	12.00	3.85
T7	192.75	12.50	6.48
T8	171.25	14.00	8.17
C.D.(0.05)	16.74	0.90	0.56
S.E.(m) ±	5.65	0.31	0.19
C.V. (%)	5.14	5.62	6.99

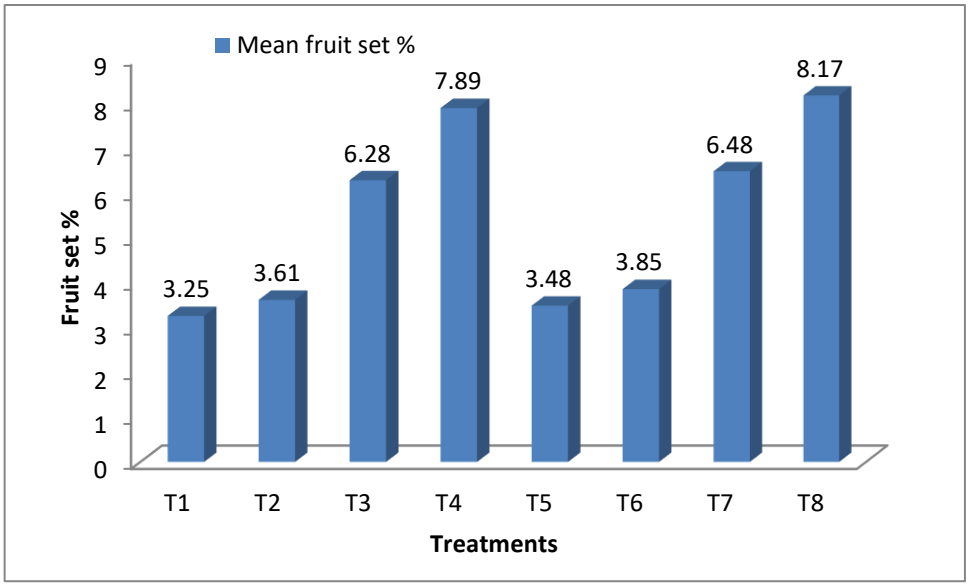


Figure 4.15. Fruit set % of sugar apple as influenced by various treatments

CHAPTER-V

DISCUSSION

DISCUSSION

Based upon the findings of the present study entitled, "Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region" an effort has been made to analyze and discuss the result on the light of reasoning as well as cause-effect relationship. The result of previous researchers on this experiment is also taken into the record while discussing the result of the study of flowering behaviour, pollen biology, stigma receptivity and the role of weather parameters in influencing the fruit set in *Annona squamosa* L.

5.1 Flowering behaviour

The data on mean number of flowers produced in different months of the flowering season are presented in table 4.1. The data revealed that Red Sitaphal variety produced highest number of flowers per tree than Arka Neelachal Vikram in every month of the study period. Flowering intensity was maximum in May followed by April in both the cultivars 'Arka Neelachal Vikram' and in 'Red Sitaphal'. It was observed that flowering comes on both older and current season growth but maximum on current season's growth and flowers are seen borne terminally or in axillary position. Few flowers are also found to grow on the main trunk itself. Most of the flowers bloom in the month of April and May due to the conducive weather such as temperature range within optimum and relative humidity less followed by bright sunshine. But in June and July months relative humidity and average rainfall increases sharply which increases pollen viability and pollen germination but decreases flowering intensity. According to Sahoo *et al.,* (2000), the two peak periods of flowering observed in Red Sitaphal in both year 1995 and 1996 were April 26th and June 26th. It was observed that high night temperature in the month of May i.e., 26.87°C and less diurnal differences were the main driving factors for peak flowering in May followed by April.

April and May being the peak month of flowering period in green sitaphal has been stated by other researchers as well. According to Nalwadi *et al.*, (1975) *A. squamosa* (green type) flowers from mid-April to mid-July with peak during May under Dharwar (Karnataka) condition. Kumar and Singh (1977) reported that in *Annona squamosa* var. Sahebganj the flowering continued from March to August

with maximum flowering during April to May under Sabour condition. Sahoo *et al.*, (2000) observed there were three peak period of flowering in green type of *Annona squamosa*- April, July, August in 1995 and April, June and August in 1996 and maximum flowering and fruiting was observed in Red Sitaphal than in Green Sitaphal in eastern coastal Odisha region . According to Kishore *et al.*, (2012), *Annona squamosa* had April and September as the major blooming periods under tropical humid eastern coastal region of India. Sanewski, (1988) found that at high temperature, 28°C, there is maximum vegetative growth with fewer flowers produced and flower drying is common in custard apple. Favuarble temperature for Annona flowering (Oct-Feb) is between 25°C to 28°C. George and Nissen, (1987) reported that the temperature above 32°C are more conducive to vegetative flushing and increased competition between fruit-lets and vegetative growth, resulting in reduced flowering and fruit set in custard apple. George and Nissen, (1988) revealed that excessive vegetative flushing and soil moisture stress (mid-day leaf water potential -2.0 MPa) reduced flowering and fruit set in *Annona*, particularly at high temperature (28°C).Reich and Borchert, (1984) reported that flowering is probably reduced by the dry-season water stress and the floral anthesis coinciding with the restoration of plant water status following the rain, that renewed root growth, and on-going dry-season root uptake after leaf abscission. Flower length was observed on the day of anthesis in both the varieties. From figure 4.2 it was observed that flower length of both the varieties had shown very little increase over the study period. Maximum average flower length in Arka Neelachal Vikram was observed in the month of July (2.65cm) and also in Red Sitaphal, average flower length was found to be maximum during July month as 2.55cm. Minimum average flower length was observed during April months in both the varieties viz., 2.12cm in Arka Neelachal Vikram and 2.00cm in Red Sitaphal. This clearly depicts that with the onset of monsoon there was slight increase in the size of flower length. Seasonal weather changes along with the genotypic differences leads to such variation in growth and development of flower. Low temperature and high humidity with abundant rainfall in July resulted in increase in flower size due to nutritional variations within the reproductive structures of plant- such as accumulation of starch, better mobilization of mineral solutes, better production of growth hormones, etc. which might have helped in better growth of flower parts. The present observations are in line with the Walse

(1984) observed that in Barbados, average flower length was 3.16cm on the day of anthesis and of Balanagar it was 2.15cm under Rahuri condition of Gujarat in kharif season. Kshirsagar *et al.*, (1976) observed average flower length on the day of anthesis was 2.24cm which is different from the present study. This might be due to differences in climatic conditions, gene differences and interaction between both.

5.2 Pollen production

It is evident from the figure 4.4 and table 4.2 that pollen production was highest in Red Sitaphal than in Arka Neelachal Vikram in every month of the study period, but the peak production was observed in the month of July which varied between 41093.54 pollen per flower in Arka Neelachal Vikram to 43628.33 pollen per flower in Red Sitaphal. Mean pollen production of Arka Neelachal Vikram was in the range of 277629.34 to 41093.54 pollen per flower whereas for Red Sitaphal it varied from 31075.21 to 43628.33 pollen per flower during the study period. Mean pollen count was lowest in April which varied between 27769.34 pollen per flower in Arka Neelachal Vikram to 31075.21 pollen per flower in Red Sitaphal. In May, mean pollen count was 33909.56 pollen per flower in Arka Neelachal Vikram to 35375.63 pollen per flower in Red Sitaphal whereas in June, mean pollen count was 36430.82 per flower in Arka Neelachal Vikram to 38756.84 pollen per flower in Red Sitaphal. Rainfall in the month of March and April was very low about 2mm and 24.60mm respectively were the main cause for such low pollen production. With rise in relative humidity and rainfall in May and June there was significant rise in pollen production per flower. There was drop in temperature from April (31.47°C) to July (29.19°C) which clearly got reflected in higher pollen production in July than in April. For *A. squamosa*, studies on this subject are limited in number. Kishore *et al.*, reported observed somewhat low pollen production per flower viz., 16280 ± 324 pollen/anther/flower and 185 ±16 anthers/flower during April to August in *Annona squamosa* L. in eastern coastal Odisha condition. It was observed that high temperatures during pollen development simultaneously affect female and male reproductive tissues, resulting in a synergistic effect on pollen production (Snider and Oosterhuis, 2011). A.E. Murneek (1937) reported that a single apple blossom may produce 70,000 to

100,000 pollen grains. According to Peet, M., & Bartholemew, M. (1996), at higher night temperatures both the amount of the pollen produced, and the percentage normal drop off causing reduction in fruit set. According to Alves Rodrigues *et al.,* (2018) there were two processes prerequisite for pollen production viz. sporogenic tissue production, and microsporocyte phase. These processes were most sensitive to seasonal variations. Higher normal pollen grain production is possible by the conducive environment by increasing the number of days for the formation of the sporogenic tissue and microspore for which preparation and accumulation of cellular reserves are done for forthcoming events. The rainy season characterized by a lower thermal amplitude and higher relative humidity, caused an increase in the duration (number of days) of almost every phases of pollen grain development, except for meiosis. In our study, higher pollen production was observed in rainy season (July) in both the varieties were due to high moisture availability with the onset of rainfall which might have increased the starch accumulation in sporogenic tissues and microspore mother cells which after meiosis gave higher number of healthy pollen grains per flower.

5.3 Pollen Viability

5.3.1 Optimization of pollen staining material

The information recorded on pollen viability by using different staining material presented in figure 4.6, table 4.3 and figure 4.7 clearly showed that Lugol's iodine solution (IKI_2) stained viable pollen black and showed maximum mean pollen viability in both the varieties of *Annona squamosa* L. that is Arka Neelachal Vikram (74.41%) and in Red Sitaphal (81.73%) whereas Acetocarmine stained viable pollen red and showed minimum mean pollen viability in both the varieties that is Arka Neelachal Vikram (58.53%) and Red Sitaphal (66.85%). The best pollen staining material is Lugol's solution because of high pollen staining capacity which is clearly recorded in both the varieties viz. Arka Neelachal Vikram and Red Sitaphal. Staining obtained with Lugol's solution was more clearer to distinguish between viable and non-viable pollens. On the contrary, pollen viability obtained by Safranin, Aniline Blue, and Acetocarmine were significantly lower than Lugol's solution stained pollens because of less pollen staining capacity. Safranin stains pollens brilliant red with pollen viability ranged from 69.60% in

Arka Neelachal Vikram and 76.82% in Red Sitaphal whereas Aniline Blue stained pollens deep blue and pollen viability per cent ranged from 64.40% in Arka Neelachal Vikram to 71.58% in Red Sitaphal. Aniline blue stains callose in pollen walls and pollen tubes (dark blue colour) and Acetocarmine stains cytoplasm of pollen (red colour) for viable pollen (Ge *et al.*, 2011). Safranin stains the nuclear membranes, chromosomes, nuclei, and lignified, suberized or cutinized cell walls whereas Lugol's solution stains only starch present in pollen. Starch is considered the food storage for pollen to survive the quiescent period of pollen germination when pollen germinates only when enough moisture and suitable temperature is provided for pollen tube growth and for further pushing and kinetics of pollen tube growth depends on this energy stored in starch of the pollen. This is in line with the several researches conducted on different plants species showing starch as a source of energy for the germination and growth of the pollen tube (Franchi *et al.*, 1996). According to Ge *et al*., (2011) Safranin and Aniline Blue stained pollen grains remain stainable even after pollen is killed at 80°C for 2 hours . *Annona* pollen can be considered under the category of slightly and non-dehydrated pollen which are vulnerable to quick desiccation. In our study, it was found that even the ruptured pollen grains which will surely not germinate or will contribute to unsuccessful fertilization, gets stained by Aniline Blue and Safranin stains, this proved the unreliability of these two stains for any further pollen viability percentage study. However Lugol's solution viability percentage was still not the same as *in vitro* germination percentage because of quick starch to water loss and thus becomes non-viable (E. Pacini, 1996). Ge *et al.*, 2011 observed that switch grass pollen could be easily stained with Aniline blue and Lugol's solution, However, none of the method could detect the difference between fresh (viable) and dead (non-viable) pollen. Sulusoglu and Cavusoglu (2014) observed that the correlation between germination tests and pollen viability were not significant in TTC and IKI tests on cherry laurel (*Prunus laurocerasus* L.). Similar observations were recorded by Parfitt and Ganeshan (1989) that the pollen stain tests are not consistent and they are not positively correlated with *in vitro* germination tests.

5.3.2 Temporal variation in pollen viability

It is evident from table 4.4 and figure 4.8 that Red Sitaphal variety (56.743%) was more viable than Arka Neelachal Vikram (52.55%) in April and

also in the subsequent months of the study period. Pollen viability percentage (%) ranged between 52.55 to 85.32 % in Arka Neelachal Vikram and 56.74 to 91.01% in Red Sitaphal. Average pollen viability was highest in the month of July with 85.32% in Arka Neelachal Vikram and 91.015% in Red Sitaphal using Lugol's solution (IKI_2) as staining material. Viable pollen were stained dark-brown or black (viable) whereas unstained (non-viable) pollen were colourless. Viability of pollen depends on the genotype of pollen, environment in which the plant grows and the interaction between them. Pollen viability of Red Sitaphal is higher than Arka Neelachal Vikram because of genetic differences. Weather conditions prevailing during pollen development are very crucial for determining viability of pollen. High temperature and low humidity in the month of April and May has caused reduction in viability percentage, with pollen grains desiccate early which hampers the later pollen-stigma interaction and further pollen tube growth. It was also found that as the percent pollen viability increasesd, germination percentage of pollens also increased within in the study period and they showed a similar growth pattern that is rise in pollen viability and germination percentage from April to July.

Similar observations were recorded by Sahoo *et al.,* (2000) under eastern coastal Odisha climate condition where the viability of pollen varied from 42.30% to 93.33 % in the green sitaphal and 45.10% to 93.75 % in the red sitaphal. The pollen viability was recorded highest from June to August in both varieties. According to Kumar *et al.*, (2015) heat stress had significant affect on pollen development and fruit set by reducing the starch accumulation in pollen grains or by inhibiting the activities of enzymes in genotypes. Reduction in the moisture content of the pollen grains below 30% due to heat stress and low humidity significantly reduced pollen viability and subsequent pollen germination (Aylor, 2003). Flowering period, environmental changes and genetic differences may contribute to the variation in pollen viability rate (Shivanna and Rangaswamy, 1992). According to Bettiol Neto et al., (2009) the pollen viability can be affected by various factors such as plant genotype, variation in moisture levels, nutritional conditions and ambient temperature. The temperature stress reduces pollen production and pollen viability of *Annona*. Extreme temperature changes have caused complete sterility of the pollen grains and/or inhibition of anther dehiscence was observed which ultimately resulted in the absence of fruitlet formation.

Hedhly, (2011) insinuated that in the post-pollination stage, temperature can reduce fertility and affect pollen tube growth of *Annona* pollens.

5.4 Pollen Germination

5.4.1 Optimization of pollen germination medium

Pollen germination and tube growth varies with genotype. Pollen of most species will germinate when placed in a solution of boron, calcium and an osmoticant and usually modifications in their concentrations must be performed in the different species and even among genotypes of the same species, although it provides a controlled *in vitro* condition for pollen germination (Taylor and Hepler 1997). Among them, sucrose plays a vital role both as osmoregulator and nutritive compound (Taylor and Hepler 1997). In addition, boron, calcium and other mineral salts are required in variable concentrations (Feijo *et al.*, 1995; Pham *et al.*, 2015). In this study, pollen germination was tested in six different treatments with four replications each. It is evident from fig 4.9 that 10% sucrose gave higher mean pollen germination (7.79% in Arka Neelachal Vikram and 9.25 in Red Sitaphal) than 15% sucrose (2.41% in Arka Neelachal Vikram and 2.54% in Red Sitaphal) in April. But when we took into consideration different mineral composition of different strengths, it was found that 10% sucrose + boric acid 400 ppm + calcium chloride 200ppm gave maximum mean pollen germination (38.72%) followed by 10 % sucrose+ boric acid (14.65%), 15% sucrose + boric acid 400ppm + calcium chloride 200ppm (11.31%), 10% sucrose(8.52%) , 15% sucrose + boric acid 400ppm (3.71%) and 15% sucrose (2.47%). In 15% sucrose + 400ppm boric acid, mean pollen germination varied from 3.40% in Arka Neelachal Vikram to 4.03% in Red Sitaphal. Pollen germination in 10% sucrose+ 400ppm boric acid solution varied from 13.40% in Arka Neelachal Vikram to 15.90% in Red Sitaphal. In 15% sucrose+400ppm boric acid+ 200ppm calcium chloride it varied from 10.35% in Arka Neelachal Vikram to 12.28% in Red Sitaphal, whereas pollen germination was found to be highest in 10% sucrose + 400ppm boric acid + 200ppm calcium chloride, it varied in the range of 35.51% in Arka Neelachal Vikram to 42.03% in Red Sitaphal. Since 10% sucrose + 400ppm boric acid and 200ppm calcium chloride gave highest pollen germination, this medium was selected for further germination studies in different months of study period.

It can be concluded that boron and calcium are an important mineral requirements for pollen germination. Daher, (2011) propounded that in the pollen tube, boric acid is involved in the cell wall formation and protein assembly in membranes through its effect on H+-ATPase activity. According to Wang *et al.,* (2003) boric acid affects pollen germination and pollen tube growth by promoting the absorption of sugar, and increasing oxygen uptake. Ahmed *et al.,* (2012) revealed that boric acid is involved in the synthesis of pectic material for the actively growing pollen tube walls. Boron combines with sugar to form sugar-borate complex which facilitates translocation of sugar molecules whereas calcium is involved in cationic balance and is essential for tube elongation. According to Zhou *et al*., (2015) calcium element plays an important role in pollen tube growth, an ionic current of calcium ions enters at the pollen tube tip which result in localized positioning of calcium ions channels in the plasma membrane by secretory vesicle fusion at the tube apex. These processes create optimal conditions for growth mediated by the cytoskeleton at the calcium (Ca^{++}) ion-entry site (Steer, 1989). Most plant species require Ca^{++} ions in the growth medium for *in-vitro* pollen germination and tip growth. Chebli and Geitmann, (2007) explained that calcium mineral plays a vital role in cell wall formation and rigidity, directing vesicle trafficking, controlling actin dynamics. Normal pollen tube growth is only possible in the presence of a calcium concentration that is situated within a certain range (Picton & Steer, 1983) that varies between species (Steer & Steer, 1989). Within this range, pollen tube tip extension rates are relatively insensitive to small changes in the calcium ion concentration (Picton & Steer, 1983), whereas outside of this range, pollen tube growth is severely hampered. Among the two varieties Red Sitaphal performed better in all the treatments taken. It implies that pollen germination is affected by the genotype of the plant, and mineral constituent present in the pollen.

5.4.2 Pollen germination in different months

Pollen germination was tested in 8 treatments with 4 replications. The data presented in table 4.5 and figure 4.10 shows that the mean pollen germination was maximum in Red Sitaphal (42.13% to 64.10%) than Arka Neelachal Vikram (35.30 to 54.51%). Peak pollen germination period was observed in the month of

July in both the varieties where it varied between 54.51% in Arka Neelachal Vikram to 64.10% in Red Sitaphal followed by June (46.69% in Arka Neelachal Vikram to 56.98% in Red Sitaphal), May (41.13% in Arka Neelachal Vikram to 49.12% in Red Sitaphal). April showed the minimum pollen germination in both the varieties where it ranged between 35.30% in Arka Neelachal Vikram to 42.13% in Red Sitaphal. Maximum and minimum mean pollen germination between two varieties could be attributed to genotypic difference whereas seasonal changes are the main cause of differential pollen germination in different months of study period. July showed higher pollen germination than in April due to lower average temperature in July (29.19°C) than in April (31.47°C), also average relative humidity was highest in July (88.00%) than in April (73.00). Rainfall in the month of July (348 mm) was also highest among the study period which might have helped in better pollen germination. Hot and dry climate not only desiccate pollen but also dries the stigmatic surface, which badly affects pollen germination. According to Cuchiara *et al*., 2012 and Taiz and Zeigler (2013) at low temperature rate of enzymatic reaction decreases due to drop in kinetic energy (K.E.), thereby pollen grains, cellular metabolism diminishes. Within optimum range of temperature i.e., 20-30°C, pollen grain germination and pollen tube elongation rate remains high but as the temperature exceeds upper lethal temperature 30°Ccellular metabolism of pollen grains falls sharply because enzyme denaturation occurs resulting in decrease in pollen grain and reduction in fruit set %.

Neitsche et al., (2009) insinuated that pollen germination of sugar apple ranges between 46.75% to 53.62% whereas Bettiol Neto et al., (2009) found germination rates ranged between 19.70% and 26.24% respectively for polle grains from flowers of 'Gefner' and sugar apple. Rodrigues, B.R.A.*et al.* (2016) found that the optimal temperature (T_{opt}) for maximum pollen germination and pollen tube growth were 26.7 °C and 27.1 °C, respectively. Rosell *et al.* (1999) observed on the effects of temperature on pollen grain germination for cherimoya and found that the percent germination was higher (47-35%) in the temperature range of 20-30°C, and at temperatures below 15 °C and above 30 °C, the rates of pollen grain germination rates decreased considerably. Bettiol Neto *et al. (*2009) insinuated that the pollen grain collection periods of this species in an orchard located in Lins, São Paulo state, and reported a mean percent germination of 37.6% for ambient

temperatures associated with a relative humidity above 80%. In contrast, when pollen grains were collected during periods of low relative humidity (53% and 43%), the mean percent germination dropped to 21.18%. Significant fluctuations in the nocturnal and diurnal temperatures, which were associated with relative humidity in dry season causes decrease in the *in vitro* pollen germination and viability in *Annona cherimola*. Matsuda *et al.* (2016), when studying pre-anthesis nocturnal temperatures in *A. cherimola*, found that pollen germination increased in the interval of 20–22 °C, whereas nocturnal temperatures below 14 °C and above 27 °C, even for a single night before the day of anthesis, reduced germination. However, a genetic component is present in germination, in addition to the environmental factor, and the requirements for in vitro pollen germination may vary not only amongst species but also amongst genotypes within species (Alcaraz *et al.*, 2011). Judd (2007) reported that the pollen viability is affected by moisture and temperature variations, and can be assessed by testing germination, enzymatic activity, and the presence of cytoplasm. The rainy season enabled the accumulation of a large volume of nutrient compounds and the greater exudation of mucilaginous compounds, which favoured the better performance of the pollen grain. Pollen grains are usually released partially dehydrated (Stanley and Linskens, 2012), but the water content in pollen usually fluctuates as a consequence of environmental variations (Pacini *et al.*, 1997; Vesprini *et al.*, 2002). Mucilage protects the pollen grain against desiccation and preserves the internal turgor pressure (Franchi *et al.*, 1996). On the other hand, the energy content of the pollen grain should be considered a relevant factor for its performance. Starch provides energy for early pollen tube growth phases and contributes to a reduction in pollen grain water potential, thus contributing to performance during germination (Herrero and Dickinson, 1981; Pring and Tang, 2004). Pre-anthesis temperatures may alter pollen vigour and affect the amount of starch accumulated during maturation and the metabolism of reserves, given that pollen is generally considered to germinate autotrophically (Baker and Baker, 1979). Lora *et al.* (2012) also observed that starch decomposition and pollen germination in *A. cherimola* were affected by pre-anthesis temperatures, with pollen starch being rapidly lost before anthesis when flowers were stored for two days at 25 °C but not when stored at 15 °C. The change in carbohydrate accumulation is likely to lead to a decrease in the energy resource availability and

to a reduction of carbohydrate osmotic power (Paupière *et al.*, 2014). Pacini *et al.* (2006) observed that carbohydrate composition is interrelated to environmental conditions; thus, pollen grains that are released partially hydrated enable a rapid pollen tube emission because the rehydration phase is shorter than in the partially dehydrated pollen (Nepi *et al.*, 2001; Franchi *et al.*, 2002).

5.5 Stigma Receptivity

The data recorded on stigma receptivity as ascertained by visual observations are presented in table 4.6 and figure 4.12. Here the data indicated that mean stigma receptivity percentage was highest in treatment 4 (44.50%) and treatment 8 (47.50%) in Arka Neelachal Vikram and Red Sitaphal respectively. In April, mean stigma receptivity was recorded lowest in the study period which varied between 31.750% in Arka Neelachal Vikram and 35.5% in Red Sitaphal. The spike in average temperature (31.47°C) and low average rainfall (24.60mm) were the major factors for low stigma receptivity in treatment 1 and treatment 5. With increase in average rainfall and slight drop in temperature during May and June months recorded significant increase in mean stigma receptivity in both the varieties. Relative humidity also played a crucial role in preventing drying of stigmatic surface for longer time so that later successful pollen germination can take place. In field conditions, although July temperatures were higher on average, relative humidity was also higher (with a mean of 88.00%) than in the April (with a mean of 73 %), indicating that the low humidity may have affected stigmatic receptivity in *A. squamosa*, resulting in a leseer number of fruits per tree. The data recorded were in line with the research done by Kishore *et al.*, (2012). He observed that stigma receptivity evaluated on the basis of fruit set, showed significant temporal difference which started at anthesis and remained receptive for 24 h with peak stigma receptivity at 09:00- 12:00 hours on the day of anthesis. Venkataratnam (1959) and Farooqi *et al.*, (1970) also reported that in *Annona squamosa* , the stigma receptivity was maximum on the day of anthesis. Walse (1984) reported that stigma receptivity ranged from 12 to 52 percent on the day of anthesis, the maximum being in Washington-107005 (52%), followed by Washington-98789 (36%), Island Gem (24%) and Atemoya Chance seedling (20%). Stigma receptivity was found lowest in Barbados and Bullock's Hear t(12%). Nalwadi *et al.*, (1975) reported that the stigma in *A. squamosa* was

receptive only two days after anthesis, while third day onwards it was non-receptive. Environmental conditions affect stigma secretions and likewise receptivity which assists *in vivo* germination and pollen tube growth. Lora *et al.,* (2009), insinuated that stigmatic receptivity in *A. cherimola* decreased when the relative humidity was reduced from 95% to 72% and was lost when no secretions were present on the stigmatic surface. Matsuda and Higuchi (2012) found that the secretion on the stigmatic surface of *A. cherimola* stopped when temperature soared high, which explain the remarkable reduction in pistil receptivity.

5.6 Fruit Set

Mean fruit set percentage in *Annona squamosa* L. ranged from 3.25% in April to 7.89% in July in Arka Neelachal Vikram whereas in Red Sitaphal it varied from 3.48% in April to 8.17% in July. Mean fruit set % in Arka Neelachal Vikram and Red Staphal were 5.25% and 5.49%. It is evident from the table 4.7 and figure 4.15 that fruit set percentage was highest in Red Sitaphal than Arka Neelachal Vikram and maximum fruit set was obtained in the month of July in both the varieties. In May, mean fruit set percentage varied between 3.61% in Arka Neelachal Vikram and 3.85% in Red Sitaphal. Mean fruit set percentage in June was in the range of 6.28% in Arka Neelachal Vikram to 6.48% in Red Sitaphal. There was significant increase in fruit set with decrease in temperature from April (31.47°C) to July (29.19°C). Increase in relative humidity and average rainfall in July showed slight higher fruit set % than other months of study period. But overall fruit set in *Annona squamosa* was quite low and subsequent fruit retention will be even lower which makes this crop less profitable. Comparing previous tables with table 4.7 gives us clear insight that fruit set % is dependent on several factors and all are interlinked to each other. As mean fruit set % was lowest in April (3.25%) when every other parameters tested such as mean pollen production per flower (27769.34), mean pollen viability (52.55%), mean pollen germination (35.30%) and mean stigma receptivity (31.75%) was lowest of all the months in the study period in Arka Neelachal Vikam whereas in July, mean fruit set % was highest (7.89%) which is the result of higher mean pollen production (41093.54 pollen per flower), pollen viability (85.32%), pollen germination (54.51%) and higher stigma receptivity (44.50%). Similarly in Red Sitaphal, it was evident that highest fruit set % (8.17%) in July than April was due to higher mean pollen production (43628.33

pollen per flower) higher pollen viability (91.01%), higher pollen germination (64.10%), and higher stigma receptivity (47.50%). Though the maximum intensity of flower production was in the months of April and May and the reason being high night temperature and less diurnal variation in the month of May whereas fruit set percent was low due to less relative humidity and high temperature which dessicated pollen and stigma. In our study, genotypic difference was the cause of maximum fruit set % in Red Sitaphal than in Arka Neelachal Vikram.

The present investigation confirms the results obtained from the experiment conducted by Sahoo *et al.,* (2000). He observed that there was only 3.33% of fruit set under natural pollination and 0.75% under controlled pollination whereas Butani *et al.,* (2020) recorded higher fruit set percent 21.37 % among different genotypes of custard apple (*Annona squamosa* L.) during kharif season under Gujarat condition. The low fruit set observed in species of the Annonaceae family is associated with aspects involving pollen load (González *et al.,* 2006), source of pollen grains and pollen grains viability (Saaveedra 1977, Rosell *et al.,* 1999) and the method and time of artificial pollination (Richardson and Anderson, 1996; Pereira *et al.,* 2003). Most of these factors are directly or indirectly affected by seasonal climatic changes which affects fruit yield% ultimately. High temperatures, low relative humidity and low average rainfall during development of sporogenic tissues affects pollen production; if it affects one day prior to anthesis then there is disruption in functioning of pollen and ovule production, desiccation of pollen, early drying of stigma, early depletion of energy source of pollen, and non-availability of water for osmoregulation and translocation of mineral solutes in reproductive tissues; all these factors contribute to low fruit set percentage in *Annona squamosa* L.

Correlation with weather parameters

It was observed from Table 5.1 that pollen viability was negatively correlated (-0.949) with temperature which means as the temperature increased there was slight drop in viability of pollen and was positively correlated with relative humidity (0.993) and average rainfall (0.953). Similarly pollen germination in both the varieties were negatively correlated with average temperature (-0.821) and positively correlated with relative humidity (0.907) and average rainfall

(0.826). Pollen production in *Annona squamosa* is negatively correlated with respect to average temperature (-0.849) and positively correlated with relative humidity (0.940) and average rainfall (0.832). It is also evident from table 5.1 that there was positive correlation between fruit set and pollen viability (0.950), pollen germination (0.942) and pollen count (0.989) and fruit set % was found to be maximum in July when average temperature was minimum (29.19°C), relative humidity (88.00%) was high and average rainfall (348mm) was also high. Thus it was clearly observed that fruit set was negatively correlated with average temperature (-0.850) and positively correlated with relative humidity (0.943) and average rainfall (0.827).

The present investigation is in confirmation with the results obtained by Lora *et al.*, (2009) insinuated that stigma receptivity in *A. cherimola* decreased when the relative humidity was reduced from 95% to 72% which ultimately result in low fruit set% due to poor fertilization According to Neitsche *et al.,* (2009) ,in field conditions, when rainy season temperatures were higher on average, relative humidity was also higher (with a mean of 63.5%) than in the dry season (with a mean of 47%), indicating that the low humidity may have affected stigmatic receptivity in *A. squamosa*, resulting in a lower number of fertilized ovules and thus fewer seeds and low yield. Reductions in fruit quantity due to increase in temperature have been reported for some species, including apricots (Rodrigo and Herrero, 2002), cherries (Hedhly *et al.*, 2007) and peaches (Kozai *et al.*, 2004). Saavedra (1977) noted that spraying water on recently pollinated cherimoya flowers increased the fruit set and the reason being increased fruit set due to reduction in the effect of dehydration on the flower's pollen and stigma. Rodrigues *et al.,* (2016) reported that at optimum temperature of 25°C, maximum germination recorded (48.13 %) and the maximum lengths of pollen tubes (536.45 μm) were obtained. The cardinal temperatures (Tmin, Topt and Tmax) for the pollen grain germination and pollen tube growth for sugar apple in *in vitro* observed by Rodrigues *et al.,* 2016 were 9.7, 26.9 and 44.2 °C respectively. A significant correlation between temperature during the bloom period and the yield has been established for the plum varieties Stanley, Monsieur Hâtif, Magna Glauca and Reine Claude d'Althan as observed by Keulemans (1984).

Table 5.1. Correlation between weather parameters and pollen viability, pollen germination, pollen count and fruit set

	Average temperature	Relative humidity	Average rainfall	Pollen viability	Pollen germination	Pollen count	Fruit set
Temperature	1.00						
Relative humidity	-0.969**	1.00					
Rainfall	-0.986**	0.964**	1.00				
Pollen viability	-0.949**	0.993**	0.953**	1.00			
Pollen germination	-0.821*	0.907**	0.826*	0.937**	1.00		
Pollen count	-0.849**	0.940**	0.832*	0.949**	0.973**	1.00	
Fruit set	-0.850**	0.943**	0.827*	0.950**	0.942**	0.989**	1.00

* Significant at the 5% level (two tailed test)

** Significant at 1% level (two tailed test)

CHAPTER-VI

SUMMARY AND CONCLUSION

SUMMARY

The present investigations on "Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (*Annona squamosa* L.) in eastern coastal region" were carried out at CHES (ICAR-IIHR) Bhubaneswar (Odisha) during 2019. Studies were conducted on five-year-old trees of Arka Neelachal Vikram and Red Sitaphal.

The results are summarized below:

1. The flowering season in *Annona squamosa* L. was considerably long from early March to August. Major flowering was observed in April -May in both the varieties of sitaphal i.e. Red Sitaphal and Arka Neelachal Vikram. Low flower intensity was observed in the July month in both the varieties.
2. Mean pollen production per flower was highest in July in both the varieties. In Red Sitaphal pollen production was 43628.33, whereas in Arka Neelachal Vikram it was 41093.54. Plant exhibited low pollen production capacity in April.
3. Best pollen staining chemical for the varieties was found to be Lugol's solution. Highest pollen viability was recorded during July followed by June. Red Sitaphal and Arka Neelachal Vikram had 91.01 and 85.32% pollen viability, respectively in July. Pollen viability was found to be minimum in April in both the varieties.
4. A combination of sucrose (10%), boric acid (400 ppm) and calcium chloride (200 ppm) was found to be the best pollen germination medium. Highest pollen germination was recorded during July followed by June. Red Sitaphal and Arka Neelachal Vikram had 64.10 and 54.51% pollen germination, respectively in July. Pollen germination was found to be minimum in April in both the varieties
5. Stigma receptivity (%) was highest during July. In Arka Neelachal Vikram and Red Sitaphal stigma receptivity was 44.5 and 47.5%, respectively.
6. Mean fruit set percentage (%) was highest in July in both the varieties. In Red Sitaphal, fruit set was 8.17%, whereas in Arka Neelachal Vikram it was 7.89%. Minimum fruit set was recorded in April.

7. Correlation studies between flower characters and weather parameters indicated that pollen viability, pollen germination, stigma receptivity and fruit set were negatively correlated with temperature and positively correlated with relative humidity. It was also evident that rainfall favoured pollen germination and fruit set in sugar apple.

Low fruit set in *Annona squamosa* during April – May was influenced by weather parameter like high temperature which affected pollen production, pollen viability, pollen germination and stigma receptivity. Conducive weather conditions during June- July facilitated better fruit set by increasing pollen germination rate. Moreover, pollen staining material and pollen germination medium were also optimized. Further studies on influence of weather parameters on reproductive biology of sugar apple are required.

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Studies on flowering behaviour, reproductive biology and fruit set of sugar apple (Annona squamosa L.) in eastern coastal region

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