DESIGN AND DEVELOPMENT OF ARTIFICIAL POLLINIZER FOR POLLINATING TROPICAL VEGETABLES UNDER PROTECTED CULTIVATION

By

RAMYA R

(2016 - 18 - 002)

THESIS

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KERALA, INDIA

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DECLARATION

I hereby declare that this thesis entitled **"Design and Development of Artificial Pollinizer for Pollinating Tropical Vegetables under Protected Cultivation"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title of any other University or Society.

Place: Tavanur
Date: 21/11/2018

RAMYA R
(2016-18-002)

CERTIFICATE

Certified that this thesis entitled **"Design and Development of Artificial Pollinizer for Pollinating Tropical Vegetables under Protected Cultivation"** is a record of research work done independently by **RAMYA R (2016-18-002)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

Place: Tavanur
Date: 19.11.2018

Dr. P. K. Sureshkumar
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We, the undersigned members of the advisory committee of **Mrs. RAMYA R (2016-18-002)** a candidate for the degree of Master of Technology in Agricultural Engineering with major in Farm Machinery and Power Engineering, agree that the thesis entitled **"Design and Development of Artificial Pollinizer for Pollinating Tropical Vegetables under Protected Cultivation"** may be submitted by **Mrs. RAMYA R** in partial fulfillment of the requirement for the degree.

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EXTERNAL EXAMINER

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Ramya .R.

RAMYA R

Dedicated

To

My parents

Dedication

This thesis is dedicated to my Mother, Father and Guide, who sacrificed much to bring me up to this level and to my lovely sisters, friends and their families for the devotion they made to make my life successful.

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SYMBOLS AND ABBREVIATIONS

Symbols		
%	:	per cent
×	:	multiplication
÷	:	division
°C		degree centigrade
±	:	plus or minus
ALA	:	Aniline blue in lactophenol staining
etc.	:	et cetera
FDA	:	Fluorescein diacetate
FeR	:	Fluorochromatic reaction
Fig.	:	Figure
G	:	Gram
Hr	:	Hour
HPLC	:	HPLC
KAU	:	Kerala Agricultural University
KCAET	:	Kelappaji College of Agricultural Engineering and Technology
Kg	:	Kilogram
Kw	:	Kilowatt
L	:	Length
ms^{-1}	:	Meter per second
MSL	:	Mean sea level
SPS	:	Sucrose phosphate synthase
TTC	:	Triphenyl tetrazolium chloride
UPOV	:	International union for the protection of new varieties of plants

INTRODUCTION

CHAPTER I

INTRODUCTION

India adores with a rich diversity of horticultural crops covering large groups of fruits, vegetables, mushrooms, flowers, plantation and spices. This is possible because of the agro climatic variations, enormous biodiversity, fertile soil, a large cultivable area etc., Vegetable crops are grown in tropical, sub-tropical and temperate region of the country. India has emerged as one of the leading vegetable producers of the world with a total annual production of 156.33 million tons from an area of 8.99 million hectares (Neeraj *et al.,* 2017). Vegetables are important constituents of Indian agriculture and nutritional security due to their short duration, high yield, nutritional richness, economic viability and ability to generate on-farm and off-farm employment. Vegetables form an integral part of staple diet in India and vegetables are the major sources of daily requirement of nutrients, vitamins and minerals. Cultivation of vegetables occupies an important place in agricultural development and economy of the country. Vegetable farming gives higher yield per unit area within the shortest possible time which ultimately increases the income and provides an opportunity for export to foreign countries and earning money.

Kerala lies in the humid tropical region belonging to warm climate and the state is blessed with an equable and pleasant climate throughout the year. The state is blessed with abundant rainfall with an average of 3000 mm every year. The temperature range is between 28^0 C and 32^0 C over the plains and around 20^0 C over the highlands. Vegetable production scenario in the state reveals that majority of the vegetable production in the state is during winter season that is from November to February. During winter season, in addition to tuber crops, vegetables like cowpea, tomato, okra, bitter gourd are grown in the state. High rainfall and high humidity limit the vegetable production in the monsoon season. Hence, Kerala depends on neighboring states for its vegetable requirements during this period.

Protected cultivation is a technique wherein the micro climate surrounding the plant body is controlled partially/fully, as per the requirement of the plant species grown (Mishra *et al.*, 2010). Protected cultivation is important for growing vegetable crops during rainy season and it has advantage of quality, productivity and good market price to the growers. Protected cultivation includes structures like polyhouse, shade net, poly-tunnel, polymulch, etc., that protects the agricultural crops from sudden changes in weather and regulates the environment inside these structures. The protected vegetable cultivation technology can be utilized for production of high value, low volume vegetables, virus free quality seedlings, quality hybrid seed production and as a tool for disease resistance breeding programs. The need of protected cultivation since last 10 years has been dramatically increased. The various advantages are reduced weed pressure, moisture conservation, reduction of certain insect pests, higher crop yields, and more efficient use of soil nutrients.

Pollination is the process of sexual reproduction in plants in which a male sexual cell, pollen grain, is transferred to a female part stigma of the flower of same species. Artificial pollination is process where humans intervene with the natural pollination process. They carry pollen or plant sperm from one flower to another allowing the pollen to fertilize the ovaries and create seeds that will develop into fruits and new plants. Vegetable cultivation under protected environment offers barriers to natural circulation of air, entry of rain water, entry of insects etc., thus prevent natural pollination by wind, water or insects. So a simple artificial pollinizer is required to ensure successful artificial pollination in protected cultivation.

There are several benefits to artificial pollination including gaining greater control over the genetic population of the crops. When insects are not pollinating a plant sufficiently, growers opt for artificial pollination. Artificial pollination increases fruit size, fruit with high seed numbers and results in a high conversion of flowers to export fruit. Vegetable production in our country is still dominating by the locally available genotype or open pollinated varieties, which are low yielding and

susceptible to various insects, pests and diseases. By using artificial pollination we can achieve fruit with high seed numbers competing for carbohydrates, increased fruit size and high conversions from flowers to export fruit.

There are techniques to pollinate manually or artificially by gently shaking the plants or tapping flowers to release pollen from male parts to female structures. Some plants like tomato have perfect flowers which contain male and female parts, disturbing flowers on these plants distributes pollen within each bloom. Collection of pollen from anther and depositing it to stigma of flower manually is a time consuming, labour intensive task. Keeping this in view, the study entitled "Design and development of artificial pollinizer for pollinating tropical vegetables under protected cultivation" was carried out at the KAU campus, Vellanikkara with the following objectives.

Objectives:

1. To study physical characteristics of anthers suitable for designing artificial pollinizer.
2. To design and develop artificial pollinizer for pollinating selected tropical vegetable crops.
3. To evaluate the artificial pollinizer and study the pollination efficiency in selected tropical vegetable crops under protected cultivation.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

A detailed prior art search related pollination, physical charectestics of flowers, reproductive organs, anthers, pollen grains etc., were carried out and brought to this chapter. Research papers and other reference material from 1932 to 2017 were collected and reviewed for studying the methods adopted in earlier artificial pollination and the changes or improvements incorporated in due course of time. The works related to both hand pollination and machine pollination were collected from various sources which include published articles, patents and commercially available designs and also from other online resources. Brief reviews of work done relevant to various aspects of the present work are reported here in the following sections.

2.1 Reproductive systems in plants

2.1.1 Flower type/ monoecious/ dioecious plants

According to Warmund (2002) flowers are the reproductive structures of angiosperms. The function of a flower is to produce the reproductive cells of the plant (eggs and pollen) and then produce seeds, the dormant young plant of the next generation. There are two types of plants, monoecious plants and dioecious plants. Monoecious plants have both male and female flowers on the same plant. Dioecious plants have only one sex of flower per plant.

Westerfield (2014) stated that both monoecious and dioecious plants require cross-pollination. Male flowers generally appear on the plants several days before female flowers. The female flower is easily recognized by the presence of a miniature fruit below the flower petals. The sequence of flower types on main shoot in squash has been described by Nitsch et. al in 1952. According them in monocious plants, staminate flower appears first and pistillate flowers at the later stages.

2.1.2 Parts of flowers

Parker(2004) described most important parts of a flower, which consists of male and female parts. The male part of the flower is called the stamen and female part is called stigma. The stamen is the pollen producing part of the plant, and it is made up of two parts: the anther and filament. The filament is the stalk that holds the anther and attaches it to the flower. The anther produces and holds the pollen, which will hopefully be transported to the female part of the flower by wind, animals, or insects. The female part of the flower is called the pistil, and it is made up of the stigma, style, and ovary. The stigma receives the pollen grains. The style is the stalk that the stigma sits on top of, and the ovary is usually at the base of the style. Fig. 2.1 shows the cut section of a flower and different parts of it.

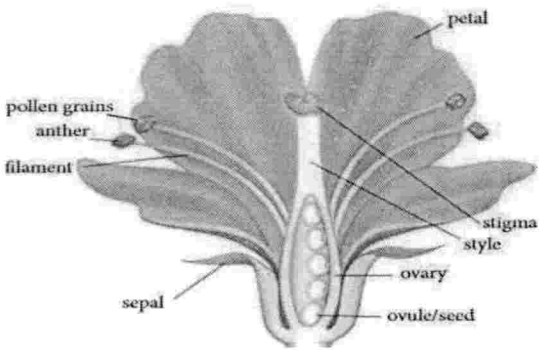


Fig. 2.1 Sectional of a flower

According to Warmund (2002) flowers can be male, female, or "complete," which means the flower has both male and female parts. Some flowers are perfect, they have both male parts and female parts in the same flower. Roses, lilies, and dandelions have perfect flowers. Other flowers are imperfect, each flower has either all male parts or all female parts. Cucumbers, pumpkins, and melons have imperfect flowers. Westerfield (200 8) explained parts of chilli flower (Fig. 2.2). Plants develop seeds through a process called pollination. Pollination is the transfer of pollen from the stamen, the male flower part, to the pistil, the female flower part. Since both the

stamen and a pistil are necessary for seed formation, they are called essential organs. Most flowers contain two other parts, the sepals and petals, which may help attract insect pollinators. A complete flower contains all four parts. If only one of the essential organs is present in a flower, it is called an incomplete flower.

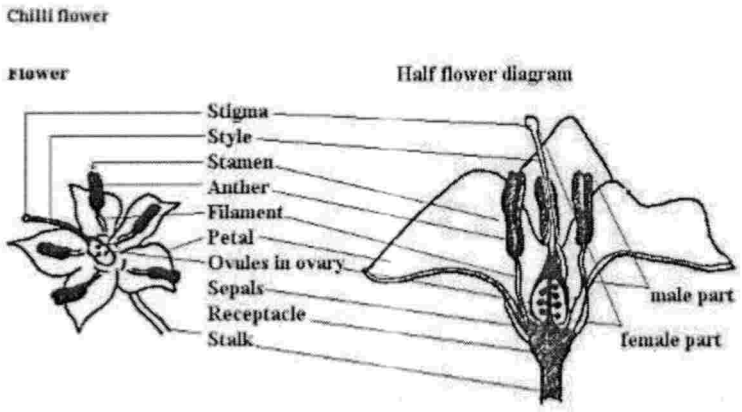


Fig. 2.2 Sectional view of chilli flower showing the different parts

2.1.3 Reproductive organs

Fryxell (1957) explained that, reproduction is a biological process by which living organisms produce more individuals of their own kind. There are two modes of plant reproduction: asexual reproduction and sexual reproduction. Sexual reproduction in plants consists of alternating, multi cellular haploid and diploid generations. In angiosperms, the female gametophyte is the embryo sac and the male gametophyte is the pollen. The haploid egg and sperm fuse to form diploid zygotes, from which new sporophytes develop. In asexual reproduction, offspring are produced without meiosis or fusion of gametes an d the plant multiplies through tubers, bulbs, corms and other vegetative parts. Sometimes a third mode of reproduction, apomixes, may be distinguished. Apomixes is the formation of new individuals from the sexual organs of a plant, without fertilization.

Stebbins (1950) stated that sexually reproducing plants can be subcategorized based on the source of the pollen that pollinates the plant. Self-pollination occurs

when the pollen from a flower pollinates the stigma of the same flower or another flower on the same plant. A species is said to be cross-pollinated if the pollen from a flower on one plant pollinates the stigma of a flower on another plant.

According to Burt (2000) there are two basic types of sexual reproduction hermaphroditism (male and female sex organs in same individual) and dioecism or gonochory (male and female sex organs on separate individuals).

Pandey (2006) reports that, the reproductive organs for angiosperms are the flowers. Most plants have bisexual flowers with all four floral parts (sepals, petals, stamens, and carpels). If only one of the sexual parts (stamens or carpels) is present, the flower is unisexual. The sepals and petals constitute the perianth and are accessory in that they are not directly involved with the sexual life cycle. The perianth functions in protecting the stamens and carpels, the sexual parts, and in attracting animal pollinators to the flower. The stamens and carpels are the essential parts of a flower because they produce the gametes for sexual reproduction

2.2 Pollination

2.2.1 Natural pollination methods/ agents

According to Kevan (2007), pollination is simply the transfer of pollen from the anthers to the stigma of a flower and is the first step in the sexual reproduction of plants. The pollination process starts with the pollen grains being discharged from small sacs in the stamen called the anthers.

Warmund (2002) states that, plants may be either cross-pollinators or self-pollinators depending on the species. Cross-pollinators require the transfer of pollen between two different plants, while self-pollinators can be fertilized by pollen transfer within the same plant. Prathap (2011) stated that there is a need of an external agent for transfer of pollen grain from anther to stigma. Examples of good abiotic agents are wind, water, gravity. Examples of biotic agents are insects, birds and mammals.

According to Weinzierl (2012) pollination is the process by which pollen is transferred from the anther (male part) to the stigma (female part) of flowers, thereby enabling fertilization an d reproduction and it is aided by wind, insects, or other animals that allows flowering plants to produce seeds and fruits. Pollinators are the organisms that carry pollen from the stamen to the stigma. They may be insects, birds, bats, or occasionally other animals. Pollinizers are plants that serve as the source of pollen for successful pollination an d fertilization.

Richards (1986) states that, pollination is a prerequisite for seed set and thus plays a critical role in reproductive success of seed plants. A majority of flowering plants make use of animals to achieve pollination while a small pro portion of them use wind or water for pollination. Plants have evolved diverse pollination strategies ranging from complete selfing to obligate out crossing.

Klein *et. al* (2007) reported that pollinating agents are essential for survival and reproduction of several wild plant species and in the recent years, there has been an increasing recognition of the importance of pollination, mostly by insects in food crops and they found that 87 of the world's leading food crops depend upon animal pollination, representing 35 percent of global food production.

Losey and Vaughan (2006) also emphasized that flower-visiting insects provide an important ecosystem function to global crop production through their pollination services. The rapid spread of human habitation is affecting the available natural habitats through urbanization and other land-use practices, putting pressure on ecosystem services delivered by wild pollinators. At the same time, the demand for pollination in agricultural production will have to keep place to sustain food production.

Brust (2011) explained that, in tomato flowers the anthers surround the female part of the flower and just about any pollen that falls on the stigma comes from the same flower (and therefore plant there is no or little cross -pollination in tomatoes).

Tomato pollen is sticky and fairly heavy as pollen grains go and it takes a good breeze or a vibration of the flower to dislodge the pollen from the anthers onto the stigma. A cut section of tomato anther and stigma is shown in Fig. 2.3.

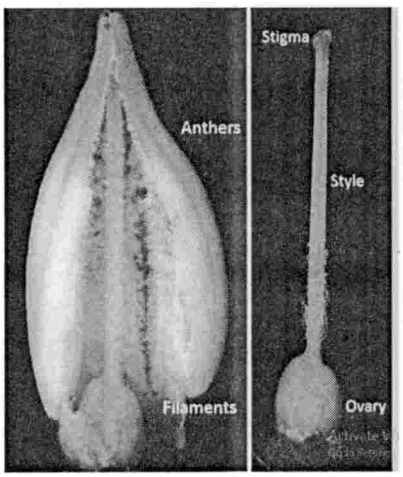


Fig. 2.3 Cut section of tomato anther and stigma

Gallai *et. al* (2009) observed that major pollinator dependent crops are fruit and vegetable crops, spices and plantation crops and pulses and oilseed crops.

Ibarra *et. al* (1999) reported on pollination, quality and yield of green beans. in presence of insect pollinators yield and seed quality of scarlet bean and runner bean is increased up to 10 fold, particularly large bodied bumble bee and carpenter bees. Honey bees are capable of working the flowers but do not actively collect pollen or facilitate cross pollination. Larger bees in P.Vulgaris can be significant benefits to cross pollination by insects, attribute to improved seed set following tripping of the flowers, which is best effected by bumble bee.

To determine the diversity of insects visiting its flowers, the time, type of provision obtained and the effect of the visits on fruit set, fruit size and weight, and number of seeds study was conducted by Nicodemo *et. al* (2009) they observed that Apis mellifera L. accounted for 73.4% of the visits made by bees. Results show that,

higher the number of visits by A. mellifera to female flowers, the greater was the fruit set, fruit size and weight, and number of seeds. In flowers visited by insects from the onset of anthesis until 9 a.m., fruit set was 35%. After 9 a.m., there was no fruit set.

An investigation on the pollinating insects of medicinally important plants was conducted by Duara and Kalita (2013). In this study, scan sampling methods were carried out to explore the insect pollinator diversity from 7.00 h up to 15.00 h by using focal sampling visiting frequency of pollinator insects. Results showed that every species of insect pollination were from order Hymenoptera, Lepidoptera and coleoptera. Total 43 species were observed during the study, three species of Lepidoptera showed highest abundance out of 43 species, The highest abundance and species richness of pollinators occurred at 8.00-12.00 h.

Shivaramu *et. al* (2012) conducted study to understand the pollinator diversity and foraging behavior of major insect pollinators on rambutan during 2008-10. Stingless bee, Trigona iridipennis and Indian honeybee, Apiscerana were the most dominant foragers with a mean visitation of 3.81 and 3.54/panicle/10 minutes. Other species included A. flora, A. dorsata, an unidentified wasp and calliphorid flies. Peak activity of all foragers was recorded between 10.00-11.00 AM. The extent of fruit set in open pollinated panicles was 29.35 fruits/panicle while bagged flowers completely failed to set fruits. The studies established the role of pollinators in rambutan fruit set and hence it is recommended to conserve natural populations or to augment them through placing honeybee colonies to realize maximum fruit set in rambutan.

Hobbs *et. al* (1961) found that the bumble bees are the suitable pollinator species to pollinate flowers with deep corolla. In many cases more effective, than manual pollination or honey bee pollination in terms of the quantity and quality of tomatoes produced

2.2.2 Art1ificial pollination

i) Manual pollination

Shivanna and Rangaswamy (1993) described procedure for pollen collection manually. Exercise mature anther just before dehiscence and allow them to dehisce under low humidity in desiccators. Anther debris are then removed with brush/forceps or pollen is sieved through mesh of suitable pore size. Pollen grains can also be collected by removing the standard and wing petals and splitting open the two keel petals along the line of fusion over a watch glass/petri dish/glossy paper. Flowers are inverted over a petri dish/glossy paper and gently tapped to dislodge the pollen from corolla tube from dehisced anthers.

Chetelat and Peacock (2013) reported that, pollination is performed early in the morning. The pollen is applied by simply brushing or rubbing stamen against stigma or with a small paint brush or a dissecting needle.

According to McGregor (1976) the tomato plant, Lycopersicon esculentum Miller, has poricidial dehiscent flowers, pollen grains were released through apical pores by gently shaking the anthers. In open areas, shaking by wind is usually sufficient to trigger this pollen release, promoting self-fertilization.

Hoping and Hacking (1983) made a comparison of pollen application methods for the artificial pollination of kiwifruit. In order to prevent both wind and insect carried pollen from staminate vines flowering kiwifruit laterals were enclosed in a terylene sleeves, freshly harvested staminate pollen was applied to the fully opened flowers by hand or by a mechanical puffer gun after dilution with talc. Pollen was also suspended in aqueous solution and applied either by hand operated pressure sprayer or by a pressurized boom sprayer using compressed air. Treated flowers were harvested and 24 hours after pollination the number of pollen grains on the stigmatic surface and number of pollen tubes that penetrated the style was determined. Results

shown that treatment of pollen with aqueous solution doesn't affect pollen viability or pollen tube growth.

ii) Machine pollination

Apparatus for vision-based pollination system was developed by Safreno (2017). It includes mobile support device to traverse crop field, storage tank containing pollination liquid, pollination node including vision system and a camera. During operation mobile support device traverse the crop field, pollination nodes repeatedly takes photographs of a portion of crop field identifying locations for delivering the pollination liquid and determine when to operate pollen applicators to the identified locations .

Atkinson D. T. and Atkinson D. L. (1990) invented apparatus for improving pollination of orchard plants. The apparatus comprises portable power unit and collection device mounted on a trailer, cyclone unit, fan, inlet, outlet, collection nozzles, hoses and dispersion nozzles. Apparatus described involves sucking pollen from maleorchard plant and blowing pollen on to the female plant, collected pollen was diverted through a cyclone which acts as both separator and pollen storage unit.

To solve the problems associated with the pollination of multiple verities and residual pollen cleaning, Jai *et. al* (2015) developed an artificial pollinating device comprises of an air blowing device for blowing air and a pollen collecting device which interns consists of connecting column, an adjusting plate, a supporting base, a pollen discharging pipe and pollen bottles. They stated that artificial pollination device was simple in structure and pollen collecting device can be detached for convenient cleaning.

An apparatus for controlled growth and pollen harvesting was developed by Science (2016) to achieve controlled rate tassels were cut from maize plants and placed in a growth chamber. An apparatus consist of growth chamber, outlet, boot and

collection point, once the anthers on a tassel begin to release pollen, the growth chamber may be vibrated and air may be directed through the growth chamber to encourage further release and collection of pollen.

Moulie (1906) designed and developed pollen collecting device consists of rectangular vessel in which severed twigs or branches-bearing blossoms from which the pollen is to be collected are held with their stems immersed in water contained in a vessel, vessel was provided at the ends with handles and loosely mounted in sockets. Small tube for emptying water, tube may be closed ordinarily by inserting a cork or plug, pair of traverse and pair of longitudinal bars are provided across the top of the vessel. Top lateral openings and flaps are also provided. In order to collect the pollen from plants, twigs or branches of these plants are detached and are then inserted through the lateral openings , the device is placed upon a sheet of paper and is located in a closed room in which the temperature is maintained constantly,. As the ends of the twigs or branches project beyond the sides of the vessel and extend over the paper upon which the device is placed, the pollen gets separates from the blossoms and fall on the paper, from which pollen might be collected time to time.

Brown (2013) studied on pollen compositions and methods for distribution on flowering plants. The invention describes composition of viable pollen suspension in a water miscible carrier. Suitable mechanical distribution system such as, electrostatic application and methods for applying droplets containing a viable pollen suspension to flowering plants are also there to increase pollination.

Michael (2013) invented a handheld vibrating pollinator and pollination methods. Generally Pollinator comprises of pollen colleting device for collecting pollens from anther of a flower and handle portion for generating vibrations. One or more wands are installed on the handle portion for transferring vibrations generated from hand held portion to the flower which causes the flower to release pollen from

the anther of the flower, released pollens were collected by the pollen collecting device.

Bullock *et. al* (2012) invented a novel apparatus for collecting increased amount of viable pollen from anthers of corn plant. The device consists of vacuum source, a cylinder capable of generating a cyclonic air moment, and a disposable receptacle to collect viable pollen from anther and a pollen deposition port which is connected to a source of vacuum at the vacuum collection port. The vacuum created a vacuum at the pollen port which is passed adjacent the anthers of the plant to draw into the cyclonic chamber pollen from the anthers that would not be shed naturally by the anthers. The collected pollen is deposited in a reservoir connected to the deposition port of cyclonic device, which is viable for the pollination of the same or another plant.

The apparatus for manual dispensing of maize pollen grains developed by Blahnik and Jaehnel (2014) apparatus consists of reusable air discharger having an internal cavity and an outlet arranged in a manner when it get actuated, air has to be expelled out from the internal cavity through the outlet. Biodegradable applicator has a first open end and a second open end, wherein the first open end is in communication with the outlet of the air discharger. The applicator is configured again in such a way that when air discharger is actuated, applicator has to receive at least one maize tassel containing maize pollen and to direct received pollen through the second end of applicator. Finally they found that single common air discharger can be used for controlled pollination of two or more maize plants.

King and Ferguson (1991) developed and evaluated mechanical system for the collection and deposition of dry kiwifruit pollen to flowers on extensive basis. Pollen was collected by twin cyclone operated by one person, which is mounted on front of a tractor. Maximum Collection rates were observed up to 140 g/h during both day and night. Germination and purity of the pollen was consistently acceptable. Application

was by impaction of dry force- feed pollen onto a rotating velcro-covered wheel which was located in an airstream. The principle was simple and its inherent flexibility permitted the evaluation of various pollen rates, delivery airflows, and machine types, fruit yields resulted was acceptable.

Jinotti (1984) developed pollen counter consists of an open ended tube that forms passage for air flow, for causing air flow at desired rate a fan was provided inside tubular member, while air is flowing through the tube glass slide was mounted and positioned within the tube in such a way that the pollen grains were deposited on its edges.

iii) Time of anthesis / stigma receptivity/ fruit set efficiency

Chetelat and Peacock (2013) experimented pollination for 24 to 72 hours after emasculation, usually early in the morning. The pollen is applied by dipping the exposed stigma into the pollen or with a small paint brush or a dissecting needle.

Aiyadurai and Koyamu (1957) after studying the anthesis of staminate and perfect flowers reported that staminate flowers opened between 9 am and 11 am while bisexual flowers between 2 pm to 4 pm.

Devar *et. al* (1981) reported that stigma was receptive a day prior to opening of flower and continued upto the day of opening of flower with maximum receptivity on the day of opening. The stigma was non-receptive one day after the opening.

Verma and Neha (2017) observed that in cucumber anthesis starts at 6AM and completes at 8AM with maximum anthesis between 6AM to 7AM in monoecious varieties whereas in gynoecious varieties it was maximum up to 6AM in both open and controlled conditions and similar pattern was observed for dehiscence. Dehiscence occurs soon after anthesis. Maximum pollen viability was recorded on the day of anthesis. Maximum stigma receptivity was noticed at anthesis time and pollination during this interval recorded maximum fruit set.

Kim *et. al* (1994) conducted experiments on influence of growth temperature on parthenocarpic fruit set in a late parthenocarpy type cucumber. He observed that low growing temperature of 15^0 C resulted in highest average rate of parthenocarpic fruit set, 78 per cent at all growth stages, at 20^0 C, 25^0 C and 30^0 C rate was below 50 per cent.

Martinez (1989) reported from an experiment on cucumber sown in summer (Aug-Oct) and winter (Nov – march) found that both seasons gave equal yield of 10-11 kg/m^2. The length of growing period increased from 90 days in summer to 150 days in winter.

Amponsha (1972) reported that the percentage of fruit set of cocoa by hand pollination was much higher than those by natural pollinations.

Study was conducted by Rajasekharan and Nandini (2015) to determine the growth of cucumber under open filed and poly house observed that number of harvest in poly house is 21.52 per cent more than open field.

Obshatko and Shabalina (1984) reported that fruiting time of cucumber mainly depends on early temperature conditions and reduction of vegetable growth by fruit load in cucumber without any changes on the ratio between weight of shoot and roots.

Drews (1979) reported that cucumber fruit set and development from February to July found that small fruits gained 25-30 g fresh weight per day. Daily growth in length and width varied between 20-30 mm and 2.5-3.5 mm respectively, increase in light intensity and temperature reduce the period of fruit development from 25-14 days. Low night air temperature enhanced fruit set whereas high air temperature at low temperature humidity encourages fruit drop.

Study on Ash gourd an entomophilic cucurbit crop was carried out by Leena and Nasser (2015) in Kerala. Observations were taken at different time intervals in the

flowering season to understand the effect of pollination on fruit production. They observed that insects belonging to the orders Hymenoptera, Coleoptera and Lepidoptera were the common visitors, during flowering season. Finally they concluded that insect pollination had a positive influence on fruit production in ash guard crop.

Hanso et. al (2003) investigated marked improvement of fruit set in Thai durian by artificial cross-pollination they selected four Thai durian cultivars and investigated the efficiency of pollination to fruit set, by performing artificial self-pollination, cross-pollination and open pollination.

He observed that open pollination treatment resulted in the lowest fruit set in all cultivars and self-pollination treatment also resulted in lowest fruit set. Finally he recommended that, artificial cross-pollination for commercial durian cultivation.

To evaluate the effects of pollination treatments that are commonly used in maize breeding and to investigate pollen effect, an experiment was conducted by Kahriman *et. al* in 2015. The results showed that pollination treatment affected the variation on all traits except for oil content, Self-pollination leads to significant reduction in kernel development. Pollen effect was found significant for most traits and this effect was evident on the related genotypes with open pollinated landrace. Results indicate that pollen effect is an important factor on kernel and ear development in small plot trials, where different types of maize are grown together.

Cruz *et. al* (2005) conducted study on Pollination efficiency of the stingless bee Melipona subnitida on greenhouse sweet pepper. Treatments of hand cross-pollination, hand self-pollination, pollination by bees and restricted pollination were performed in Northeastern Region of Brazil. Results showed that M. subnitida can be considered an efficient pollinator of greenhouse sweet pepper.

iv) Pollination in protected cultivation

Banda and Paxton (1991) stated that artificial mechanical vibration, using hand-held electrical shakers, is commercially employed for the pollination of tomato flowers in greenhouses, which results in tomatoes of higher quality than fruits derived from self-fertilization.

Kevan *et. al* (1999) reported on pollination, quality, and yield of greenhouse tomatoes. There is a relationship between the quality of pollen delivered and pollen distribution on the stigma with the marketability of fruit in terms of size and shape. They also states that quality of pollen and stigma is related to the rate of development, size and shape of the fruit and on the number of seed produced.

In an experiment conducted by Sabara and Winston (2003) on pollination recommendations of greenhouse sweet and hot peppers. Wind is unavailable under greenhouse conditions, insect activity is generally required to facilitate both self and cross pollination in pepper crops. The use of honey bees in green house is possible , but it is difficult because they do not like the still air and tend to attempt to leave forage outside.

Sushil *et. al* (2013) conducted experiment on impact of planned honey bee pollination on the seed production of three vegetables like Broccoli, kohl Rabi, Chinese cabbage. He concluded that planned honey bee pollination was found to inflict maximum impact on seed production of broccoli with an increased seed yield and net profit.

Carlos *et. al* (2012) had evaluated Mexican native bumble bee as a potential pollinator of greenhouse tomatoes. Maturation time was significantly longer and sugar content, fresh weight and seed count were significantly higher for bumblebee pollinated flowers than for flowers pollinated manually or with no supplemental pollination.

2.3 Pollen studies

2.3.1 Pollen physical characteristics

Shivanna and Rangaswamy (1993) described pollen is the male partner in the fertilization process and it is reduced in to three cells, two are male gametes encapsulated in the protecting, decay- resistant wall.

Sosnoskie *et. al* (2009) conducted study on pollen grain size, density, and settling velocity for Palmer Amaranth (Amaranthus palmeri) that influence pollen flight. The mean diameter for Palmer amaranth pollen, as determined by light microscopy, was 31 mm (range of 21 to 38 mm).

Storme *et. al* (2013) conducted experiment on volume-based pollen size analysis. They found that volume-based pollen size measurements are not biased by the pollen shape or position and substantially reduce non-biological variation, allowing a more accurate determination of the actual pollen size.

2.3.1 Pollen storage

Iwanami (1972) demonstrated the feasibility of storing pollen grains in organic solvents. This method has been tested with many pollen systems especially two celled systems had been found suitable.

Saoji and Rewatkar (2015) conducted studies on pollen storage at different temperature and humidity conditions in oryza sativa. The experimental results reveal that the pollen grains of some varieties could be well presented for a period of 20 days at high relative humidity and low temperature (3^0C). The germination ability of pollen was observed to be good up to 6th day of storage and during the remaining days there appears to be slow decline in the germination ability of stored pollen grains.

Kwon *et. al* (2005) conducted study on collection, germination and storage of water melon pollen for pollination under temperate conditions. They adopted

adequate pollen collection and storage techniques to improve fruit set in watermelon and they tested water melon by using organic solvents pentane, ethyl ether, benzene and acetone. Results shown that pentane and benzene solvents collected higher number of pollen compared to other solvents, pentane has higher pollen germination rate compared to other solvents. They noticed that with increasing temperature from 15, 20, 25, 30 and 350C has increases the pollen tube growth. During experiment they stored pollen for 4 weeks at room temperatures -10, 4, +100 c. and observed pollen stored at -10 0C had better germination rate. They recommended that storage of pollen at 4 0C would give better results.

Shin *et. al* (2007) had investigated effect of pollination methods on development and sugar content of oriental melon fruits. Three treatments Honey bee pollination, Bumble bee pollination and control treatment were applied to oriental melon fruits and they compared the results of three treatments. Results shown that fruits pollinated by honey bee and bumble bees has lesser length and width compared to fruit pollinated by control treatment (fruit setting growth regulator) but fruits pollinated by both bumble bee and honey bee has increased and soluble solids compared to controlled pollination.

2.3 .3 Pollen viability

Aref and Baki (1992) conducted experiment on determination of pollen viability in tomatoes. He described a procedure for determining pollen viability in tomatoes by growing pollen in a growth medium containing 0.29 sucrose, 1.27 mMca(No3)29, 0.16mM H3BO39 and mMKNO3 to which 0.001% of FDA (flurescein diacitate) is added. He concluded that neither the germination medium nor FDA has any adverse effect on germination and pollen tube growth. and also he suggested that flurescence is a good measure of pollen viability.

Yaxin *et. al* (2011) investigated pollen viability, pollen longevity, and pollen size using different materials. He concluded that pollen size was in the range of 42.5 to 54.0 μm. No significant difference was observed in average pollen size between transgenic and control plants, pollen viability and longevity can be affected by increasing temperature and ultraviolet-B irradiation, but relative humidity had only limited impact. Weather conditions had a large impact on pollen longevity. Under sunny atmospheric conditions, pollen longevity of both cultivars decreased rapidly, with a half-life of <4.9 min and a complete loss of viability in 20 min. Under cloudy atmospheric conditions, the half-life of pollen was more than fivefold longer than under sunny conditions, and it took approximately 150 min to lose viability completely.

Mayer and Gottsberger (1999) had reported on pollen viability in the genus Silene (Caryophyllaceae) and its evaluation by means of different test procedures. In this experiment they conducted and compared the results of both direct and indirect tests to estimate pollen viability. After comparing the results of both direct and indirect test they concluded that germination percentages obtained by conducting in vivo test was ranged from 67 to 77%, compared to FCR , ALS have high pollen viability ranging from 82 to 93%.

Donald *et. al* (2000) conducted study to determine viability of pollen after suspension in cold water from 0-34 days. Linear regression analysis of in vivo and in vitro tests were conducted and results shown that filled seed efficiency and pollen viability decreased about 3 percent per day.

2.3.2 Pollen mixers

Kim *et. al* (1997) utilization of various charcoal powders as new economical pollen diluents for kiwifruit pollination. They studied the effect of artificial pollination using wood charcoal powder as a diluents in place of commercial imported diluents. They noticed no significant differences in fruit set, fruit weight and

size at harvest and observed the cost of the pollen diluent was greatly reduced when charcoal powders were used.

Zahra and Abbadi (2007) conducted experiment on effects of artificial pollination on Pistachio (Pistaciavera L.) fruit cropping. In the experiment they selected six shots of different pollinizers and applied them on each tree, they applied four mixtures of pollen (2, 4, 6 and 8%) with wheat flour. Results shown that artificial pollination of pistachio has both positive and sometimes negative effects on number of fruits per cluster. Artificial pollination increase number of fruits per cluster, total yield per cluster and also improved nut size and kernel dry weight in pistachio trees.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

In this chapter the procedure followed in the selection of tropical vegetable crops, study of floral characteristics and the physical characteristics of flower are explained in detail. Methods followed to study physical characteristics of anther and pollen which is suitable for designing artificial pollinizer was explained briefly. Functional requirements of an artificial pollinizer and a conceptual design of it are explained. Methods adopted for the design and development of two models of artificial pollinizers are detailed. Procedure followed for the validation of the developed artificial pollinizer for pollinating selected tropical vegetable crops under protected cultivation is also narrated.

3.1 Geographical location of experimental site

The field experiments were conducted at Department of Agricultural Engineering and Department of Olericulture of College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur distinct, Kerala. The site is located at 10^0 31'N latitude and 76 ^{0} 13'E longitude at an altitude of 22.25 m above MSL. The area experiences typical humid tropical climate and receives average annual rainfall of 3006.2 mm. The average minimum and maximum temperature was 23.20 ^{0}C and 32.36 ^{0}C respectively.

3.2 Pollination in plants

Pollination is the process by which pollen from the male part of a plant, called stamen is transferred to the female reproductive organ of a plant, called stigma, and thereby enabling fertilization and production of seeds. It may take place naturally or artificially.

3.2.1 Natural pollination

When pollination is caused due to the action of natural agents such as insects, birds, water or wind or even plants themselves and when self-pollination occurs within closed flower, process is termed the Natural pollination. Natural pollination often occurs within the same species of plants.

3.2.2 Artificial pollination

Artificial pollination is the process where humans intervene with the natural pollination process. They carry pollen, or plant sperm, from one flower to another, allowing the pollen to fertilize the ovaries and create seeds that will develop into fruits and new plants. Artificial pollination is carried out by manual means or by using machines.

i) Manual pollination

Manual pollination is a technique that can be used to pollinate plants when natural pollination is either undesirable or insufficient. This method of pollination is done by manually transferring pollen from the stamen of one plant to the pistil of another. This is often done with a cotton swab or small brush, removing the petals from a male flower and brushing it against the stigmas of female flowers, or by simply shaking flowers in the case of bisexual flowers.

ii) Machine pollination

When artificial pollination is done by some mechanical means, it is termed as machine pollination. Machine pollination can be done with the help of pollination machines like puffer gun, hand operated pressure sprayer, boom sprayer and pressure vessel, battery powered portable duster, Turbo Bee and Robotic Bees (Barnett *et al.*, 2010). Pollen shakers or vibrators are used for artificial pollination in a lower scale.

3.3 Selection of tropical vegetable crops

Six tropical vegetable crops which were available during the study period at the study area are selected to study the floral and physical characteristics. The crops selected are tomato, chilli, pumpkin, ash guard, cucumber and water melon.

3.4 Floral characteristics

Floral characteristics of flower influences pollen collection and pollen deposition. They give clear idea about time at which pollen grains are collected from male flower and deposition of collected pollens on to the stigma of female flower. Some important floral characteristic of flower are type of flower, breeding system, sex expression, sex ratio and time of anthesis.

3.4.1 Type of flower

Flower is the reproductive structure found in flowering plants. The biological function of a flower is to effect reproduction, usually by providing a mechanism for the union of sperm with eggs. Generally, flowering begins in cucurbits with the production of staminate flowers, and depending on the species, pistillate or hermaphrodite flowers later appear on the plant. Tomato and chilli have both staminate and pistillate part in a single flower. Cucurbits have separate staminate and pistilate flowers either on the same plant or on different plant.

3.4.2 Breeding system

The breeding system explains how pollination is taken place in flowers. Tomatoes and chili are generally self-pollinating vegetables because they have relatively small, inconspicuous flowers that can shed pollen directly on to the stigma, they have both male and female part on the same flower and they do not need assistance of bees insects or wind for pollination through cross pollination was also

possible in tomato and chili. Pumpkin, ash guard, water melon and cucumber are cross pollinated in nature where bees act as pollinating agent.

3.4.3 Sex expression

Sex expression refers to the contrasting features of male and female individuals of the same species. Sex is usually of two types, male and female. Cucurbit plants provide different types of sex expression and most commercial cultivars of these crops are monoecious, andromonoecious, or gynoecious. Monoecious cucurbits produce separate staminate (male) or pistillate (female) flowers on the same plant. Andromonoecious cucurbits have both staminate and hermaphrodite (perfect) flowers on the same plant, while gynoecious plant types produce only pistillate flowers. Sex type was recorded at flowering stage and parental line were grouped into monoecious, gynocieous, andromonocious and dioecious.

3.4.4 Sex ratio

Sex ratio is the ratio of male flowers to female flowers in a population. Staminate flowers are generally produced every day or every other day and normally develop singly or in clusters in leaf axils, while pistillate flowers are only produced singly and at much less frequent intervals than males. The time required from seedling emergence to the onset and termination of flowering, as well as the time that elapses between the appearance of staminate and then either pistillate or hermaphrodite flowers vary with cucurbit species/cultivars and also depend on environmental conditions during the growth period.

Number of male and female flowers was counted starting from the commencement of flowering till its completion to determine sex expression.

$$\text{Sex ratio} = \frac{\text{No. of male flowers}}{\text{No.of female flowers}}$$

3.4.5 Time of anthesis/ flower opening

Time at which the flowers were fully opened and anthesis takes place was recorded. Most cucurbit flower opens early morning and remains opened until afternoon. Stigmas are receptive during anthesis but stigmas are most receptive soon after the flower opens.

The floral characteristics of Tomato, chilli, pumpkin, ash guard, cucumber and water melon are consolidated and presented in Table 3.1.

3.5 Physical properties of flower and their measurements

Some important physical characteristics of anther, flower and pollen that are useful for designing artificial pollinizer were studied. Number of anthers, length, pedicel girth and weight of flower, length of anther, thickness of anther, quantity of pollen, length and diameter of pollen are some important physical characteristics which influence design of artificial pollinizer. Procedure followed for these are described in the following sub sections.

3.5.1 Number of anthers

Number of anthers are different for different selected tropical vegetable crops. It can be counted manually.

3.5.2 Length of flower

Length of a flower can be measured by using steel rule or vernier calipers by keeping one end of scale at the tip of petal and other end of scale at the stem of flower as shown in plate 3.1(a).

3.5.3 Pedicel girth of flower

Pedicel girth of flower was measured at lower pedicel portion with help of vernier calipers.

Flower was held between the lower jaws of the calipers and corresponding reading is recorded as shown in the plate 3.1(b).

3.5.4 Weight of flower

Collected flowers and anthers could be weighed using precision electronic balance model GP-332 (Plate 3.2). The sensing capacity range of the balance is from 0.0018 g to 320 g within a temperature range of 15-45^{0}C and requires power of 230 watts. Ten flowers of each of all the selected tropical vegetable crops are weighed separately and the average weight of one flower was calculated and recorded. Similar procedure in adopted for measuring the weight of anthers also.

Table 3.1 Floral Characteristics of selected tropical vegetable crops

Crop	Cucumber	Water melon	Pumpkin	Ash guard	Tomato	Chilli
Flower type	Staminate and pistillate or only pistillate	Staminate and pistillate or staminate and perfect	Staminate and pistillate	Staminate and pistillate	Staminate and Pistillate	Staminate and Pistillate
Sex expression	Monoecious or gynoecious	Monoecious or andromonoecious	Monoecious	Monoecious	Monoecious	Monoecious
Breeding system	cross-pollination	cross-pollination	cross-pollination	cross-pollination	Self-pollination	Self-pollination and often cross pollination
Sex ratio	10:1 or all or predominantly pistillate flowers	5:1–13:1	3.5–10:1	34:1	1:1	1:1
Time of pollination	6 – 8 am	5:30- 7am	5-7am	4:30 -7:30 am	7 to 9 am	5 to 7 am

(Bomfim *et al.*, 2016)

3.5.5 Length of anther

Length of anthers of cucumber, water melon, pumpkin and ash guard were measured by using vernier calipers. Anther is held horizontally between the lower jaws of verneir calipers and corresponding reading were noted. Ten replications for each crop is done and average is calculated and tabulated. In case of tomato and chilli the anther is too small so their length was measured by keeping 10 anthers in line and the total length was measured with a vernier calipers and divided by 10 to get the length of one anther. Ten replications of these were done and average value is tabulated.

3.5.6 Thickness of anther

Thickness of anther was measured by using vernier caliper. Anther was held vertically between the lower jaws of vernier calipers corresponding reading is noted as shown in plate 3.3. Ten replications of each crop is measured and the average thickness is calculated.

3.6 Pollen studies

Size and shape of pollen and pollen output influences the design of artificial pollinizer. Pollen viability, pollen storage and pollen mixers affects the fruit set efficiency. So investigations on these aspects were carried out and the procedures were detailed here.

3.6.1 Pollen collection

Pollen grains from each selected crops were collected manually for conducting the pollen studies and the procedure is explained below:

- Well-developed male flowers that are not yet opened are selected for pollen collection.

- Two or more sepals and petals are removed to expo se the petals on one side of flower in case of tomato and chilli.
- Collect pollen from male parent in tomato and chilli the anther is removed and it is opened by using dissecting needle along length, collect the pollen on the tip of needle by dragging upwards through the side of one of anther.
- In case of pumpkin, ash guard, cucumber and water melon small brush is used to collect pollen grains from male flower.
- The collected pollen grains were taken in separate petridishes for studies.

3.6.2 Pollen measurements

Pollen grains from freshly dehisced anthers were collected manually after anthesis. Pollen size and shape were observed by using Olympus Bx43 light microscope and the measurements were taken by using the software ultrascope version 9.1. The procedure is detailed below:

- A slide of pollen grains is prepared by gently dropping the pollen on a glass slide using the needle or laboratory wire loop
- Add 1 or 2 drops of glycerol to it carefully
- Gently place the cover slip on the sample at an angle to remove air bubbles,
- Place the slide on the microscope for viewing.
- Connect the microscope to the laptop. Pollen images were captured and measurements were taken using ultascope software.

Ten replications each were done, to ensure precision, and the average values are tabulated.. The experiment set up is shown in Plate 3.4

(a) (b)

Plate 3.1 Measurement of length and pedicel girth of flower

Plate 3.2 Electronic balance

Plate 3.3 Measurement of anther thickness

Plate 3.4 Setup for dimension measurements of pollen grai

3.6.3 Assessment of shape of pollen grains

Shape is the external form or outward appearance of an object. According to the document published by International Union for Protection of new Varieties of Plants (UPOV, 2007) the term shape is defined as length/width ratio to develop quantitative characteristics related to it, rather than considering shape as a single qualitative characteristic. In that respect, it is possible to define a plane shape using the ratio of length/width, position of broadest part and lateral outline. A chart is also prepared to ensure the ratio of length to width for three different sets of shapes such as parallel, rounded and angular as shown in Table 3.2.

The dimensions of the pollen grains of all the selected crops were measured separately as explained in section 3.6.2 and the shape of each pollen grains were found out by using the chart. The shape category obtained from the chart is presented in tabular form.

3.6.3 Pollen output from a single flower

Pollen output refers to number of pollen grains collected from a single male flower.

- Ten ml of water is added to the pollen collected in petridish or glass container
- Gently place one pollen mixed with water on a slide by using syringe.
- Place the cover slip on the sample at an angle to remove air bubbles
- Place the slide under the microscope for viewing
- Take a photograph of pollen grains using ultrascope software - 9.1.
- Count the number of pollen grains present in each drop by observing the photograph
- Total number of pollens collected from one flower can be calculated by counting number of pollen grains in each drop and number of drops in one ml,

Total quantity of water in ml added to pollen grains collected from single flower.

Total number of pollen grains in a flower = No. of pollen grains present in each drop × No. of drops contained in one ml × total quantity of water added to pollen grains collected from single flower in ml.

Table 3.2 Chart for simple symmetric plane shapes

Sl. No.	Ratio length/width	>6:1	6:1 to 3:1	2:1 to 1.5:1	1.2:1	1:01	01:01.2	1:1.5 to 1:2	1:3 to 1:6
			very elongated	moderately elongated	slightly elongated	medium	slightly compressed	moderately compressed	very compressed
1	Parallel set Oblong								
2	Rounded set Ovate								
3	Elliptic								
4	Obovate								
5	Angular set Triangular								
6	Trullate								
7	Rhombic								
8	Obtrullate								
9	Obtriangular								

1. narrow deltate 2. medium deltate 3. broad deltate 4. quadrate rhombic 5. Circular 6. narrow oblate 7. medium oblate 8. broad oblate 9. Square 10. transverse broad oblong 11. transverse medium oblong 12. transverse narrow oblong 13. narrow obdeltate 14. medium obdeltate 15. broad obdeltate.

3.6.4 Pollen mixers

Pollen is a fine to coarse powdery, sticky substance so it has to be mixed with some inert, nontoxic substance for artificial pollination. General pollen mixers are talc powder, wheat flour, nylon powder, thermo plastic poly amide, charcoal and water (Weiguang *et.al.,* 2013). Water is selected as pollen mixer for this study as water is easily available and more suitable for pollens which are stickier in nature. Also the pollen samples collected by water medium can be directly used for pollination without any obstruction.

3.6.5 Pollen viability

Pollen viability refers to the ability of the pollen to germinate on stigma and perform its function of delivering male gametes to the embryo sac. Viability of pollen was determined by following two methods

i) Acetocarmine staining method.

Pollens were taken from staminate flowers selected randomly stained with 1:1 glycerin to acetocarmine and observed under light microsope. Unstained, under stained and partially stained pollen grains were scored as non-viable and the uniformly stained and properly filled pollen were scored as viable. Viability was estimated as percentage of the number of viable pollen grains to the total number pollen grains scored

$$\text{Pollen viability percentage} = \frac{\text{No. of viable pollen grains}}{\text{Total no.of pollen grains}} x100$$

ii) Invitro pollen germination method.

Fresh pollen grains were put in to culture medium containing 1 % sucrose in cavity slides and kept them covered in a petridish and incubated for 15 min at room temperature. Slides were made for each pollen sample and were examined under a

light microscope to observe pollen tube growth. The total grains present in each sample and the no. of grains germinated grains were counted.

The pollen fertility was calculated using the following formula

$$\text{Pollen fertility percentage} = \frac{\text{No. of pollen grains germinated}}{\text{Total no.of pollen grains}} x100$$

3.6.6 Pollen storage

Pollen grains are stored in suitable medium to enable artificial pollination for longer periods. So pollen grains collected on a particular day can be stored for one or two days and could be used for artificial pollination on preceding days. In order to check storage qualities of pollen grains viability tests are conducted after 24 and 48 hours of storage in different media. Storage of pollen grains were done in two media viz. pure water and 1 per cent sucrose solution. Procedure is described below.

i) Pollen grains stored in pure water:

Pollens collected in the container by spraying clean water were sucked with the help of a syringe. One drop of this collected sample were transferred to cavity slides for storage studies. Cavity slides were kept at room temperature and viability test were conducted after 24 hrs. and 48 hrs. as explained in section 3.6.5 and data were recorded.

ii) Pollen grains stored with 1% of sucrose solution:

In this method pollen grains are stored in 1 % sucrose. 1 % sucrose solution is prepared by adding 1 g of sucrose in 100 ml of pure water and dissolving it well. This solution is used for spraying to the male flowers through the nozzle of hand pump. The sample thus collected is used to prepare cavity slide. Cavity slides were kept at room temperature for storage studies. Viability tests were conducted after 24 hr. and

48 hr. of storage as explained earlier. The results are tabulated and also represented graphically. Views of the sample slides prepared is shown Plate 3.5.

3.7 Design considerations for artificial pollinizer

The strategies and parameters for the design of artificial pollinizer were evolved based on the observations made on physical properties of flower and pollen. Based on the minimal requirements and literature review it was learnt that the artificial pollinizer essentially comprise of two devices other for separation of pollen from anthers which can be termed as pollen collection device and one is for deposition of collected pollen on to the stigma which can be termed as pollen deposition device.

3.7.1 Design of artificial pollinizer

The steps followed in the design of artificial pollinizer is shown in fig 3.1 in the form of flow chart. The main objective of the study is to enable artificial pollination under protected cultivation. The functional requirements considered in the development of artificial pollinizer are furnished below

- It should enable artificial pollination in selected tropical vegetable crops under protected cultivation. Protected cultivation offers barriers to natural pollination and artificial pollination by manual means are time consuming and less efficient.
- It should be a simple hand held device operated by pneumatic or fluid pressure.
- Pollen grains from anthers of male flowers could be collected with ease.
- The collected pollen grains should not have any damage in terms of viability.
- The medium for collection of pollen grains could be air or water.
- In case of pollen collection through air. There should be some suitable filtering element to separate pollen grains from the air pollen mixture.

- Collected pollen could be easily mixed with some inert pollen mixers, preferably water to enable artificial pollination.
- In case of pollen collection through water medium, there should be provision for spraying water at sufficient pressure to the flowers for separating the pollen from the anther.
- There should be a water sealed container which can be opened and closed easily to accommodate flowers on plants without damaging it.
- Sprayed water along with pollen grains should be collected in the container without any wastage.
- The collected pollen water mixture could be sucked easily and sprayed to the stigma of female flower for artificial pollination.

In view of the above, two models of pollen collection devices are designed, using different media for collection, to check the suitability for different crops.

3.7.2 Conceptual design model – I

Pollen collection device model - I is designed based on the functional requirements as described earlier by using air as the medium for pollen collection. The air is sucked by using a vacuum pump through pollen collection tip and a collection chamber. Pollen collection unit has got a brush at the tip to detach pollen grains from the flowers and same is sucked through hallow tubes to the collection chamber. The collection chamber is provided with a suitable size screen mesh to separate pollen grains and air. The air is then sucked by a vacuum pump and released to atmosphere. A schematic view of conceptual design is shown in Fig. 3.2

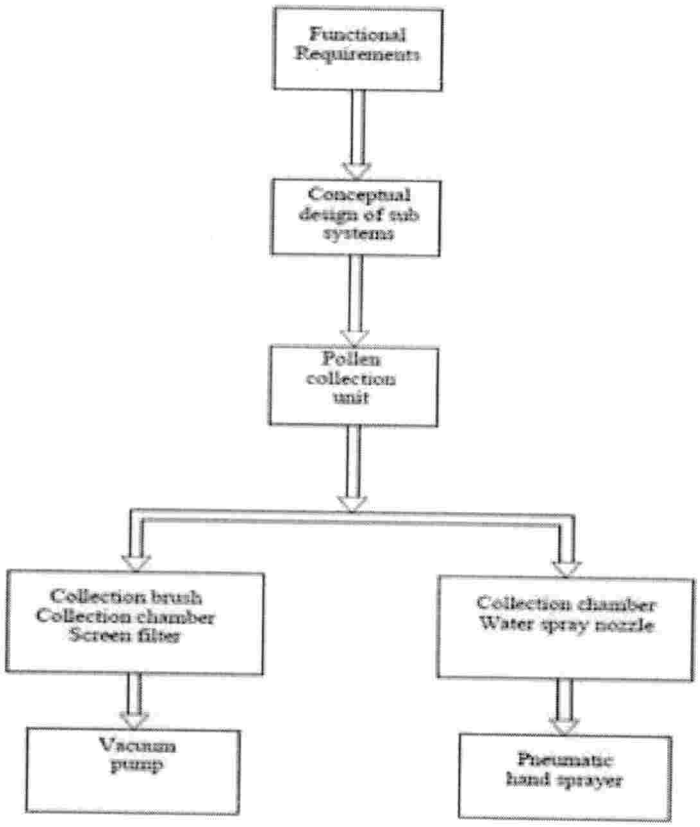


Fig. 3.1 Flow chart of the design process

3.7.2 Conceptual design model – I

Pollen collection device model - I is designed based on the functional requirements as described earlier by using air as the medium for pollen collection. The air is sucked by using a vacuum pump through pollen collection tip and a collection chamber. Pollen collection unit has got a brush at the tip to detach pollen grains from the flowers and same is sucked through hallow tubes to the collection chamber. The collection chamber is provided with a suitable size screen mesh to separate pollen grains and air. The air is then sucked by a vacuum pump and released to atmosphere. A schematic view of conceptual design is shown in Fig. 3.2.

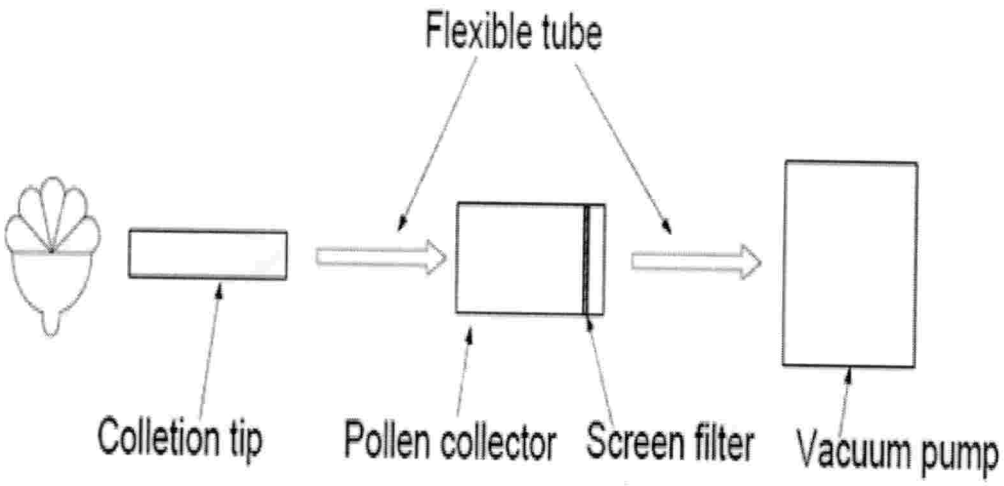


Fig. 3.2 Conceptual design(Model – I)

3.7.3 Conceptual design Model – II

The second model uses water as the medium for pollen collection. Pollen collection unit uses water spray through the nozzle of a pneumatic hand sprayer. The nozzle of the sprayer and the male flower for pollen collection are placed at two ends of a water tight container without damaging the flower. Water is sprayed to the opened flower and the spray wash out the pollen grains and the mixture is collected in the water light container. This water pollen mixture can be directly used for artificial pollination. The diagram of conceptual design of pollen collector Model – II is shown in Fig. 3.3.

3.8 Designs for sub systems for Model – I

Pollen collection unit Model –I is based on pneumatic pressure created by a vacuum pump. The other components of the model are pollen collecting brush, pollen collecting tube, pollen collecting chamber, screen mesh, an adopter for pollen tube

and adaptors and fixtures to the pump. Design and development of these components are detailed in the following subsections

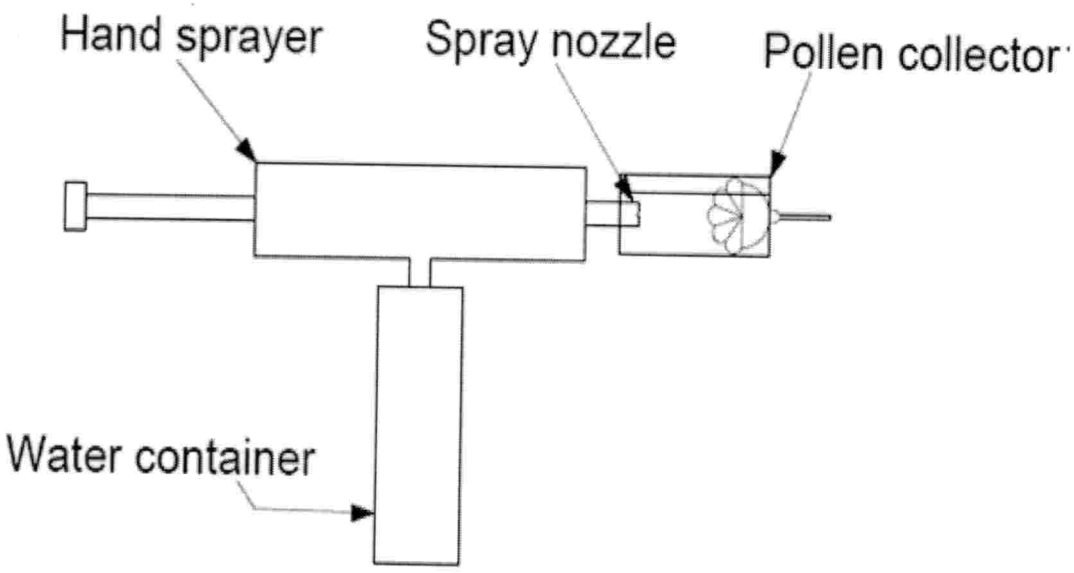


Fig. 3.3 Conceptual design of (Model – II)

3.8 Designs for sub systems for Model – I

Pollen collection unit Model –I is based on pneumatic pressure created by a vacuum pump. The other components of the model are pollen collecting brush, pollen collecting tube, pollen collecting chamber, screen mesh, an adopter for pollen tube and adaptors and fixtures to the pump. Design and development of these components are detailed in the following subsections

3.8.1 Pollen collecting unit

A hollow rectangular cross section tube made of light weight plastic material is used for pollen collection. A brush is provided at one end of rectangular tube so

that the pollen grain could be wiped off to the center where vacuum pressure created by pump acts. So the pollen grains can be easily carried by the air, sucked by the vacuum pump, which goes to the pollen collecting chamber. The design sketch of the pollen collecting unit are shown in Fig 3.4 and Plate 3.6.

3.8.2 Pollen collecting tube

Pollen collecting tube is a flexible tube of 11 mm inner diameter and has a length of 400 mm. One end of the this flexible tube is connected to the rectangular pollen collecting tube and the size of the tube is selected in such a way that an air tight connection is obtained between the two tubes. The dimensional sketch is shown in Fig.3.5 (a) and the view of tube is shown in Plate 3.7.

3.8.3 Adapter for pollen collecting chamber

Pollen collecting flexible tube is attached to the pollen collecting chamber by using a suitable adopter, so that the chamber can be attached and detached with ease using threaded joint. The adapter consists of tube connector, an air tight washer and a cup with internal threads to suit the pollen collecting chamber. The sketch of the adapter unit is shown in Fig. 3.5 (b).

3.8.4 Pollen collecting chamber

A longitudinal, cylindrical chamber made of transparent plastic material having a length of 115 mm and diameter 24 mm is used as the chamber for pollen grains collection. One end of the chamber is provided with external threads to suit to the internal threads of the adapter cap. The other end of the cylindrical chamber is covered with a screen mesh of 15 micron size. The mesh is fixed to the chamber in such a way that only air is allowed to pass through the mesh and all pollen grains are collected in chamber when this is connected to the vacuum pump. Pollen collecting chamber is shown in Plate 3.8 and dimensional view of pollen collecting chamber is shown in Fig. 3.5 (c)

i) Micro mesh screen

Screen size is selected based on the studies of physical characteristics of pollen grains of selected tropical vegetable crops. The minimum pollen size observed in the study was 17 micro-meters which is equivalent to 0.017 mm. So a stainless steel micro mesh screen of 15 micro-meter is selected for separating pollen grains and air in the pollen collecting chamber as shown in plate 3.9 and Fig. 3.5. .

3.8.5 Fixtures and adaptors for vacuum pump

Pollen grain collecting chamber is attached to the vacuum pump by using some fixtures and adaptors. It includes an air tube, air tube adaptor and vacuum pump connector.

i. **Air tube :** One end of the cylindrical pollen collecting chamber, where the micro mesh screen is fixed is inserted to the flexible transparent polyethylene tube. The size of air tube is selected as 22mm (internal dia) so that an air tight joint is obtained without any bonding materials. The tube is having a length of 500 mm and the other end is connected to the vacuum pump using suitable adaptor and connectors. The dimensional view of air tube is presented in Fig. 3.6 (a) and Plate 3.10.

ii. **Air tube adaptor**: Air tube is attached to the connector for vacuum pump by using an adaptor as shown in Fig. 3.6 (b).

iii. **Vacuum pump connector:** Vacuum pump connector is used for connecting the air tube and vacuum pump. The connector is attached to the inlet side of vacuum pump by a quick fix coupling and rubber washer. The pump connector is a conical hollow tube having 225 mm length and 22 mm and 50 mm diameter on either sides. Vacuum pump connector is shown in Fig. 3.6 (c) and Plate 3.11.

3.8.6 Vacuum pump:

A vacuum pump is selected based on preliminary studies conducted to access the pollen sucking velocity of air. Three different velocities viz. 10 m/s, 20 m/s and 30 m/s were used and pollen sucking ability at different air velocities are observed by trial and error method. According to this study an electric powered vacuum pump capable of developing wind velocity above 20 m/s is selected. The specifications of the selected pump is presented in Table 3.3 and dimensional features are shown in Fig.3.7. Different views of the vacuum pump are shown in Plate 3.12.

Table 3.3 Specifications of Vacuum pump

Sl. No.	Parameter	Range
1	Voltage	220 volts
2	Frequency	50 HZ
3	Input power	550 watts
4	Rated speed	13000rev/min
5	Wind velocity	20-22 m/s

3.9 Design of sub systems of Model – II

The pollen grains which are too sticky in nature cannot be collected easily by vacuum pump method as done in model – I. The pollen grain will not detach completely and there are chances of adhering to the tube and collector surfaces. So the model – II tried as per the conceptual design for using water as the collecting medium. The Model– II consists of pollen collecting chamber, water c container,

water spraying. nozzle and a hand sprayer working on pneumatic pressure. Design and development of the different sub systems are explained in the following sub sections.

Plate 3.5 Sample cavity slides prepared for storage studies

Plate 3.6 Pollen collection unit for model- I

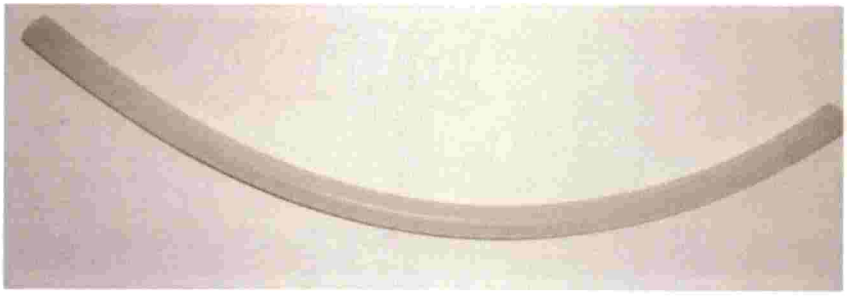


Plate 3.7 Pollen collection tube

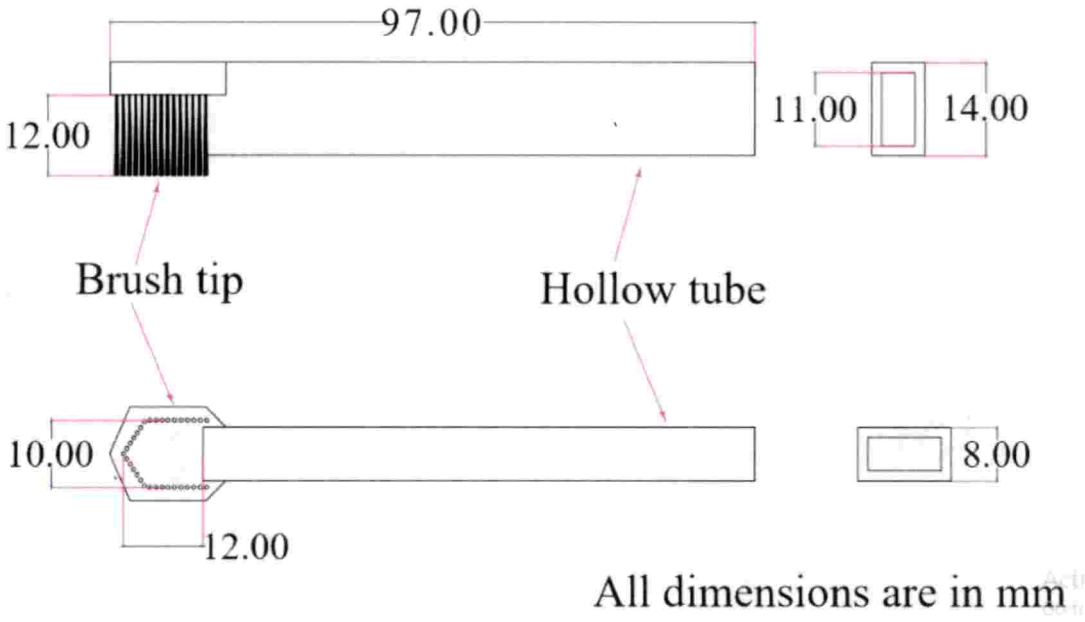
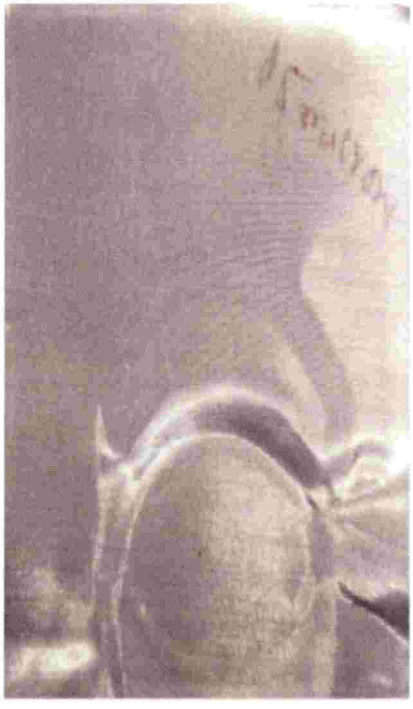
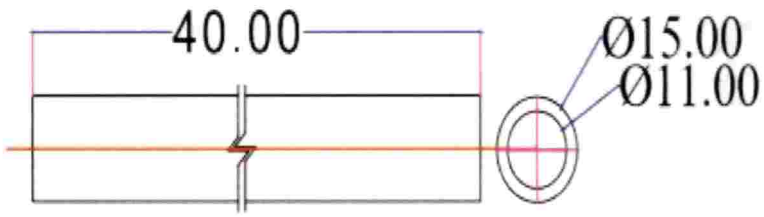


Fig. 3.4 Dimensional views of pollen collecting tip for artificial pollinizer model- I

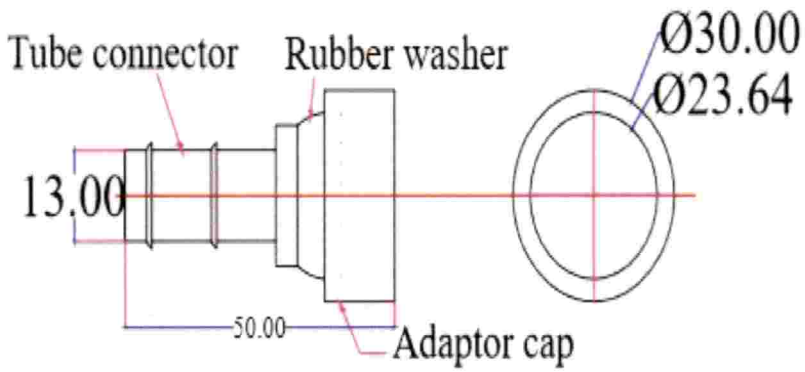
3.8 Pollen collection chamber



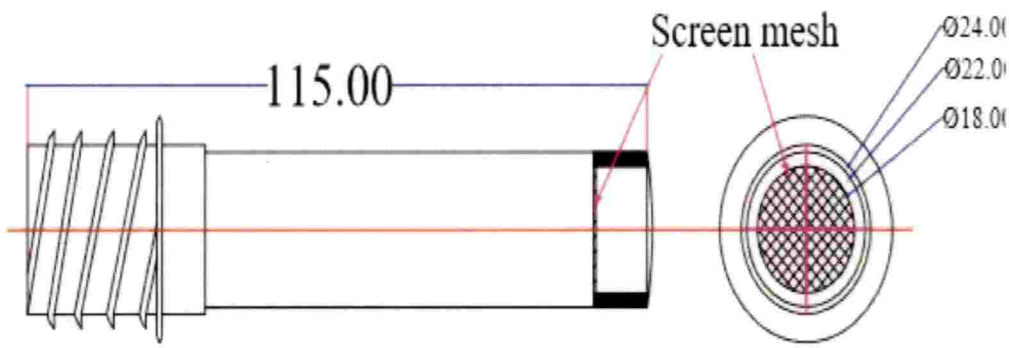
3.9 Micro mesh screen



a) Pollen collecting tube - 1



b) Adaptor for pollen collecting chamber

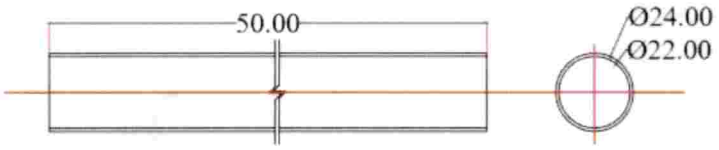


c) Pollen collecting chamber

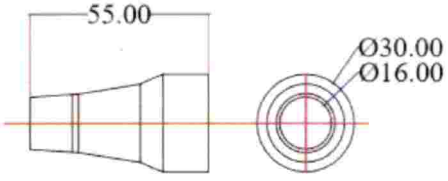
Fig. 3.5 Dimensional views of pollen collecting tube and chamber for artificial pollinizer model -II

Plate 3.10 Air tube connecting chamber and vacuum pump

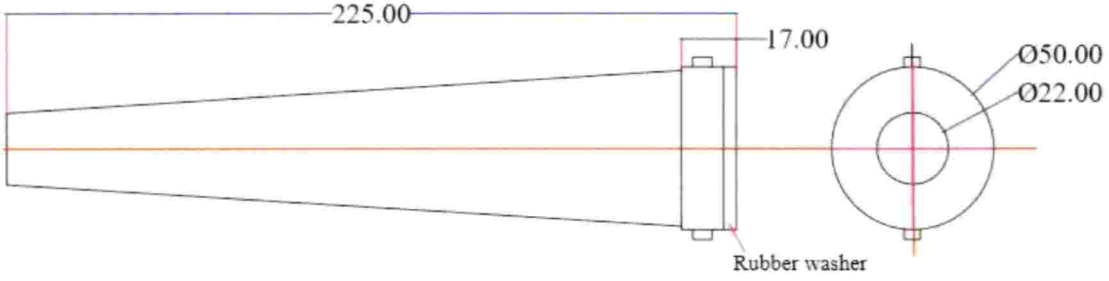
3.11 Vacuum pump connector



a) Air tube -2



b) Air tube adaptor



c) Vacuum pump connector

All dimensions are in mm

Fig.3.6 Dimensional views of fixtures and adapters for artificial pollinizer model - I

Plate 3.12 Vacuum pump unit

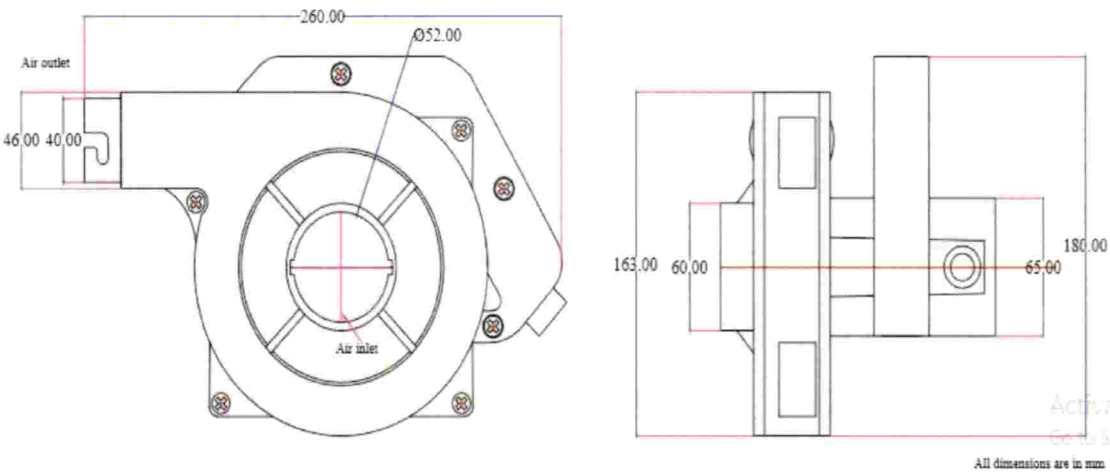


Fig. 3.7 Dimensional views of vacuum pump for artificial pollinizer model -I

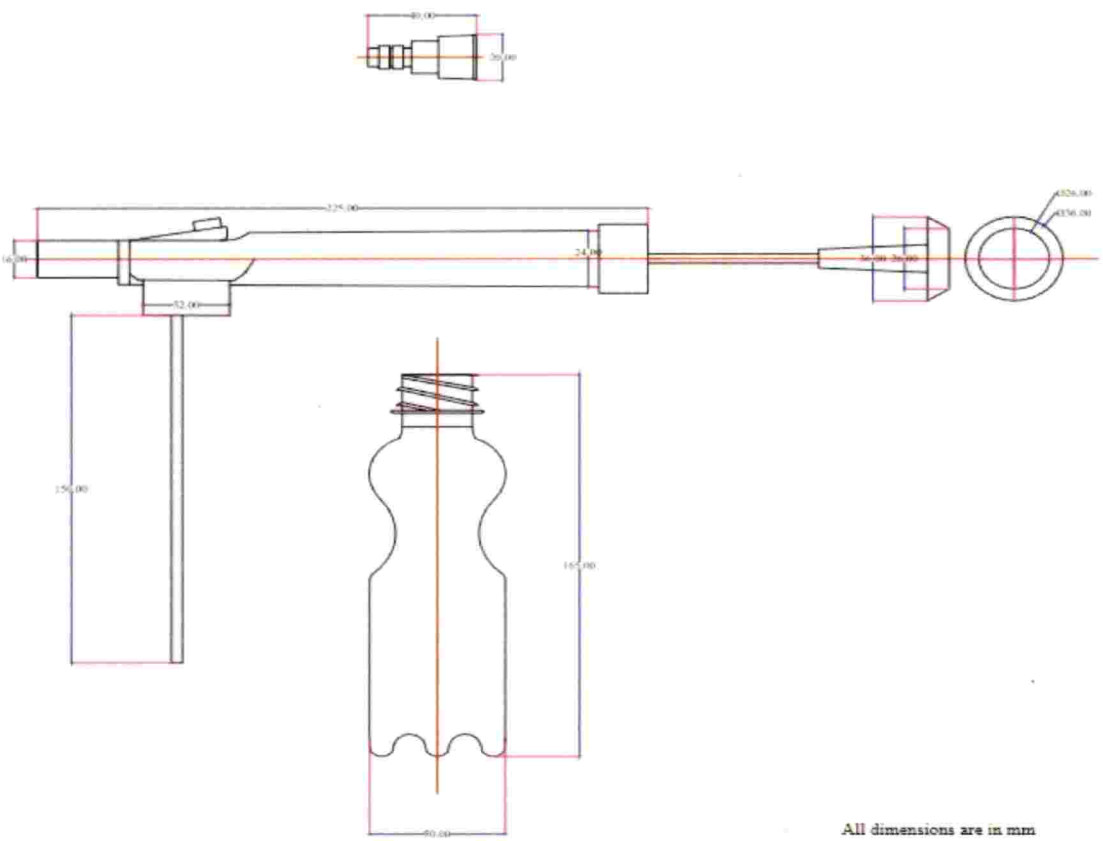


Fig. 3.8 Dimensional views of components for artificial pollinizer model –II

3.9.1 Pollen collecting chamber

A water tight container made of good quality plastic material with an openable lid and of size 92 x 68 x 24 mm available in the market is taken as pollen collecting chamber. An orifice of 7 mm for fixing the water spraying nozzle is given at one side of the container and a V-shaped slot for flower insertion of 15 mm depth and 4 mm width at top is made on opposite side as shown in Plate 3.13.

The length of pollen collecting chamber is selected to get an appropriate spray pattern with enough pressure to collect all the pollen grains from the flower is washed off and collected in the chamber. Fig. 3.9 shows the dimensional sketch of the pollen collection chamber.

3.3.2 Spray nozzle

A water spray nozzle which gives a uniform solid cone spray pattern and made of brass material is fixed at one shorter side of the chamber. An orifice of size 7 mm was given on the chamber to fix the nozzle on the opposite side of slot given for inserting flower. This arrangement enable the water sprayed to reach uniformly on the flower and was off all the pollen grains which can be collected in the pollen collecting chamber. Spray nozzle is shown in Plate.3.14 and Fig 3.8 (a). The fixing of nozzle to the container and position of V-shaped slot are shown in Plate 3.14 (b).

3.9.3 Pneumatic hand sprayer

A plunger type hand sprayer which forces the liquid on compressed air pressure is used for spraying water. The sprayer has got a plunger and barrel, pressuring handle, provision for fixing standard container for taking water, suction pipe, a needle valve to release air pressure and a valve operating knob. The details of hand sprayer are shown in Fig. 3.8 (b) and Plate 3.15.

3.9.4 Water container

A transparent poly ethylene bottle of 200 ml capacity is taken as the water container as shown in Fig. 3.8 (c) and Plate 3.16.

Plate 3.13 Pollen collecting chamber (Model – II)

(a) **(b)**

3.14 Spray nozzle and method of fixing it on the collecting chamber

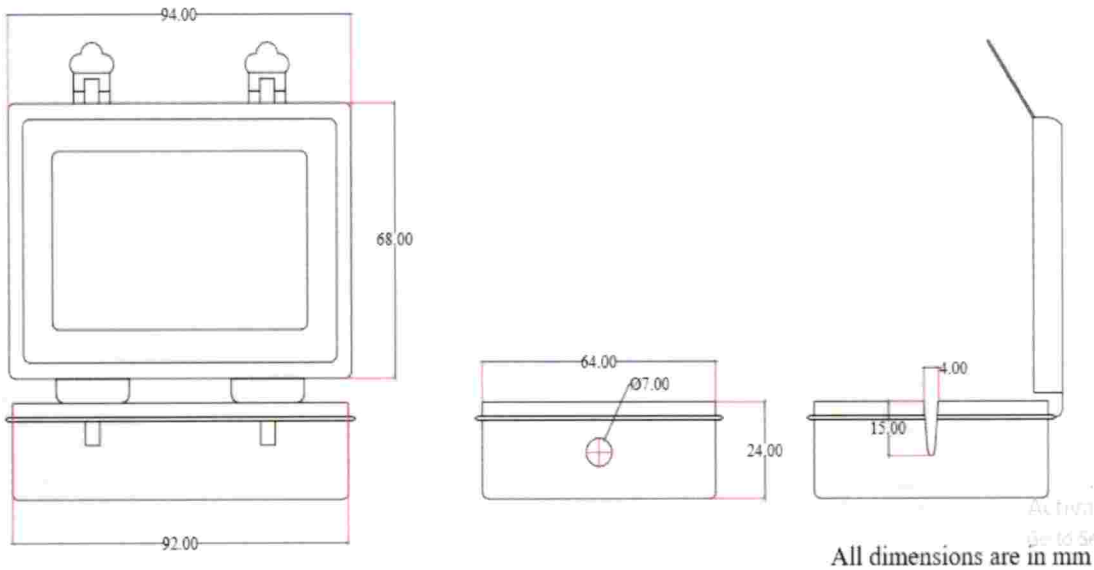


Fig. 3.9 Dimensional views of pollen collecting chamber for artificial pollinizer model – II

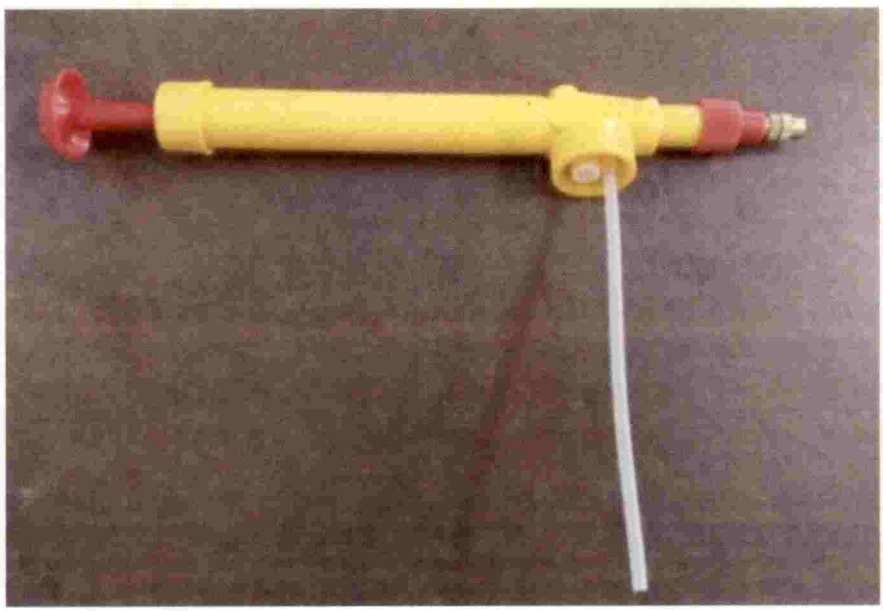


Plate 3.15 Pneumatic hand sprayer

3.16 Water conatiner

Plate 3.17 Assembled view (Model – II)

3.10 Procedure for pollen collection

The pollen grains from the selected tropical vegetable crops were collected for conducting studies and artificial pollination. The procedure followed for pollen collection by using the developed two models of pollinizers are described below.

3.10.1 Pollen collection using pollen collector Model – I

This model is used for collecting dry pollen by using vacuum pump. Pollen grains from crops like pumpkin and ash guard which are non-sticky in nature can be collected by the procedure as explained below. Selection of flower is done first as explained in section 3.6.1

- Pollen collector Model – I is connected to the electric power and the blower switch is put on.
- Pollen collection brush at the tip of the collector is inserted into the flower by holding flower in one hand.
- Brush out the pollen grains from the flower. As the pollen grains come out it will be sucked and carried in the air stream to the pollen collecting chamber as in the case of a vacuum cleaner.
- The dry pollen grains are deposited at near the micro mesh screen in the pollen collection chamber, and only the air is forced out through the vacuum pump.
- Pollen grains from collection chamber can be taken out after dismantling the chamber. For easy collection water can be sprayed to the chamber and the pollen grains collected can be used for artificial pollination.
- Artificial pollination is done by simply sucking the pollen water mixture by a syringe and sprays it to the stigma of female flower.

3.10.2 Pollen collection using pollen collector Model – II

Pollen grains in the crops like water melon and cucumber are more sticky in nature and is found difficult to collect by using model – I. So pollen collector model - II is used for collecting pollen grains using water as the medium. The procedure is as simple as explained below:

- Fill the water in the water container to its ¾ th of the height and fit it to the suction side of barrel of hand sprayer and tighten the threads. Then actuate the plunger to pressurize the water in the container.
- Lid of the pollen collection chamber of model –II i s opened and the flower is inserted to the V – shaped slot provided on the opposite side of the nozzle of hand sprayer.
- Close the lid firmly and press the valve knob to spray the sufficient quantity of water to the flower.
- The sprayed water will wash off all the pollen grains which will be collected in the collection chamber.
- A syringe is used to suck the pollen and water mixture and same can be used for artificial pollination.

3.11 Fruit set efficiency

Fruit set efficiency is the ratio of number of flowers converted in to fruit to the total number of flowers pollinated. Fruit set efficiency of two selected tropical vegetable crops such as cucumber and water melon only could be experimented due to the non-availability of the other crops during the experiment period. Fruit set efficiency of these two crops were observed by collecting the pollen grains using the developed artificial pollinizer.

Experiment was conducted by keeping the pollen grains in two medium and at three time interval. The procedure of the experiment is explained below.

- Pollen grains were collected from the male flowers using artificial pollinizer model-II by taking pure water as medium for collection.
- Collected pollen grains in water medium were deposited to the stigma of the freshly opened female flower on the same day.
- The pollen grains were stored in two separate containers in room temperature for pollinating after 24 hours and 48 hours.
- Artificial pollination is conducted with the stored pollen on the next two days and the pollinated flowers were tagged with separate distinguishing tags.
- Fruit set efficiency was assessed by counting the number of flowers converted into fruit on 3^{rd}, 7^{th} and 15^{th} days after pollination.
- The same procedure is repeated by using 1 % sucrose solution for collecting pollen grains and storing them for two days.
- Fruit set efficiency observed for pollen stored in plain water and sucrose solutions after 24 and 48 hours of storage and for fresh pollen were observed separately.

Views of the field experimental procedure are shown in Plates 3.17 to 3.21. The fruit set efficiency for cucumber and watermelon for two storage media and at three time intervals are consolidated in tabular as well as graphical forms.

Plate 3.18 View for pollen collection using Model –II

Plate 3. 19 View of transferring water pollen mixture to syring

Plate 3.20 Artificial pollination by spraying pollen to female flower

Plate 3.21 View of tagged flower pollinated for fruit set efficiency experiment

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Artificial pollinizer for pollinating some selected tropical vegetable crops grown under protected cultivation was designed, fabricated and evaluated. The results of the preliminary studies on flower, anther and pollen, design and development process and field evaluation are presented in this chapter.

4.1 Selection of tropical vegetable crops

Six tropical crops viz. tomato, chilli, water melon, cucumber, pumpkin and ash guard were selected as explained in the section 3.3 for conducting the preliminary studies. The floral characteristics of the flowers were collected from literature and presented in table 3.1. Out of the six vegetable crops, two crops viz. cucumber and water melon, which are widely cultivated under protected environment were selected for the validation of developed artificial pollinizer.

4.2 Physical characteristics

The physical characteristics of the six tropical vegetable crops which influences the design of artificial pollinizer were measured as described in section 3.5 and the results are tabulated and presented in table 4.1.

4.2.1 Number of anthers

The anther present in the flower of different crops varies in number. For tomato and chilli it varies from 4 to 6 and for all other crops it was observed that the number of anthers is three only.

4.2.2 Length

Among the six crops studied, highest length is observed for pumpkin flower with 60.338 mm and chilli was the smallest flower with a length of 12.222 mm.

Pumpkin also has the highest anther length of 21.38 mm and chilli has the least anther length compared to other five crop flowers. Length of flower and length of anther are important parameters which influence the design of pollen collection unit of artificial pollinizer.

Table 4.1 Dimensions of flower, anther of selected vegetable crops

Sl. No.	Crop	Measurements					
		Flower			Anther		
		L (mm)	G (mm)	W (g)	L (mm)	T (mm)	Nos
1	Tomato	15.313 ± 0.47	3.206 ± 0.19	0.0689 ± 0.01	8.437 ± 0.17	0.484 ± 0.02	4 -6
2	Chilli	12.222 ± 0.25	2.604 ± 0.17	0.0695 ± 0.01	2.428 ± 0.13	0.957 ± 0.03	4 -6
3	Water melon	18.313 ± 0.16	6.234 ± 0.17	0.1718 ± 0.03	7.47 ± 0.23	1.535 ± 0.03	3
4	Cucumber	18.79 ± 0.25	3.664 ± 0.32	0.1174 ± 0.01	4.768 ± 0.04	0.754 ± 0.03	3
5	Pumpkin	128.04 ± 0.52	41.116 ± 1.46	8.5414 ± 0.14	21.38 ± 2.19	4.242 ± 0.0.3	3
6	Ash guard	60.338 ± 2.42	23.33 ± 0.47	1.506 ± 0.21	12.013 ± 0.56	1.146 ± 0.16	3

L- Length, G- Pedicel girth, W- fresh weight, T- Thickness

4.2.3 Pedicel girth

From table 4.1 it was observed that pumpkin, has the maximum pedicel girth of 41.116 mm and chilli has the minimum of 2.604 mm. Pedicel girth of flower decide the size of pollen collection unit of artificial pollinizer.

4.2.4 Weight of flower

Pumpkin flower has the maximum weight of 8.5424 g and tomato has minimum flower weight 0.0695 g.

4.2.5 Length and thickness of anther

Pumpkin has got the highest length and thickness for the anther with values of 21.38 mm and 4.242 mm respectively. The anther length was found minimum in chilli with 2.428 mm and the thickness is minimum in tomato with 0.48 mm. It is important to note that the flower anthers of tomato and chilli are of too small in size. So devices which can collect pollen grains from these crops require highly precise parts to suit such small dimensions. Also the anthers of chilli and tomato are developed in a closed manner and it required to cut open for collecting pollen grains by external means.

4.3 Pollen studies

The different characteristics of pollen grains such as size, shape and pollen output which affects the design artificial pollinizer studied as explained in section 3.6. The results of the study are tabulated and presented in Table 4.2 and microscopic view of six selected tropical vegetable crops like tomato, chilli, pumpkin, ash guard, water melon and cucumber were presented in plates 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6 respectively.

Table 4.2 Dimensions of pollen grains of selected tropical vegetable crops

Sl. No.	Crop	Length (µm)	Width (µm)
1	Tomato	30.5937 ± 0.77	27.7732 ± 1.49
2	Chilli	28.0056 ± 1.17	26.4642 ± 0,96
3	Water melon	17.3691 ± 0.98	15.9108 ± 0.81
4	Cucumber	73.0208 ± 1.21	64.7337 ± 1.53
5	Pumpkin	46.8194 ± 1.72	42.9438 ± 1.67
6	Ash guard	21.7768 ± 0.77	21.1474 ± 0.77

4.3.1 Pollen measurements

Measurements of pollen grains were determined by taking photographs using Olympus Bx43 light microscope and Ultrascope - 9.1software as explained in section 3.6.2 and the results are tabulated and presented in the table 4.2

i) Length of pollen

From the table 4.2 it is clear that average length of pollen grains were maximum in ash guard with a dimension of 73.0208μm and is found to be minimum for pumpkin with 17.3691 μm compared to other flowers.

ii) Width of pollen

The average widths of pollen grains of tomato, chilli, watermelon, cucumber, pumpkin and ash guard were presented in Table 4.2. Ash guard has maximum width of 64.7337μm and pumpkin has minimum width of 15.9108μ m. The length and diameter of pollen grains are important in the selection of filter element for the design of artificial pollinizers. The size of opening filter screen should be r than the size of pollen grains.

4.3.2 Shape of pollen

The external appearance or shape of pollen grains were accessed with the help of the chart suggested by UPOV and is described in section 3.6.3. According to the UPOV document, the shape is defined as quantitative assessment of ratio of length and width. A shape category chart is also there in the document. The shape of the pollen grains of the selected crops were found out by considering the length/width ratio and with the help of chart presented in table 3.2. The shape category of the pollen grains obtained and the parameters considered are presented in a table 4.3.

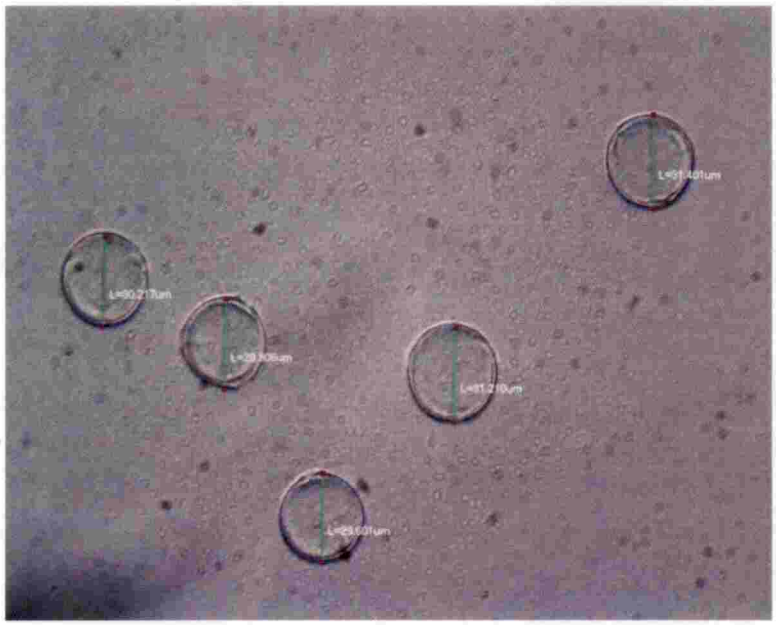


Plate 4.1 Microscopic view of tomato pollen grains

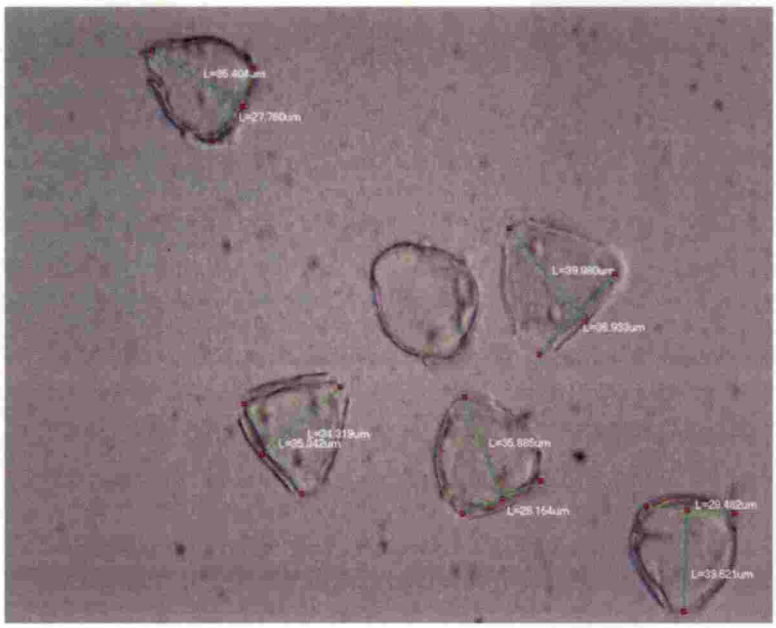


Plate 4.2 Microscopic view of chilli pollen grains

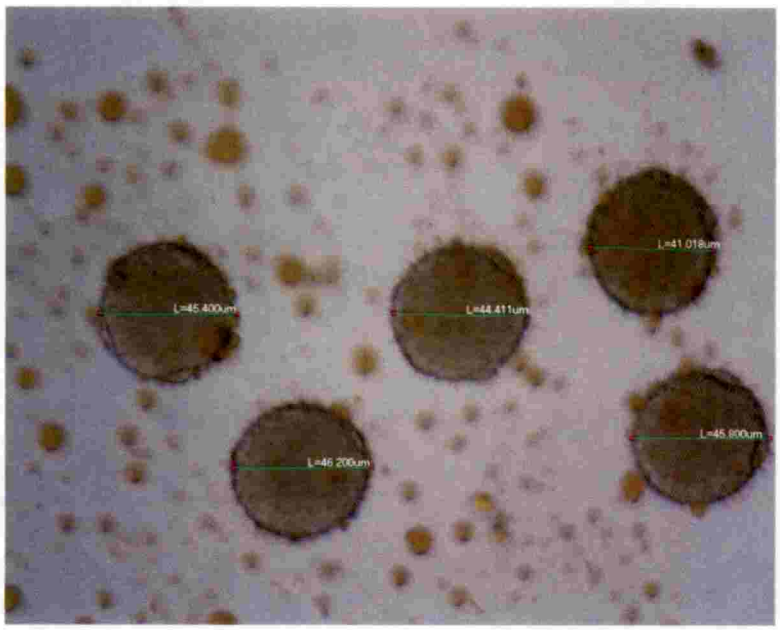


Plate 4.3 Microscopic view of pumpkin pollen grains

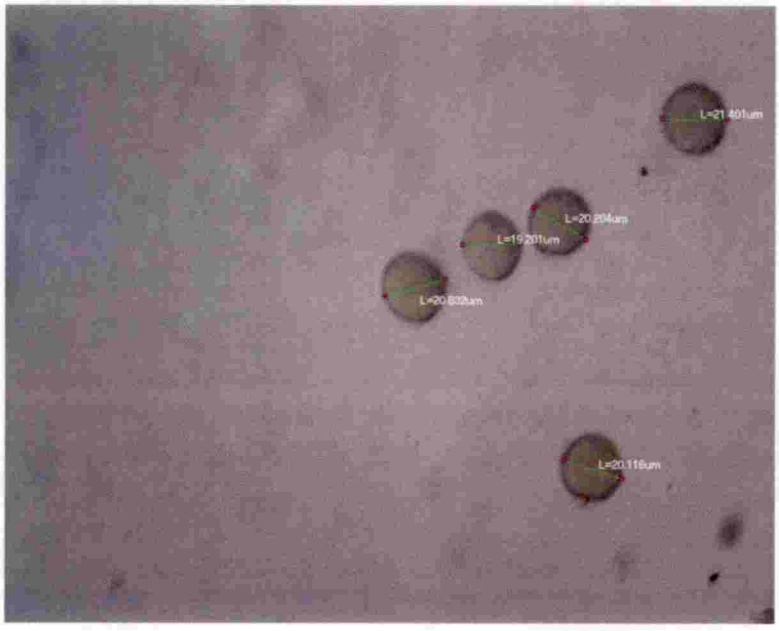


Plate 4.4 Microscopic view of ash guard pollen grains

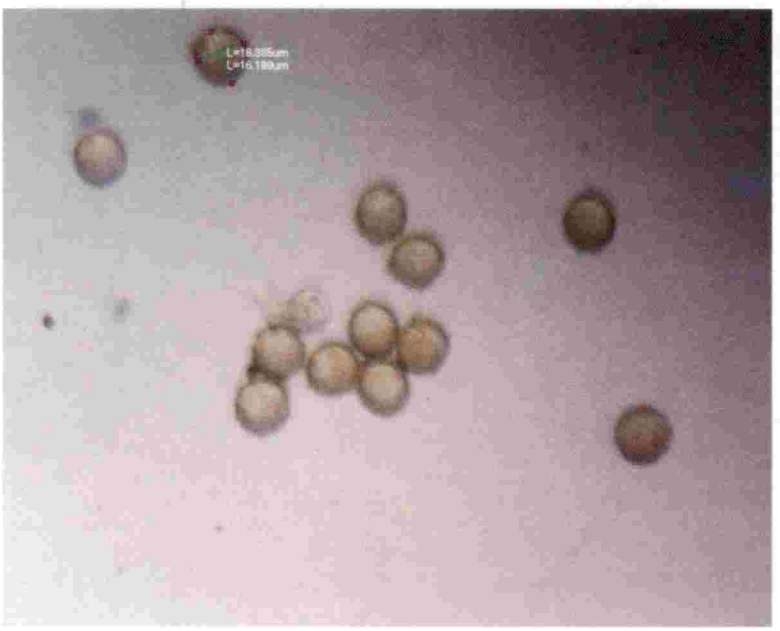


Plate 4.5 Microscopic view of watermelon pollen grains

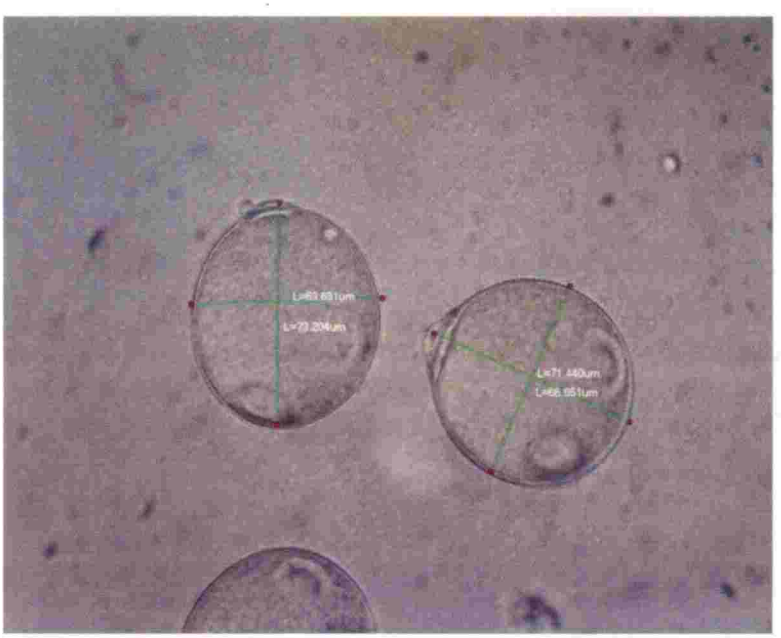
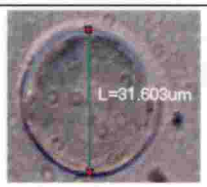
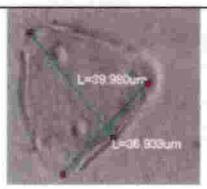
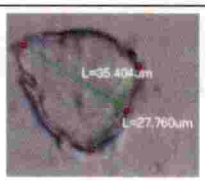
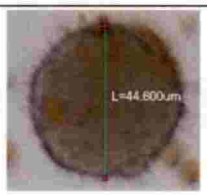
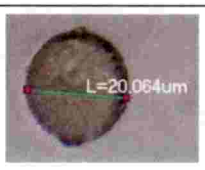
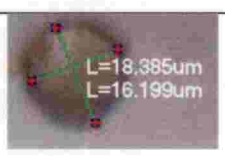
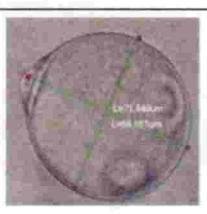
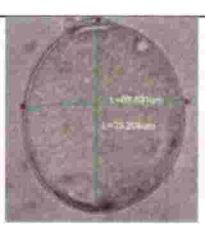


Plate 4.6 Microscopic view of cumber pollen grains

Table 4.3 Categorization of shape of pollen grains of selected vegetable crops

SI No.	CROP	Pollen view and dimension under microscope		L/W Ratio*	Shape category
1	Tomato			1:1.1	Circular
2	Chilli			1:1.2	Broad deltate
3	Pumpkin			1:1	Circular
4	Ash guard			1:1.1	Circular
5	Watermelon			1: 1.13	Narrow oblate
6	Cucumber			1:1.15	Narrow oblate

* Length/ width ratio

(Source: UPOV document, 2007)

The shape of pollen grains of tomato, pumpkin and ash guard are found to be 'circular' in the rounded set category with L/W ratio ranging from 1: 1 to 1: 1.1. Pollen grain of chilli comes under angular set category and shape is 'broad deltate' with L/W ratio of 1: 1.2. Pollen grains of water melon and cucumber comes under rounded set category and is 'narrow oblate' with L/W ratio of 1 : 1.1.13 to 1:1.15.

4.3.3 Pollen output

The quantity of pollen grains contained in a single flower and that can be collected for artificial pollination is considered as pollen output. The approximate pollen output from the different vegetable crops is calculated as explained in section 3.6.3 and in table 4.4. The quantification of pollen grains in single flower is presented in table form in Appendix –I.

Approximate quantity pollen grains found in water melon is the highest with 2923 numbers where as it is minimum in chilli with 284 numbers. Quantity of pollen grains in a single flower decides the quantity of pollens used for pollination at a time. The number of pollen grains used for pollination also decides the number of seeds present in the fruits

. Table 4.4 Quantity of pollen grains present in single flower of crops

Sl. No.	Crop	Average no. of pollen grains present in 1 flower
1	Tomato	471
2	Chilli	284
3	Water melon	2923
4	Cucumber	2188
5	Pumpkin	838
6	Ash guard	2441

3.4 Pollen mixers

Water is selected as a pollen mixer for the experiment as explained in section 3.6.4. Water is an effective pollen mix because it is nontoxic. The use of water as pollen mix would be highly beneficial, particularly with pollen that tends to clump, it is an effective media which didn't affect pollen viability, pollen germination, pollen fertilization and not interfere with pollen-stigma interactions. Water retains the viability of pollens as it neither increase nor decrease the viability of pollens and it just acts as a storage media. Water can improve flow and uniformity of pollen grain distribution which intern helpful in seed production and breeding programs where controlled pollination is used to produce hybrids.

4.4 Artificial pollinizer for tropical vegetable crops under protected cultivation

Artificial pollination is an unavoidable and important process when crops grown under protected environment as it offers barriers to natural agents for pollination like insects, wind, water etc. Popularization of polyhouse cultivation of vegetables increases the scope of artificial pollination in large. Artificial pollination can be done manually or mechanically. Manual artificial pollination is widely practiced in the state and is less efficient and time consuming process. Also collecting pollen grains from male flower is a difficult operation which requires high degree of precision. Artificial pollination by mechanical means is limited to the use of pollen vibrators or shakers that too is not common in the state. So, a simple device which can be used for artificial pollination can help the farmers doing vegetable cultivation in poly houses. In view of this, a simple device for artificial pollination is designed and developed.

4.4.1 Design and development of artificial pollinizer

The main objective of the study is to design and develop artificial pollinizer for pollinating tropical vegetable crops. Artificial pollination requires two activities

like collection of pollen from the male flower and deposition of the same in to the female part of flower with precision. In these, collection of pollen from the male flower is done at most care. Two models of pollen collection unit were developed based on the media used for pollen collection.

Based on the functional requirements and conceptual design two models of artificial pollinizers were designed and developed as explained in section 3.7.

4.4.2 Salient features of artificial pollinizer model – I

Artificial pollinizer model – I uses air as the medium for collection of pollen grains from the male flower. Air suction developed by a vacuum pump collects the pollen grains and deposited in a chamber. The artificial pollinizer model – I is designed and developed as explained in section 3.7.2 and the sub systems are developed as explained in section 3.8. Overall dimensions and components of the artificial pollinizer are tabulated in table 4.5. The dimensional sketch of the assembled artificial pollinizer model – I is presented in Fig. 4.1 and the assemble d view is shown in plate 4.7.

4.4.3 Salient features of artificial pollinizer model – II

Artificial pollinizer model – II uses water as the medium for collection of pollen grains from the male flower. Water is sprayed by a pneumatic hand sprayer to the male flower carefully placed in a collection chamber. Sprayed water wash out the pollen grains from the flower without damaging it and collected in the chamber. The artificial pollinizer model – II is designed and developed as explained in section 3.7.3 and the sub systems are developed as explained in section 3.9. Overall dimensions and the main components of the artificial pollinizer are tabulated in table 4.6. The dimensional sketch of the assembled artificial pollinizer model – I is presented in Fig. 4.2 and the assembled view is shown in plate 4.8.

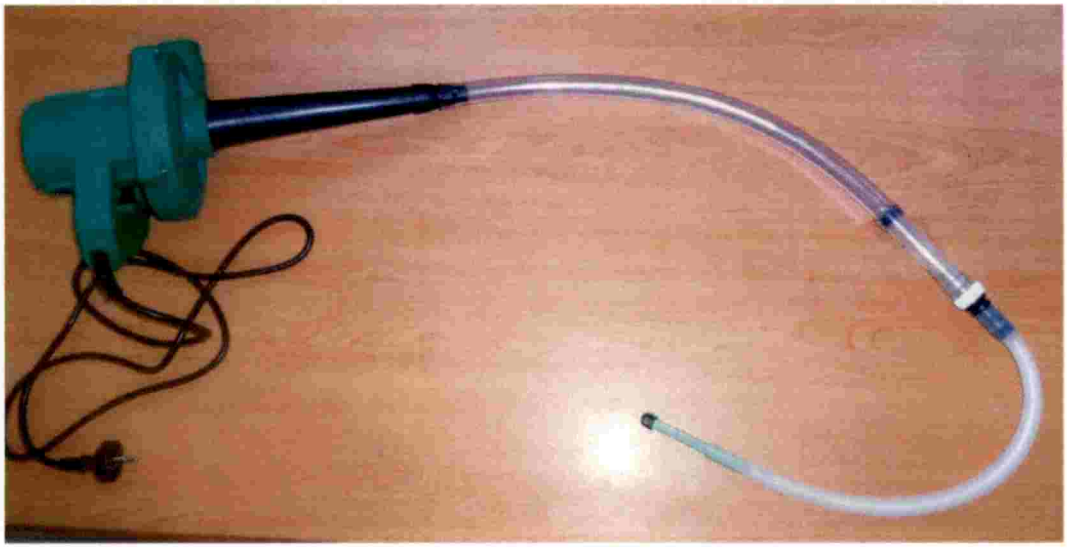


Plate 4.7 Assembled view of Air assisted aftificial pollinizer Model – I

Plate 4.8 Assembled view of water assisted aftificial pollinizer Model –II

Table 4.5 Dimensional specifications of Air assisted artificial pollinizer model - I

Sl. No.	Particulars	Values
Overall dimensions		
1	Length, mm	1280.00
2	Breadth, mm	260.00
3	Height, mm	180.00
4	Weight, g	1475.00
Pollen collection unit		
5	Length, mm	97.00
6	Sectional dimensions Width x Height, mm	14.00 x 8.00
7	Brush tip bristle length, mm	12.00
Pollen collection tube		
8	Length, mm	400.00
9	Outer diameter, mm	15.00
10	Inner diameter, mm	11.00
Adaptor for pollen collection chamber		
11	Length, mm	50.00
12	Adaptor cap diameter, mm	30.00
13	Diameter of tube connector, mm	13.00
Pollen collection chamber		
14	Length, mm	115.00
15	Inner diameter, mm	24.00
16	Screen size, µm	15.00
17	Screen area diameter, mm	18.00
Air tube		
18	Length, mm	500.00
19	Inner diameter, mm	22.00
20	Outer diameter, mm	24.00
Air tube adaptor		
21	Length, mm	55.00
22	Small end diameter, mm	16.00
23	Large end diameter, mm	30.00
Connector to vacuum pump		
24	Length, mm	225.00
25	Small end diameter, mm	22.00
26	Large end diameter, mm	50.00
Vacuum pump		
27	Overall Length, mm	260.00

28	Overall width, mm	150.00
29	Inlet diameter, mm	52.00
30	Outlet diameter, mm	40.00

Table 4.6 Dimensional specifications of artificial pollinizer model - II

Sl. No.	Particulars	Values
Overall dimensions		
1	Length, mm	265.00
2	Breadth, mm	30.00
3	Height, mm	195.00
4	Weight, g	60.00
Spray nozzle		
5	Length, mm	40.00
6	Orifice diameter	1.00
7	Sectional diameter at big end, mm	20.00
Hand sprayer		
8	Length excluding plunger handle, mm	265.00
9	Small end diameter, mm	16.00
10	Big side diameter, mm	30.00
11	Length of suction tube, mm	150.00
12	Diameter at water container , mm	32.00
Water container		
13	Length, mm	165.00
14	Diameter, mm	50.00
Pollen collecting chamber		
15	Length, mm	92.00
16	Width, mm	64.00
17	Height, mm	24.00
18	Nozzle orifice diameter, mm	7.00
19	Length of flower slot, mm	15.00
20	Top width of flower slot, mm	4.00

4.5 Evaluation of artificial pollinizer

Performance of artificial pollinizer was evaluated in terms of viability of pollen grains collected using the pollinizer. Pollen viability is evaluated for all the six

selected vegetable crops under laboratory conditions. Also the fruit set efficiency is another parameter to evaluate the artificial pollinizer under field conditions. Experiment on fruit set efficiency was conducted for watermelon and cucumber only due to the non-availability of other crops at the time of conducting experiment.

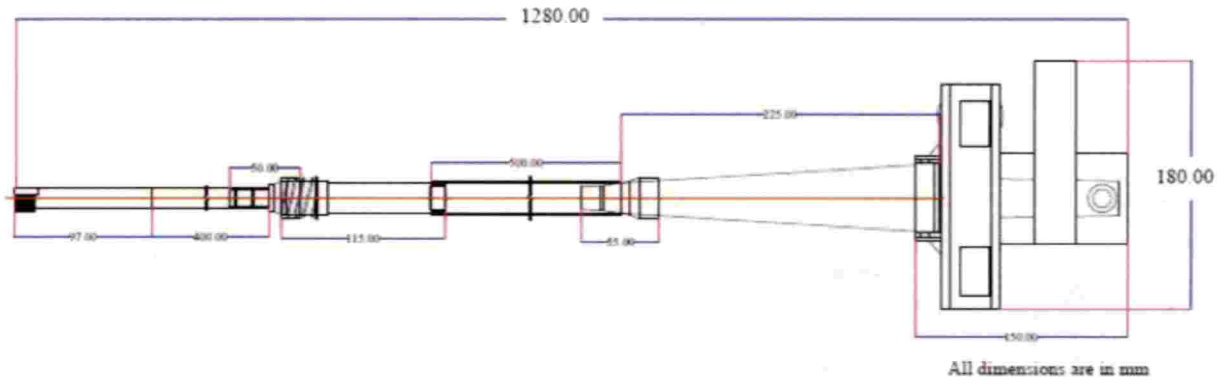


Fig.4.1 Assembled view of air assisted artificial pollinizer model- I

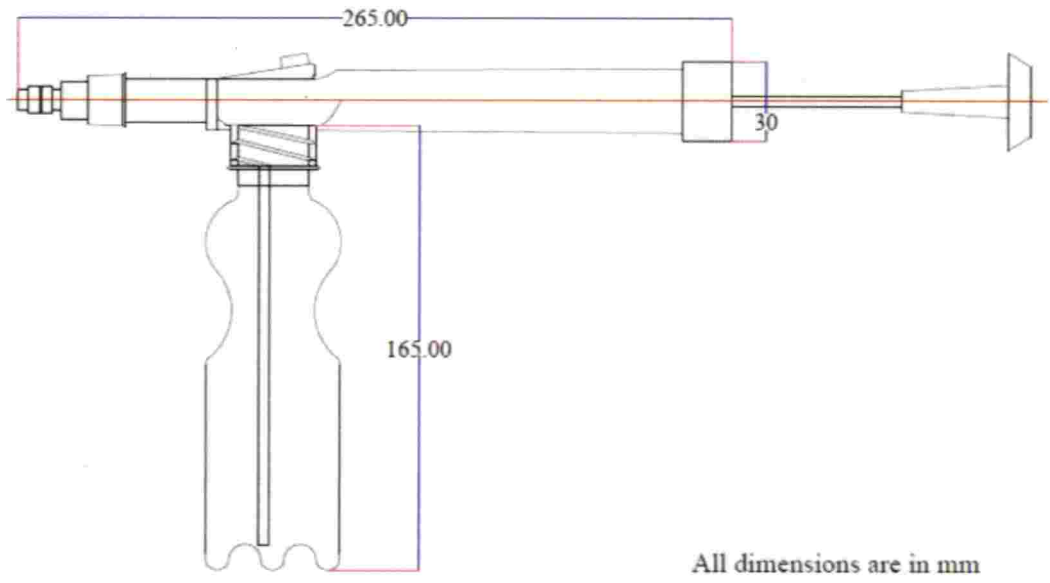


Fig.4.2 Assembled view of water assisted artificial pollinizer model – II

4.5.1 Pollen viability

Pollen viability refers to the ability of the pollen to perform its function of delivering male gametes to the embryo sac. Pollen viability is an index of its quality and vigor. Pollen viability varies from minutes to years, and which primarily depends on the taxonomic status of the plant and on the environmental conditions. Pollen viability is determined by following two methods like, Acetocarmine staining method and Invitro pollen germination method as explained in section 3.6.5. Accordingly, pollen viability percentage of pollen grains collected using the developed artificial pollinizer from cucumber and water melon crops were done in laboratory at different time intervals and stored in two media. The results of the experiment are presented in table 4.7.

Table 4.7 Pollen viability percent in different storage media at different storage period

Sl. No.	Storage period	Storage Medium	Viability per cent in	
			Cucumber	Watermelon
1	Fresh	Fresh water	93.76	92.14
2	After 24 hrs.	Water	49.12	48.76
3		1 % sucrose solution	70.18	72.88
4	After 48 hrs.	Water	44.3	44.64
5		1 % sucrose solution	58.56	60.08

Microscopic views of viability test by acetocarmine staining method for cucumber and watermelon are presented in Plates 4.9 and 4.10 and viability test by germination method for cucumber and watermelon is presented in Plates 4.11 and 4.12.

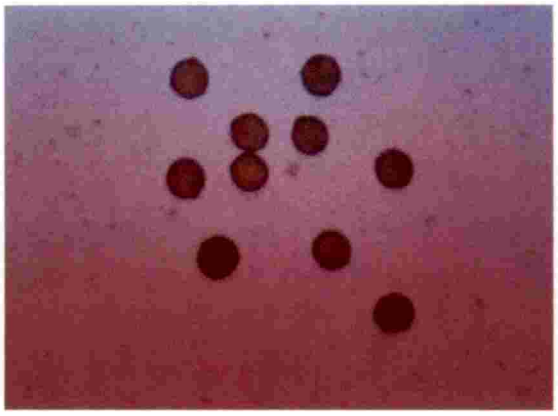


Plate 4.9 Microscopic view of viability by acetocarmine staining in cucumber

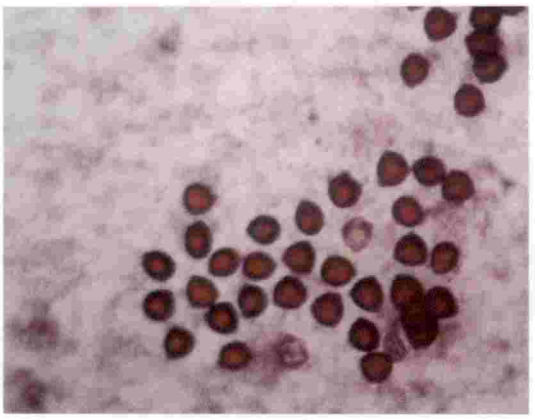


Plate 4.10 Microscopic view of viability by acetocarmine staining in water melon

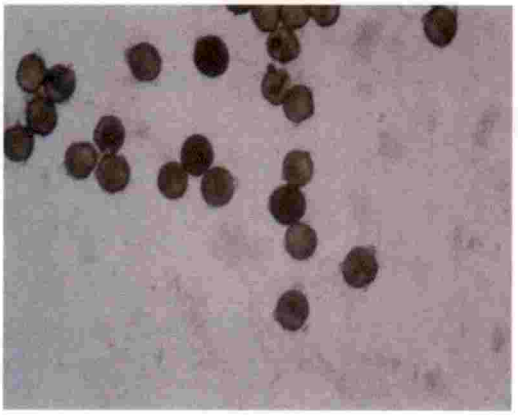


Plate 4.11 Microscopic view of germination in water melon

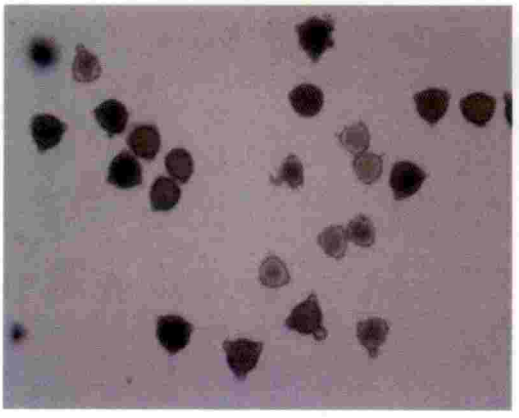


Plate 4.12 Microscopic view of germination in cucumber

Pollen viability was found highest in cucumber with a value of 93.76 per cent and is obtained for pollinating with fresh pollen. For water melon it is observed as 92.14 per cent for fresh pollen. The viability seems to be decreasing with increase in storage period. Also pollen stored in 1 per cent sucrose solution is more viable than pollen stored in water. In cucumber, pollen viability is reduced by 23.58 per cent after 24 hours of storage and 35.2 per cent after 48 hours of storage in sucrose

solution. Similarly in watermelon the reduction in viability is observed as 19.26 per cent and 32.06 per cent respectively after storage in sucrose solution. Pollen viability is reduced by 44.64 per cent and 49.46 per cent when stored in plain water for 24 and 48 hours for cucumber respectively. Similarly, the reduction in pollen viability for watermelon is 43.38 per cent and 47.5 per cent when stored in plain water for 24 and 48 hours respectively.

The pollen viability in different storage media for different time interval are graphically represented in Fig. 4.3 and 4.4.

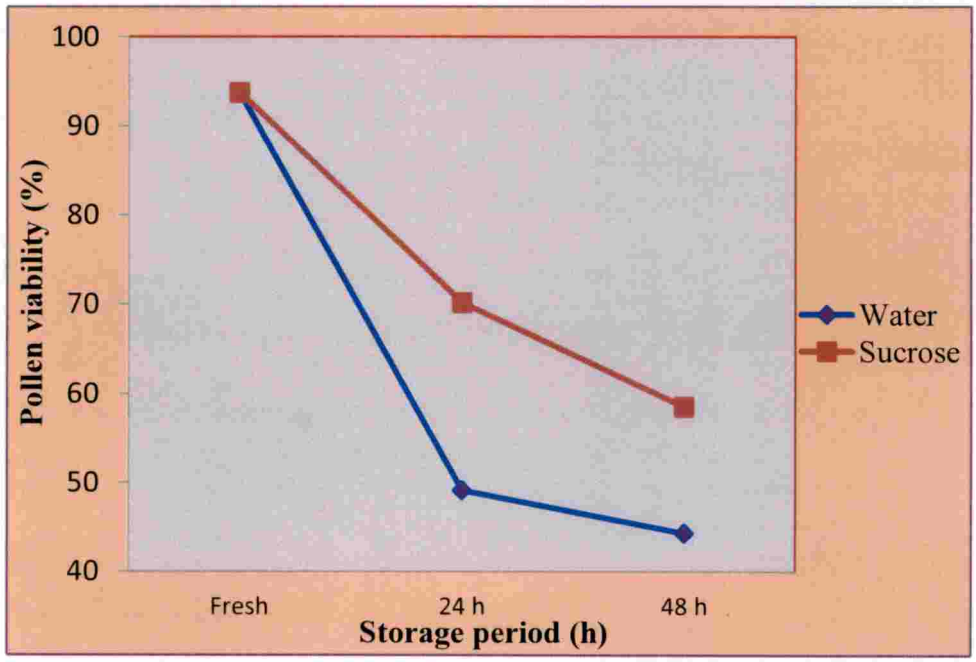


Fig. 4.3 Effect of storage period on pollen viability in cucumber under different storage media

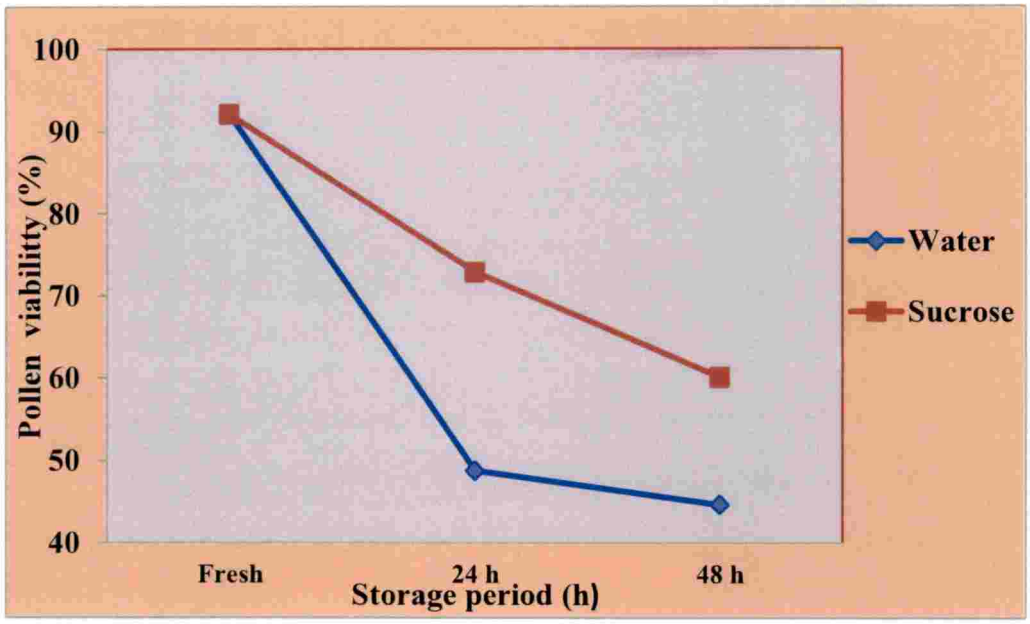


Fig. 4.4 Effect of storage period on pollen viability in water melon under different storage media

It is evident from these graphs that pollen viability is decreasing with increase in storage period for both the crops. Maximum pollen viability is observed when artificial pollination was done with fresh pollen grains. Also, the viability is found higher for pollen stored in 1 % sucrose solution than pollens stored in plain water in both crops.

5.2 Fruit set efficiency

Fruit set efficiency is also one parameter for accessing the performance of artificial pollinizer. It refers to the number of flowers developed into fruits after artificial pollination. Fruit set efficiency in cucumber and watermelon crops are evaluated at three intervals and pollen stored in two media as explained in section 3.11. Results of the experiment are tabulated in Table 4.8 and in Fig. 4.5 and Fig. 4.6. Maximum fruit set efficiency per cent is observed in watermelon with 80 % for

pollinating using fresh pollen grains and is reduced by 20 % for pollen grains stored in 1% sucrose solution and pollinated after 24 hours.

Table 4.8 Fruit set efficiency in different storage media at different storage period

Sl. No.	Storage period	Storage Medium	Fruit set efficiency per cent in	
			Cucumber	Watermelon
1	Fresh	Fresh water	70	80
2	After 24 hrs.	Plain water	40	40
3		1 % sucrose solution	60	60
4	After 48 hrs.	Plain water	20	20
5		1 % sucrose solution	40	40

Another 20 % reduction is observed when pollinated after 48 hours with the same sample. The reduction in fruit set efficiency when stored in plain water is 40 % and 60 % respectively for 24 and 48 hours of storage. In case of cucumber, fruit set efficiency per cent is 70 % for fresh pollen. The same is reduced by 10 % for pollen grains stored in 1% sucrose solution and pollinated after 24 hours. Another 20 % reduction is found when pollinated after 48 hours with the same sample. The reduction in fruit set efficiency when stored in plain water is 30 % and 50 % respectively for 24 and 48 hours of storage.

The results of the field evaluation of artificial pollinizer are also presented in the form of graphs in Fig. 4.5 and Fig. 4.6. These figures shows that, fruit set efficiency is also decreasing with increase in storage period for both the crops. Maximum fruit set efficiency is observed when artificial pollination was done with fresh pollen grains. Also, the fruit set efficiency is found higher for pollen stored in 1 % sucrose solution than pollens stored in plain water in both crops. Also the fruit set efficiency values are on par with manual pollination.

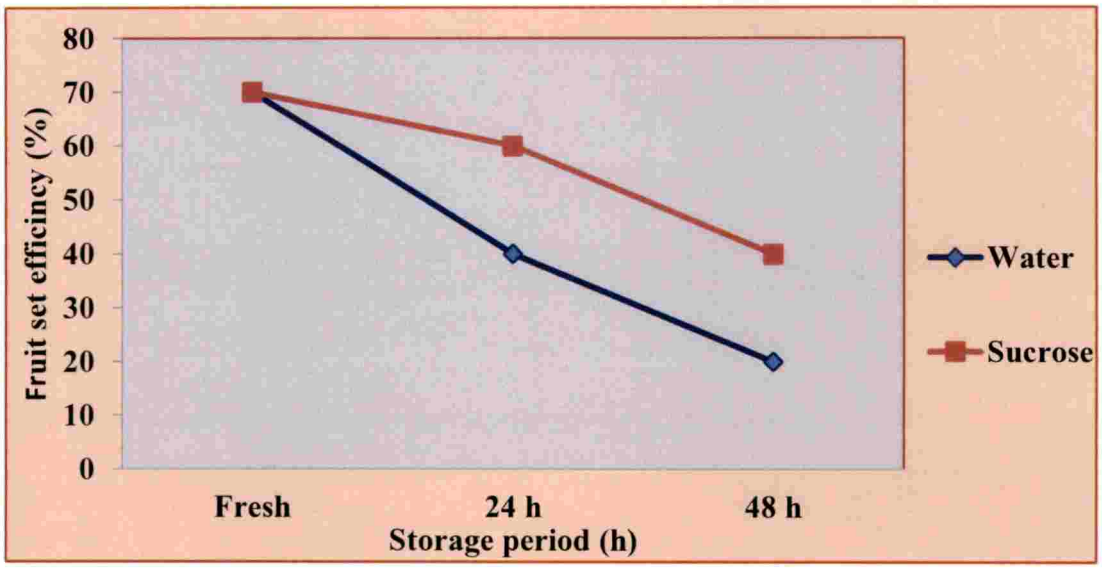


Fig. 4.5 Effect of storage period on fruit set efficiency in cucumber under different storage media

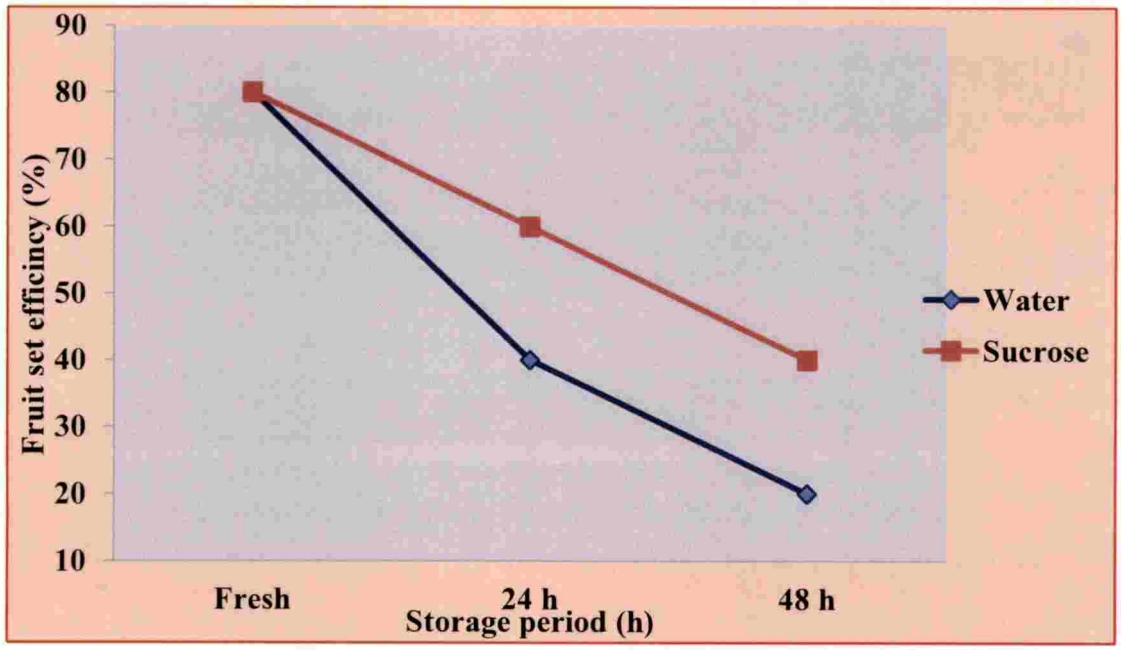


Fig. 4.6 Effect of storage period on fruit set efficiency in cucumber under different storage media

Views of various stages of fruit development in cucumber and watermelon crops on the 3rd, 7th and 15th days after artificial pollination is presented in Plate 4.13 to Plate 4.18.

Stages of fruit development at different time intervals in cucumber

Plate 4.13 Third day after artificial pollination

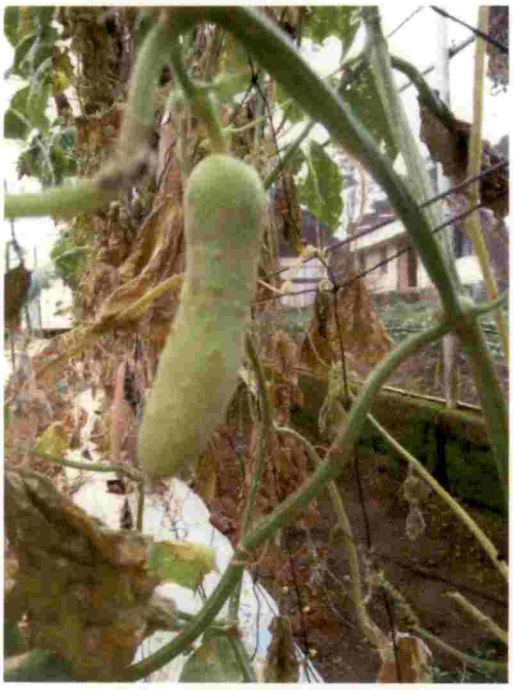


Plate 4.14 Seventh day after artificial pollination

Plate 4.15 Fifteenth day after artificial pollination

Stages of fruit development at different time intervals in watermelon

Plate 4.16 Third day after artificial pollination

Plate 4.17 Seventh day after artificial pollination

Plate 4.18 Fifteenth day after artificial pollination

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

A study was undertaken to design and develop an artificial pollinizer for pollinating tropical vegetable crops under protected cultivation. To obtain preliminary data required for the design, floral and physical characteristics of flower, anther and pollen of selected tropical vegetable crops were studied in laboratory conditions. Accordingly a conceptual design was evolved and two models of artificial pollinizer were developed. Suitability of the developed pollinizer was evaluated in laboratory as well as field conditions. Results of the evaluation have been presented and analyzed.

5.1 Floral and physical characteristics of selected tropical vegetable crops

Six tropical vegetable crops such as tomato, chilli, cucumber, watermelon, pumpkin and ash guard were selected for the preliminary studies. Primary data such as flower type, sex expression, breeding system, sex ratio and time of pollination were collected and tabulated. Physical characteristics of flowers such as number of anthers, length of flowers, pedicel girth, flower weight, length of anther and thickness of anther were measured in the laboratory and recorded.

5.2 Physical characteristics of pollen

Pollen studies like pollen collection, pollen size measurements, shape of pollen grain, pollen output, pollen mixers and pollen viability are studied in laboratory. Pollen size and shape were observed by using Olympus Bx43 light microscope and the measurements were taken using the software Ultrascope version 9.1.

5.2.1 Size and shape of pollen grains

Shape is the external form or outward appearance of an object and is accessed

according to the document published by International Union for Protection of new Varieties of Plants (UPOV) in 2007. The shape is considered as length/width ratio to develop a quantitative characteristics rather than considering shape as a single qualitative characteristic. A shape category chart is also suggested by UPOV with respect to the length/width ratio. The dimensions of the pollen grains of all the selected tropical vegetable crops were measured separately using the software and the shape of each pollen grains were found out by using the chart. The dimensions and shape category of pollen grains were presented in tabular form. Size and shape of pollen grains are important parameters in the design of pollen collection unit for artificial pollinizer.

5.2.2 Pollen output

Pollen output refers to the total quantity of pollen grains collected from a single male flower. Average pollen output from the flowers of all the selected tropical vegetable crops were found out in laboratory.

5.2.3 Pollen mixers and pollen storage

Pollen grains are mixed with some inert, nontoxic substance for artificial pollination. Water is used as pollen mixer and is universally available and helps to improve the pollen collection in case of stickier pollen grains.

Pollen grains are stored in suitable medium to enable artificial pollination for longer periods. In order to check storage qualities of pollen grains, viability tests are conducted after 24 and 48 hours of storage in different media. Storage of pollen grains was done in two media such as pure water and 1 per cent sucrose solution.

5.2.4 Pollen viability

Pollen viability refers to the ability of the pollen grains to perform its function of delivering male gametes to the embryo sac.

Pollen viability refers to the ability of the pollen grains to perform its function of delivering male gametes to the embryo sac. Viability of pollen was determined by acetocarmine staining method and invitro pollen germination method.

5.3 Design and development of artificial pollinizer

Based on the preliminary studies, functional requirements and conceptual design two models of artificial pollinizers were designed and developed.

5.3.1 Artificial pollinizer model – I

Artificial pollinizer model – I uses air as the medium for collection of pollen from flowers. Suction pressure developed by a vacuum pump suck the pollen grains from the male flower to a pollen collection chamber. The pollen collection unit of model – I is provided with a brush tip which detach the pollen grains from the flower. The detached grains are sucked by a vacuum pump and are deposited in a pollen collection chamber. A screen mesh filter of 15 μm aperture provided at one end of the pollen collection chamber prevents the pollen grains to escape along with the air.

Components of artificial pollinizer model – I includes pollen collection unit, pollen collection tube, adaptor for pollen collection chamber, pollen collection chamber, air tube, air tube adaptor, connector to vacuum pump and a vacuum pump. An electric powered air blower of 220 V, 50 Hz and 0.55kW is used as vacuum pump. The blower produces a suction velocity of 20-22 ms^{-1} at 13,000 rpm.

5.3.2 Artificial pollinizer model – II

Artificial pollinizer model – II uses water as the medium for collection of pollen from flowers. Water is sprayed from a nozzle to the male flower kept carefully in a pollen collection chamber. Water spray is produced by a pneumatic hand pump and pure water is taken in a water container attached to the pump. Water spray from the nozzle wash out the pollen grains from the flower and is collected in the water

tight container in which the male flower is placed. The collected water-pollen mixture can be used directly for artificial pollination as water is a good pollen mixer. Components of artificial pollinizer model – II includes a spray nozzle, pneumatic hand sprayer, water container and pollen collecting chamber.

Collected pollen can be sucked by a syringe for spraying to the female flower for artificial pollination. Dry pollen collected by model – I is also mixed with water and used for artificial pollination using a syringe.

5.4 Evaluation of artificial pollinizer

Pollen viability and fruit set efficiency are the two parameters used to evaluate the artificial pollinizer. Viability of pollen was observed under laboratory condition and fruit set efficiency was observed under field conditions.

Fruit set efficiency is the ratio of number of flowers converted in to fruit to the total number of flowers pollinated. Fruit set efficiency of two selected tropical vegetable crops such as cucumber and water melon only could be experimented due to the unavailability of the other crops during the experiment period. Fruit set efficiency of these two crops were observed by collecting the pollen grains using the developed artificial pollinizer. Experiments were conducted by keeping the grains in two media like plain water and 1 % sucrose solution and at three time intervals like fresh pollen, 24 hours of storage and 48 hours of storage. The results were presented, analyzed and conclusions were drawn.

CONCLUSIONS

The following are the important conclusions from the study.

1. Pollination in tropical vegetable crops is possible by natural or artificial methods. When pollination is caused due to the action of natural agents such as insects, birds, water or wind or even plants themselves and when self-pollination occurs

within a closed flower, is the Natural pollination. Artificial pollination can be either by manual means or machine pollination.

2. Floral and physical characteristic such as flower type, sex expression, breeding system, sex ratio and time of pollination of six tropical vegetable crops like tomato, chilli, cucumber, watermelon, pumpkin and ash guard were collected and tabulated.
3. The number of anthers present in tomato and chilli varies from 4 to 6 and for all others it is three only.
4. Physical characters of the flower and anther were measured using precision vernier calipers. Highest flower length was observed for pumpkin with 60.338 mm and chilli has the smallest flower with a length of 12.222 mm. Also pumpkin has the highest anther length of 21.38 mm and chilli has the least anther length compared to other crop flowers.
5. Pedicel girth was maximum for pumpkin with 41.116 mm and is minimum for chilli with 2.604 mm. Pedicel girth of flower decides the size of pollen collection unit for artificial pollinizer.
6. Highest length and thickness of anther is found in pumpkin with values of 21.38 mm and 4.242 mm respectively. Also the anther length was found minimum in chilli with 2.428 mm and the thickness is minimum in tomato with 0.48 mm.
7. Weight of flower was found in precision electronic balance model GP-332 with a sensing capacity range from 0.0018 g to 320 g within a temp. range of 15-450C and power requirement was 230 watts. Flower weight is found maximum for pumpkin with 8.5424 g and minimum for tomato with 0.0695 g.
8. The different properties of pollen grains such as size, shape and pollen output are important in the design of artificial pollinizer. The pollen properties of six selected tropical vegetable crops such as tomato, chilli, pumpkin, ash guard, water melon and cucumber were studied and presented.
9. Pollen grains from freshly dehisced anthers were collected manually after anthesis. Pollen size and shape were observed using Olympus Bx43 light

microscope and the measurements were taken by using the software Ultrascope version 9.1.

10. Pollen sizes are found maximum in ash guard and minimum in pumpkin with dimensions of length and width of 73.0208µm × 64.7337µm in ash guard and of 17.3691 µm × 15.910 µm respectively.
11. The length and diameter of pollen grains are important in the selection of filter element for the design of artificial pollinizers. The size of aperture of filter screen should be lesser than the size of pollen grains.
12. Shape is the external form or outward appearance of an object. Shape of the pollen grains was accessed according to the document published by International Union for Protection of new Varieties of Plants (UPOV, 2007). The term shape is defined as length/width ratio to develop quantitative characteristics related to it, rather than considering shape as a single qualitative characteristic. A standard chart is also suggested by UPOV to ensure the ratio of length to width for three different sets of shapes such as parallel, rounded and angular. The dimensions of the pollen grains of all the selected crops were measured separately and the shape of each pollen grains were found out using the chart.
13. The shape of pollen grains of tomato, pumpkin and ash guard are found to be 'circular' in the rounded set category with L/W ratio ranging from 1: 1 to 1: 1.1. Pollen grain of chilli comes under angular set category and the shape is 'broad deltate' with L/W ratio of 1: 1.2. Pollen grains of water melon and cucumber come under rounded set category and the shape is 'narrow oblate' with a L/W ratio of 1: 1.13 to 1:1.15.
14. Pollen output refers to the quantity of pollen grains contained in a single flower and that can be collected for artificial pollination. The approximate pollen output from single flowers of the different vegetable crops was found out. Approximate quantity pollen grains found in water melon is the highest with 2923 numbers where as it is minimum in chilli with 284 numbers. Pollen output of other crops were tomato - 471, cucumber – 2188, pumpkin – 838 and ash guard – 2441 nos.

Quantity of pollen grains in a single flower decides the quantity of pollens used for pollination at a time. The numbers of pollen grains used for pollination affect the number of seeds present in the fruits.

15. Water is used as the pollen mixer for the experiments as it is an effective medium which didn't affect pollen viability, pollen germination, pollen fertilization and not interfere with pollen-stigma interactions and can improve flow and uniformity of pollen grain distribution.
16. Based on the functional requirements and conceptual designs two models of artificial pollinizers were designed, developed and evaluated
17. Artificial pollinizer model – I uses air as the medium for collection of pollen from flowers due to the suction created by a vacuum pump and collected in a chamber. The overall dimensions of the pollinizer are length - 1280 mm, width - 260 mm, height - 180 mm and weight - 1475 g.
18. Artificial pollinizer model – II uses water as the medium for collection of pollen from flowers by spraying water from a nozzle of pneumatic hand sprayer and the pollen-water mixture is collected in a chamber. The overall dimensions of the pollinizer are length-265mm, width-30mm, height-195mm and weight-60 g.
19. Performance of artificial pollinizer was evaluated in terms of pollen viability and fruit set efficiency. Pollen viability is evaluated for all the six selected vegetable crops under laboratory conditions. Experiment on fruit set efficiency was conducted for watermelon and cucumber only due to the non-availability of other crops at the time of conducting experiment.
20. Pollen viability is seems to be decreasing with increase in storage period. Maximum viability is observed for fresh pollen is 93.76 % in cucumber and 92.14 % in watermelon.
21. Pollen stored in 1 per cent sucrose solution is more viable than pollen stored in plain water.
22. In cucumber pollen viability is reduced by 23.58% after 24 hours of storage and 35.2 % after 48 hours of storage than fresh pollen when stored in 1 % sucrose

solution. Similarly in watermelon the reduction in viability is observed as 19.26% and 32.06% respectively than fresh pollen.

23. Pollen viability is reduced by 44.64 % and 49.46 % than fresh pollen while stored in plain water for 24 and 48 hours in case of cucumber. But in watermelon, it is reduced by 43.38 % and 47.5 % when stored in plain water for 24 and 48 hours respectively.
24. Fruit set efficiency is also one parameter for accessing the performance of artificial pollinizer. It refers to the number of flowers turn into fruits after artificial pollination.
25. Fruit set efficiency is decreasing with increase in storage period for both cucumber and watermelon. Maximum fruit set efficiency is observed when artificial pollination was done with fresh pollen grains. Also, the fruit set efficiency is found higher for pollen stored in 1 % sucrose solution than pollens stored in plain water in both crops. The values of fruit set efficiency are on par with manual pollination.
26. Maximum fruit set efficiency per cent is observed in watermelon with 80 % when pollinated with fresh pollen grains and is reduced by 20 % for pollen grains stored in 1% sucrose solution pollinated after 24 hours. Another 20 % reduction is found when pollinated after 48 hours with the same sample. The reduction in fruit set efficiency when stored in plain water is 40 % and 60 % respectively for 24 and 48 hours of storage.
27. In cucumber, fruit set efficiency is 70 % for fresh pollen and is reduced by 10 % for pollen grains stored in 1% sucrose solution pollinated after 24 hours. Another 20 % reduction is found when pollinated after 48 hours with the same sample. The reduction in fruit set efficiency when stored in plain water is 30 % and 50 % respectively for 24 and 48 hours of storage.

Suggestions for future study:

1. Extensive field trials and standardization are required with artificial pollinizer model – I for collecting dry pollen grains from crops like pumpkin, ash guard etc.
2. Pollen collection from small flowers like chilli, tomato etc. require minute pollen collection units and mechanism for opening anther needs

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APPENDICES

Appendix I
Flower and anther measurements of Tomato, Chilli and watermelon

	TOMATO					CHILLI					WATER MELON				
	Flower			Anther		Flower			Anther		Flower			Anther	
	L	G	W	L	T	L	G	W	L	T	L	G	W	L	T
1	15.64	3.34	0.068	8.44	0.46	11.74	2.40	0.063	2.50	0.91	18.34	5.98	0.215	7.26	1.52
2	15.88	2.92	0.065	8.52	0.48	12.48	2.48	0.072	2.48	0.99	18.24	6.38	0.134	7.68	1.56
3	14.79	3.32	0.064	8.49	0.46	12.48	2.76	0.081	2.41	0.97	17.98	6.36	0.176	7.28	1.54
4	14.86	3.12	0.067	8.37	0.50	12.14	2.62	0.062	2.46	0.98	18.62	6.12	0.162	7.24	1.52
5	15.92	3.34	0.067	8.14	0.52	11.92	2.70	0.064	2.44	0.98	18.44	6.26	0.145	7.66	1.50
6	14.84	2.96	0.087	8.14	0.50	12.24	2.40	0.067	2.16	0.90	18.24	5.96	0.201	7.68	1.56
7	14.74	2.98	0.088	8.56	0.48	12.48	2.40	0.061	2.64	0.97	18.34	6.38	0.142	7.26	1.58
8	15.56	3.26	0.060	8.66	0.46	12.36	2.82	0.062	2.25	0.96	18.27	6.36	0.159	7.22	1.52
9	15.28	3.42	0.059	8.53	0.50	12.14	2.76	0.075	2.45	0.95	18.32	6.18	0.210	7.78	1.54
10	15.62	3.40	0.064	8.52	0.48	12.24	2.70	0.088	2.49	0.96	18.34	6.36	0.174	7.64	1.51

L-Length in millimeters, G-Pedicel girth in mm , T-Thickness in millimeters, W-Weight in grams .

Appendix II

Flower and anther of measurements of cucumber , pumpkin and Ash guard

	CUCUMBER					PUMPKIN					ASH GUARD				
	Flower			Anther		Flower			Anther		Flower			Anther	
	L	G	W	L	T	L	G	W	L	T	L	G	W	L	T
1	18.98	3.92	0.109	4.78	0.78	128.5	42.84	8.321	23.11	3.68	64.82	23.18	1.359	12.64	1.18
2	18.22	3.66	0.109	4.76	0.78	127.3	40.62	8.604	24.52	4.48	59.42	22.50	1.467	12.68	1.12
3	18.96	3.32	0.114	4.76	0.76	128.2	39.98	8.635	19.82	4.42	58.96	23.48	1.889	12.56	1.18
4	18.56	3.94	0.114	4.68	0.74	128.4	40.82	8.622	18.24	4.12	58.32	23.98	1.683	11.78	1.12
5	18.82	3.98	0.125	4.80	0.72	127.4	42.88	8.664	22.63	4.50	60.58	23.64	1.232	11.98	1.48
6	18.92	3.94	0.121	4.78	0.78	128.4	43.42	8.265	22.36	3.82	64.64	23.68	1.526	12.16	0.98
7	18.92	3.98	0.126	4.80	0.74	127.2	38.98	8.457	22.56	4.10	58.94	22.62	1.461	12.36	0.98
8	18.94	3.28	0.126	4.76	0.72	128.4	39.96	8.622	19.56	4.40	58.64	23.22	1.531	11.48	1.18
9	18.96	3.34	0.118	4.74	0.74	128.2	40.84	8.604	18.36	4.42	60.24	23.52	1.231	11.28	0.96
10	18.62	3.28	0.112	4.82	0.78	128.4	40.82	8.620	22.64	4.48	58.82	23.48	1.681	11.21	1.28

Appendix III

Pollen measurements of Tomato, Chilli, Pumpkin, Ash guard , Water melon, Cucumber.

	TOMATO		CHILLI		PUMPKIN		ASH GUARD		WATERMELON		CUCUMBER	
	L	W	L	W	L	W	L	W	L	W	L	W
1	31.223	28.666	27.865	26.447	49.633	41.633	21.001	22.045	17.974	15.559	72.735	64.462
2	30.865	27.003	26.448	25.678	46.615	43.212	21.004	21.802	16.199	15.986	75.045	67.619
3	29.600	27.601	29.603	27.603	46.643	43.617	21.402	22.015	19.180	17.421	73.204	63.631
4	31.212	28.813	27.607	27.250	47.600	42.402	22.672	21.224	17.256	15.454	71.440	66.651
5	31.402	28.106	29.403	27.407	43.800	40.802	22.608	21.224	17.528	15.225	71.665	63.665
6	30.201	28.216	27.607	25.279	48.635	44.206	20.604	20.064	16.199	14.968	73.278	66.258
7	30.616	29.332	27.865	26.447	46.621	43.023	21.422	21.423	16.180	14.998	72.665	63.564
8	30.206	24.627	26.448	25.678	47.621	40.402	22.622	20.064	17.986	16.451	72.564	64.256
9	29.206	26.143	29.603	27.603	44.624	44.411	22.201	20.209	17.224	16.754	75.046	63.244
10	31.406	29.225	27.607	25.250	46.402	45.730	22.232	21.404	17.965	16.292	72.566	63.987

L-Length in micrometers, W- Width in micrometers

Appendix IV

Quantification of collected pollen from Tomato flower

Trail No.	Number of pollen in 1 one drop	Number of drops in 1 ml.	Quantity of collected sample from 1 flower (ml)	Average no of pollen in 1 flower (No's)
1	2	30	8	480
2	2	29	7	406
3	3	28	7	588
4	2	30	8	480
5	2	29	7	406
6	2	30	8	480
7	2	30	7.5	450
8	2	30	8	480
9	2	29	8	464
10	2	30	8	480
Avg.	**2**	**29**	**7.75**	**471**

Appendix V

Quantification of collected pollens from chilli flower

Trail No.	Number of pollen in 1 one drop	Number of drops in 1 ml.	Quantity of collected sample from 1 flower (ml)	Average no of pollen in 1 flower (No's)
1	1	29	8	232
2	2	30	8	480
3	1	30	8	240
4	1	30	8	240
5	2	30	8	480
6	1	30	8	240
7	2	30	7.5	450
8	1	30	8	240
9	0	29	8	0
10	1	30	8	240
Avg.	1	29	7.9	284

Appendix VI

Quantification of collected pollen from Pumpkin

Trail No.	Number of pollen in one drop	Number of drops in 1 ml.	Quantity of collected sample from 1 flower (ml)	Average no of pollens collected from 1 flower (No's)
1	4	30	8	960
2	4	29	7.5	870
3	3	29	8	696
4	4	30	8	960
5	4	29	8	928
6	3	30	8	720
7	3	30	7	630
8	4	30	8	960
9	3	29	8	696
10	4	30	8	960
Average	**3.6**	**29.6**	**7.8**	**838**

Appendix VII

Quantification of collected pollen from Ash guard flower

Trail No.	Number of pollen in one drop	Number of drops in 1 ml.	Quantity of collected sample from 1 flower (ml)	Average no of pollens in 1 flower (No's)
1	10	30	8	2400
2	12	29	7.5	2610
3	10	29	8	2320
4	13	29	8	3016
5	10	29	7.5	2175
6	12	30	8	2880
7	9	30	7	1890
8	10	30	8	2400
9	10	29	8	2320
10	10	30	8	2400
Average	**10.6**	**29.5**	**7.8**	**2441.1**

Appendix VIII

Quantification of collected pollen from water melon flower

Trail No.	Number of pollen in 1 one drop	Number of drops in 1 ml.	Quantity of collected sample from 1 flower (ml)	Average no of pollen in 1 flower (No's)
1	15	30	8	3600
2	15	29	7	3045
3	14	28	8	3136
4	12	30	8	2880
5	12	29	7	2436
6	13	30	8	3120
7	12	30	7.5	2700
8	12	30	8	2880
9	11	29	8	2552
10	12	30	8	2880
Avg.	12.8	29.5	7.75	2922.9

Appendix IX

Quantification of collected pollens from Cucumber flower

Trail No.	Number of pollen in 1 one drop	Number of drops in 1 ml.	Quantity of collected sample from 1 flower (ml)	Average no of pollen in 1 flower (No's)
1	10	29	8	2320
2	9	30	7.5	2025
3	9	30	8	2160
4	8	30	8	1920
5	9	30	8	2160
6	9	30	8	2160
7	10	30	7.5	2250
8	10	30	8	2400
9	10	29	8	2320
10	9	30	8	2160
Avg.	9.3	29.8	7.9	2187.5

ABSTRACT

DESIGN AND DEVELOPMENT OF ARTIFICIAL POLLINIZER FOR POLLINATING TROPICAL VEGETABLES UNDER PROTECTED CULTIVATION

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Artificial pollination is a process that is highly require in vegetable crops grown under protected cultivation since, protected environment offers barriers to natural agents for pollination such as insects, wind or water.

A study was undertaken to design and develop an artificial pollinizer for pollinating tropical vegetable crops under protected cultivation. To obtain preliminary data required for the design, floral and physical characteristics of flower, anther and pollen of selected tropical vegetable crops were studied in laboratory conditions. Accordingly a conceptual design was evolved and two models of artificial pollinizers were developed. Suitability of the developed pollinizer was evaluated in laboratory as well as field conditions. Results of the evaluation have been presented and analyzed.

Six tropical vegetable crops were selected for the preliminary studies like floral characteristics, physical and dimensional measurements of flowers, anther and pollen grains were conducted in the laboratory. Sizes of pollen grains were measured by using Olympus Bx43 light microscope and the measurements were taken using the software Ultrascope version 9.1. Shape was determined from standard shape charts suggested by UPOV, 2007. The shape of pollen grains of tomato, pumpkin and ash guard are found to be 'circular' with a Length/Width ratio ranging from 1: 1 to 1: 1.1. Pollen grain of chilli is 'broad deltate' with L/W ratio of 1: 1.2. Pollen grains of water melon and cucumber are 'narrow oblate' with a L/W ratio of 1: 1.13 to 1:1.15.

Based on the preliminary studies, functional requirements and conceptual designs two models of the artificial pollinizers were designed and developed.

Artificial pollinizer Model – I uses air as the medium for collection of pollen from flowers. Suction pressure developed by a vacuum pump suck the pollen grains

from the male flower to a pollen collection chamber. The pollen collection unit is provided with a brush tip which detach the pollen grains from the flower and sucked by a vacuum pump are deposited in a pollen collection chamber. A screen mesh filter of 15 μm aperture is used in the chamber to prevent the pollen grains from carry away. Components of artificial pollinizer model – I includes pollen collection unit, pollen collection tube, adaptor for pollen collection chamber, pollen collection chamber, air tube, air tube adaptor, a connector and a vacuum pump. An electric powered air blower of 220 V, 50 Hz and 0.55kW is used as vacuum pump. The blower produces a suction velocity of 20-22 ms^{-1} at 13,000 rpm.

Artificial pollinizer Model – II uses water as the medium for collection of pollen from male flowers. Water is sprayed from a nozzle to the male flower kept carefully in a pollen collection chamber. Water spray produced by a pneumatic hand pump from the nozzle wash out the pollen grains from the flower and is collected in the water tight container. Components of artificial pollinizer Model – II includes a spray nozzle, pneumatic hand sprayer, water container and pollen collecting chamber.

Collected pollen can be sucked by a syringe for spraying to the female flower for artificial pollination. Dry pollen collected by Model – I is also mixed with water and used for artificial pollination using a syringe.

Evaluation of artificial pollinizer was done by accessing pollen viability and fruit set efficiency. Viability of pollen was observed under laboratory condition and fruit set efficiency was observed under field conditions.

From the studies it is observed that, pollen viability is decreasing with increase in storage period for both the crops. Maximum pollen viability is observed when artificial pollination was done with fresh pollen grains. Also, the viability is found higher for pollen stored in 1 % sucrose solution than pollens stored in plain water in both crops.

Study on fruit set efficiency is also seems to be decreasing with increase in storage period for both the crops. Maximum fruit set efficiency is observed when artificial pollination was done with fresh pollen grains. Fruit set efficiency is higher for pollen stored in 1 % sucrose solution than pollens stored in plain water in both crops. Also the fruit set efficiency values are on par with manual pollination. Stages of fruit development in cucumber and watermelon crops are also observed on the 3rd, 7th and 15th days after artificial pollination and found that the artificial pollination done using the artificial pollinizer was successful.