

**INVESTIGATIONS FOR THE DEVELOPMENT OF ELECTROSTATIC
POLLINATOR**

By
RINJU LUKOSE
(2017-18-005)

THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Technology
In
Agricultural Engineering
(Farm Power and Machinery)

Faculty of Agricultural Engineering and Technology
Kerala Agricultural University



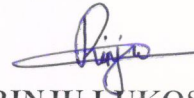
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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND
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2019

DECLARATION

I, hereby declare that this thesis entitled “INVESTIGATIONS FOR THE DEVELOPMENT OF ELECTROSTATIC POLLINATOR” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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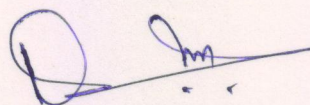
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Certified that this thesis entitled "INVESTIGATIONS FOR THE DEVELOPMENT OF ELECTROSTATIC POLLINATOR" is a bonafide record of research work done independently by Ms. Rinju Lukose under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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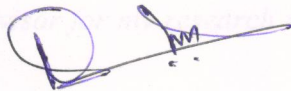
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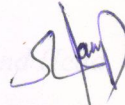
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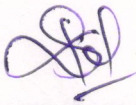
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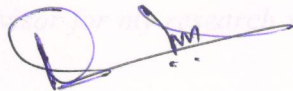


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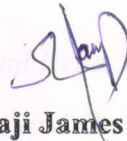
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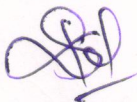
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LIST OF SYMBOLS AND ABBREVIATIONS

Ø	: Diameter
%	: Percentage
µA	: micro ampere
µm	: micro meter
ANOVA	: Analysis of Variance
DMRT	: Duncan's Multiple Range Test
etc.	: Etcetera
<i>et al.</i>	: And others
fC	: femto coulomb
HVDC	: High voltage direct current
kPa	: kilo Pascal
kV	: Kilo Volt
mm	: milli meter
MOS	: Metal Oxide Semi- conductor
MOSFET	: Metal Oxide Semi-conductor Field Effect Transistor
N	: Newton
No.	: Number
pC	: pico coulomb
RH	: Relative humidity
V	: Volt

Introduction

CHAPTER I

INTRODUCTION

Pollination is an important phase in the production of many fruits and vegetables. Angiosperm plants are typically pollinated in three sequential mass-transfer phases viz., release of pollen grains from the anthers, conveyance of pollen by biotic or abiotic carriers and placement of pollen on receptive surfaces of the stigma. In modern agriculture, with competitive markets for quality fruits, vegetables and hybrid seeds, the role of pollination can influence economic profit. Natural pollen transfer is effected by a number of vectors, of which the most important are wind, animals (usually insects), and plant bio-mechanisms which deposit pollen onto the stigma of the same flower.

Angiosperms have basically two mating systems viz., outcrossing (xenogamy) in which pollination occurs between plants with different genetic constitutions, or selfing (autogamy) in which no mixing of different genetic material occurs other than through recombination (Johri *et al.*, 2001). Outcrossing is achieved by cross pollination, resulting from the transfer of pollen between different flowers of different plants of the same species, while selfing is the outcome of pollen transfer within the same flower (self-pollination) or between different flowers of the same plant (geitonogamy). The extent to which an angiosperm responds to pollination and the fraction of that pollination that is selfed or out-crossed vary greatly by plant species or variety, and in any particular case a flower visitor must meet specific needs to qualify as a legitimate pollinator.

Pollination success is often measured in terms of percentage fruit - or seed - set, the ratio of ripe fruit or seeds relative to initial number of available flowers or ovules, respectively. This ratio is rarely 100 per cent owing to such factors as normal levels of fruit abortion, suboptimal pollination conditions, herbivory, or cultural problems

(Vidal *et al.*, 2010). The degree to which a plant species depends on a particular pollinator is determined in part by the mating and breeding system of the plant.

Protected cultivation is a technique which provides favorable environmental condition and reduces the stress levels for maximizing the yield potential of crop plants. Limitation of the cultivation area promotes the scope of protected cultivation as it can achieve self - sufficiency in vegetable sector with less area. This technology has great potential in tropical areas where open field cultivation of vegetables is restricted by heavy rains. Extreme hot and high intensity of UV rays in summer season affect the quality of products in open field condition which can be overcome by the protected structures. These structures include polyhouse, shade net, polytunnel etc. which can be utilized for round the year cultivation of different vegetables with reduced incidence of pests and diseases.

Protected cultivation creates a barrier for the wind pollination by restricting the entry of free air into it. Bee pollination is also not possible as the protected conditions prevent the entry of bees and bees cannot survive inside it due to the high temperature. Thus plants growing under protected cultivation demands artificial pollination for the better yield.

Artificial pollination occurs when human intervene with the natural pollination process. Artificial pollination done with the help of mechanical devices is known as mechanical pollination. There are so many mechanical pollinators are available in the market according to the type of plant species. But most of the mechanical pollination methods are contact in type and hence they may cause mechanical injury to the flower from which the pollens are being collected which leads to the decrease in fruit set efficiency. In this juncture, application of electrostatic force in non - contact type mechanical pollen collection is gaining more importance.

Pollen transfer in natural pollination appears to involve electrostatic forces (Corbet *et al.*, 1982; Erickson and Buchmann, 1983). Several researchers have reported

that a cloud of pollen arises from the flower anthers and is attracted to bees that hover above the flower. This observation leads to a hypothesis that the bee is electrically charged when flying and flopping its wings.

Electric fields are present in the environment and wind dispersed pollen grains are electrically charged (Bowker & Crenshaw, 2006). Consequently, as charged airborne pollen grains encounter the electric fields in the environment around plants they may experience an electrostatic force to influence deposition. Many flowers are morphologically adapted to take advantage of electrostatic forces during pollination; these flowers generally have a longer pistil than other flowers so that, when a charged pollen cloudlet is introduced by an insect or by artificial means, the flowers with the longer pistil are able to collect more pollen grains than those with shorter pistils Vaknin *et al.* (2001). Keeping this in view, a study entitled “Investigations for the development of an electrostatic pollinator” was carried out with the following objectives:

1. To study the morphological characteristics of vegetable flowers under protected cultivation.
2. To design and develop an electrostatic non-contact pollinator for collection and deposition of pollen.
3. To evaluate the electrostatic pollinator for fruit set efficiency under protected cultivation.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

In this chapter, a brief review of research works carried out in relation to the various aspects of electrostatic pollination is presented.

2.1 POLLINATION

Johri *et al.* (2001) studied about the reproductive biology of angiosperms. In flowering plants, reproduction occurs as a result of either sexual, adventive embryony or through parthenogenetic. Flowers undergoing sexual reproduction were classified into two types: unisexual and bisexual which was based upon the presence of carpels (female) and stamens (male). Pollen – pistil interaction was the major aspect in the sexual reproduction. Generally, stigma receptivity was at maximum soon after anthesis.

Warmund (2007) reported that, type of pollination varies according to the plant species. The main two types of pollination methods were: self-pollination and cross pollination. Self-pollination is the transfer of pollens within the same plant or even same flower, while cross pollination is the transfer of pollen grains between two different plants. Poor pollination lead to the abortion of flower which resulted in the poor fruit set and ultimately low yield.

Oriani *et al.* (2009) assessed the efficiency of self-pollination, insect pollination and wind pollination in *Syngonanthus elegans* by comparing the percentage of seed germination. Results showed that insect pollination was the most efficient system. The highest frequency of visit of floral visitor species was found to be more during the anther dehiscence time. Anther dehiscence for *S.elegans* was observed between morning 0900 and 0930 hrs.

Kevan (2017) defined pollination as the transfer of pollen grains from the anthers to the stigma of same flower or a different flower in the same plant or in a different

plant. After pollination, pollen germinates in the stigma, which grows through the style and releases its sperm cells into the ovule. Pollinator is an agent that causes this transfer. Natural pollinating agents are mainly wind or biotic agents such as birds, insects, bats and other animals.

2.1.1 Floral phenology (Tomato and Bitter guard flowers)

Cooper (1927) presented a detailed description about the tomato flower. Tomato flower is perfect, regular, hypogynous, pendant, and six-merous. The size of calyx increases with the development of the fruit. Flower consists of six or more orange – yellow coloured stamens which are attached to the corolla tube. The stamens are syngesious and coalesced to form a cone around the stigma, at the central portion of the flower. The enlarged basal portion of the pistil is the ovary, which is six – celled. Other parts of the pistil are elongated style and flattened stigma.

Picken (1984) explained about the floral characteristics of tomato. Flowers of tomato cultivars consist of five or more bi-lobed anthers which are united together with the help of interlocking hairs to form a cone round the style. The stigma is closely surrounded by the sterile extensions of the anther which are present in the neck of this hollow cone. Each anther consists of several hundreds of pollen and anther dehiscence takes place by its longitudinal splitting. This released pollen falls directly on the stigma and fertilization takes place by self- pollination. But in green houses pollination does not happens until it was disturbed by wind or artificial means.

Alleemullah *et al.* (2000) described that anthesis, anther dehiscence and stigma receptivity have a significant role in the final yield and quality. The study conducted on chilly variety and observed maximum fruit length, diameter, weight and no of seeds on the flowers which were pollinated on the day of anthesis. Anthesis was maximum at morning. There is a gap between anthesis and anther dehiscence. Anther dehiscence usually takes place by the longitudinal splitting of anther. Stigma receptivity period was determined by hand pollinating the flower at 2 hour intervals. The stigma

receptivity continues for nine days, i.e. 5 days before anthesis to three days after anthesis. It was also reported that changes in the temperature and light intensity have affected the floral phenology.

Behera (2005) explained that bitter melon is a monoecious crop having both male and female flowers in the same plant. In the optimum environmental conditions, the first male flower appears at 45-55 days after sowing. The anthesis and anther dehiscence occurs early in the morning. The stigma receptivity timing is drawn-out to 24 h before and 24 h after anthesis.

Deyto and Cervancia (2018) studied about the floral traits of bitter melon. The ratio of male to female flowers was observed as 19:1. Number of pollen grains in one flower was estimated as 9296 ± 623 . Female flowers consist of ovaries which resembles like small fruits. The pistil was 5 mm long with six stigmas attached on a single style. The receptive stigma was observed as moist and bright green. The flowers were fully opened between 0530 to 0600 h. The anther dehiscence was 1 h before the blooming of male flower. Female flowers were also bloomed synchronously with the male flowers and stigma was found to be receptive for 24 h. Successfully pollinated flowers were started to set fruit on the 2nd to 5th day whereas unpollinated flowers dried up and the ovaries became yellow on 5th day. They also reported that foraging period was peaked at 0700 to 0800 h.

2.1.2 Influence of environmental factors on pollination

Picken (1984) explained about the environmental factors affecting fruit set in tomato. Low light intensity reduces the production of carbohydrates. This deficiency is detrimental to the flower initiation, flower development and ultimately the fruit set. Due to reduction in the pollen development, pollen germination and pollen tube growth, temperatures below 10°C and above 30°C have adverse effect on the fruit set. Under high humidity, pollen tend to adhere on the anthers and it didn't adhere on the stigma at low humidity.

Munoz and Cuartero (1991) studied about the effect of temperature and irradiance on stigma exertion, ovule viability and embryo development in tomato. Three varieties of tomato such as Marroqui, Moneymaker and Red Top were grown under four experimental conditions: high temperature (33/23°C. day/night) with summer light ($467 \mu\text{mol m}^{-2} \text{s}^{-1}$), high temperature (33/23°C) with low light ($280 \mu\text{mol m}^{-2} \text{s}^{-1}$), low temperature (27/4 °C) with winter light ($291 \mu\text{mol m}^{-2} \text{s}^{-1}$) and low temperature (27/4 °C) with low light ($175 \mu\text{mol m}^{-2} \text{s}^{-1}$). Analysis of data from these three cultivars indicated that there was a significant difference in the stigma level between high temperature and low temperature conditions. For low temperature conditions, the mean value of stigma was -1.3 mm and negative sign indicates that the stigma insertion. For high temperature conditions, the mean value of stigma level was 0.5 mm which indicates the stigma exertion. They concluded that low light intensity combined with high temperature favor stigma exertion.

Huyskens *et al.* (1992) investigated about the optimization of agro techniques for the cultivation of bitter melon (*Momordica charantia* L). Germination was maximum at temperatures between 25 to 35°C and no germination was found at 8, 12, 40 and 45°C. More number of pistillate flowers were produced during spring –summer under long days and high temperatures than in autumn – winter under short days and low temperatures.

Rylski *et al.* (1994) studied about the flowering, fruit set, fruit development and fruit quality under different environmental conditions in tomato and pepper crops. Cracked fruits, deformed fruits, color spots were developed on the pepper fruit as a result of low temperature during winter season. Low temperature combined with high humidity suppressed pollen production in tomato. Temperature during winter season decreased the quality of tomato fruit. Low temperature with less light intensity caused deterioration in fruit taste.

Lohar and Peat (1997) investigated the changes in the floral characteristics of heat-sensitive and heat-tolerant tomato cultivars in response to the high temperature. Earlier anthesis, reduction in the no of flower buds, stigma exsertion, empty flowers, persistent flowers, absence of anther dehiscence etc. were the problems observed in the tomato cultivars as the result of high temperature.

Karapanos *et al.* (2008) described the environmental factors affecting tomato (*Lycopersicon esculentum* Mill.) cultivation. High temperature induces flower abscission in tomato by restricting the supply of assimilates to the developing flower buds. Along with that, it also negatively affects the fruit set, fruit size and quality. Extreme temperatures (low or high) decreases the fruit set efficiency due to the limited pollen production and low viability. Night temperatures less than 10°C affect the pollen fertility. But the favorable temperature and light conditions during day time can recover the problems caused by the low night temperature. High temperatures greater than 32°C, seriously affect tomato species by limiting the pollen release from the anthers, prevention of anther cone formation and reduction in stigma receptivity. Moderate day temperatures less than 32°C and night temperatures less than 22°C reduces the fruit set since the short anthers, style exsertion and poor anther dehiscence results in the poor pollination.

Behera *et al.* (2010) explained about the favourable environmental conditions of bitter melon species. They could grow tomato well in different environments such as hot humid areas, sub-tropical climates etc. It could be cultivated during summer, winter and rainy seasons and the plants were day neutral. The optimum temperature for better development was observed as 25 to 28°C. But the plants growth and flowering performance was well under very high temperature.

2.1.3. Pollination under protected cultivation

Buchmann and Hurley (1978) reported that there was no nectar production as a reward for pollinators in the case of flowers with poricidal anthers such as tomato. So nectar seeking females and male bees does not generally visit these flowers.

Banda and Paxton (1991) carried out experiments on the tomato pollination. Plants were exposed to six treatments such as no pollination, pollination by honey bees, pollination by electric vibration, pollination by honey bees and electronic vibration, pollination by bumble bees and pollination by bumble bees and electric vibration. These treatments were evaluated in terms of fruit set, size of fruit, weight of fruit and number of seeds per fruit. Results shown that electronic vibration and bumble bees produced a high percentage of fruit set efficiency. Pollination by honey bees yielded large and heavy fruits as that of vibration pollination. The combined use of bumble bees and vibration leads to the increase in fruit weight.

During the study of pollen collection by bees, Thorp (1999) observed that honey bees were not enough to pollinate some commercial crops and bees were unable to sonicate flowers. Thus, considerable interest has to be given for commercial pollination of buzz pollinated crops especially greenhouse tomatoes. Sabara and Winston (2003) reported that more honey bees leave greenhouses to forage outside in summer season compared to that of winter season.

Whittington *et al.* (2004) found out the identity of plant species of pollen collected by bumble bees placed in green houses for tomato production. Pollen loads were removed by keeping bees immobile under freezing conditions. They concluded that bumble bees forage their major time on outside floral resources and it lead to the decrease in the amount of pollination of tomato. That reduction was not because of the decrease in the number of tomato flowers, but was due to the decrease in the amount of pollens in flowers according to the seasonal variations. During the examined period,

some of the flowers were not pollinated by bees which lead to the decrease in the financial benefit of greenhouse tomato growers.

2.2 ARTIFICIAL POLLINATION

2.2.1 Mechanical Pollination

Moulie (1907) developed a pollen collection device for flowers which are using in the manufacture of perfumes, medicines etc. The device consists of a vessel and was provided at the ends with handles, loosely mounted in sockets and rigidly secured to the sides. A pair of lateral and transverse bars was provided at the top of the vessel. To collect pollen from the twigs or branches, vessel was filled with water and twigs were immersed into the water by inserting through the lateral openings in a diagonally downward direction. A sheet of paper was placed below the vessel. As the branches were projecting beyond the sides of the vessel, pollens which were separating from the blossoms could be collected into the paper.

Hosaka (1974) developed a hand held pollinator which discharges the pollen by using a pump. It consists of a pollen storage container, a power source, pump, conduit and actuator. Housing was a pistol shaped casing which have an elongated upper section and a hollow lower section. Pump generates air into the pollen storage tank. Conduit was connected to the pollen storage and it discharges pollen to the outside through a discharge nozzle. Actuator actuates the pump to cause air to be supplied to the pollen storage tank. It includes a manually actuated electrical switch.

Atkinson and Atkinson (1990) developed an apparatus for improving pollination of orchard plants. It consisted of a portable power unit and collection device mounted on a trailer, cyclone unit, fan, inlet, outlet, collection nozzles, hoses and dispersion nozzles. It suck pollen from male orchard plant, the collected pollen passes through a cyclone which can acts both as a separator and pollen storage unit and deposits the pollen into the female part of the flower.

Bullock *et al.* (2012) developed a method for vacuum pollen collection from haploid and doubled haploid plants. It consists of a vacuum source, a cylinder capable of generating cyclonic air movement and a disposable receptacle. Vacuum was generated at the vacuum port because of the cyclonic air movement inside the cylinder. By placing the vacuum collection port near to the flowers, anthers were drawn into the cyclonic chamber. Pollens get separated from the anthers by spinning of the anthers in the cyclonic system. Those pollens were collected inside the disposable receptacle. This method of pollen collection improved the seed production in plants which produces a limited amount of viable pollen.

Michael (2013) developed a pollination device which comprises of a pollen capture device for capturing pollen from anthers, a handle which can generate vibrations and at least one wand on the handle portion to transfer the vibrations generated by the handle portion to flower. Handle portion could generate vibrations which range from 29,000 vibrations per minute to 44,000 vibrations per minute.

Blahnik and Jaehnel (2014) developed a method for manual dispensing of maize pollen. It consists of a reusable air discharger and a single use biodegradable applicator. Reusable air discharger had an internal cavity and an outlet and it expels air through this outlet into applicator which have a first and second end. Applicator was designed in such a way that it can receive at least one maize tassel containing maize pollen. Pollens get released through the second end of the applicator according to the operation of the air discharger.

Jai *et. al* (2015) invented an artificial pollinating device to solve the problems associated with the pollination of multiple varieties. It comprises of an air blowing device for blowing air and a pollen collecting device which consists of connecting column, a supporting base, an adjusting plate, 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.

Safreno (2017) developed an apparatus for vision-based pollination system. It consists of 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.

2.2.2 Manual Pollination

According to McGregor (1976), tomato plants have poricidal dehiscent flowers. Pollination in these flowers can be achieved by gently shaking the flowers.

Hopping and Hacking (1983) conducted a comparison study of the pollen application methods in kiwi fruit. Flowering kiwifruit laterals were covered by using terylene sleeves to avoid both insect and wind pollination. Freshly harvested pollens were applied by hand or by mechanical puffer gum after mixing with talc. Pollens diluted in aqueous solution were applied by using hand operated pressure sprayer or by a pressurized boom sprayer using compressed air. Evaluation of application method was done by determining the number of pollen grains on the stigmatic surface and number of pollen tubes that penetrated into the style.

Shivanna and Rangaswamy (1993) described procedure for pollen collection manually. Exercise mature anther just before dehiscence and allow them to dehiscence under low humidity in desiccators. Anther debris was then removed with brush or forceps or pollen was sieved through mesh of suitable pore size.

Kwaasi (2003) explained that as conventional method, date pollens were applied by inserting one to three branches of male inflorescence in the fully opened female inflorescence. This method has been replaced by hand pollination, in which pollen was placed in porous sacs or dusters and spread as dusts on female flowers or by mechanical spraying.

Guzman and Zara (2012) described the artificial hand pollination in vanilla flowers. In the method, the stamen cap, which encloses the pollen, was removed and rostellum, which covers the stigma, was pushed up by using a bamboo stick or any similar object like a toothpick. Then, the pollen and stigma were brought into contact by hand manipulation.

Chetelat and Peacock (2013) had given guidelines for the pollination in tomatoes. Pollen collection from flowers could be done by cutting a slit in the anther cone using a dissection needle and scrap the contents in it. In the case of very humid conditions, remove the flower from the plant and dry anthers under sun or by using dehiscent lamp. Using dry – cool atmosphere, pollen could be stored for 1-2 months. Pollen application could be done by using dissecting needle or by dipping the stigma into the pollen.

2.3 ELECTROSTATIC POLLINATION

2.3.1 Electrostatics

Electrostatics is the branch of engineering science, deals with the study and applications of static electricity. It is the surface phenomenon governing electric charges accumulated on object body.

Coulomb's law is an experimental law formulated in 1785 by the French colonel, Charles Augustin de Coulomb. It deals with the force which a point charge exerts on another point charge. It states that the force (F) between two point charges Q_1 and Q_2 acts along the line joining them and directly proportional to the product of charges and inversely proportional to the square of distance (R) between them. It can be expressed mathematically as,

$$F \propto \frac{Q_1 \cdot Q_2}{R^2}$$

$$F = \frac{K \cdot Q_1 \cdot Q_2}{R^2}$$

Where, K is the proportionality constant. In SI units, charges Q_1 and Q_2 are in coulombs (C), the distance R is in metre (m), the force F is in newton (N) and $K=1/4\pi\epsilon_0$. The constant ϵ_0 is known as the *permittivity of free space* (in farads per meter) and has the value,

$$\epsilon_0 = 8.854 \times 10^{-12} \text{ Fm}^{-1}$$

Therefore,

$$K = \frac{1}{4\pi\epsilon_0} = 9 \times 10^9 \text{ m/F or N.m}^2/\text{C}^2$$

2.3.2 Particle charging methods

There are mainly three methods for particle charging.

- Corona charging
- Tribo-electric charging
- Electrostatic induction charging

Banarjee and Law (1996) observed that pollen suspensions can be successfully metered and charged by using electrostatic induction. For this, almond pollen was suspended into the liquid which possess low electrical resistivity and an osmotic pressure which was equal to that of the cytoplasmic pressure of the pollen grains. Induction charging voltage for electrode was varied between 230 to 1000 V. With the varying voltage, spray cloud current increased from $-2.6\mu\text{A}$ to $-13.3\mu\text{A}$ with a corresponding charge to mass ratio of -9.9 mC/kg at 1000 V. While the variation in the pollen mass loading over a range of 0.5 g/L to 10 g/L resulted in a constant spray cloud current.

Banarjee and Law (1998) investigated the triboelectric chargeability of pecan pollen and lycopodium spores by using nylon and Teflon. The process of charging two dissimilar bodies by contact and/or rubbing is known as tribo-electrification. The charge to mass ratio of lycopodium and pecan pollens were increased positively after

charging with teflon and negatively after charging with nylon. So they concluded that both nylon and Teflon were feasible for pollen charging of electrostatic pollinators.

Mayr and Barringer (2006) compared corona charging with triboelectric charging in electrostatic powder coating. Graham crackers were coated with food powders which varied from 13 to 138 μm sizes. The powder particles were charged at 50 and 95 kV by corona charging system and charged by tribo-charging with nylon and teflon. Non - electrostatic coating was done at 0 kV. In corona charging system, an electrode wire was placed just beyond the gun tip in corona gun. Charging in triboelectric charging system was done by rubbing the powder along a nylon or teflon barrel. Charge to mass ratio of the powders was measured by using a Faraday cup which was attached with an electrometer. Dust produced during coating was measured by placing filters behind the grounding block containing the crackers. Results showed that transfer efficiency in teflon charging and corona charging was high compared to nylon charging and non-electrostatic coating. It was also observed that corona charging is the most effective method to reduce the dust.

Corona charging includes corona discharge for pollen charging by using very high voltage. Since the pollen grains of the vegetable flowers are very fragile and to avoid corona discharge, electrostatic induction method is most suitable for the pollen collection and deposition.

In electrostatic induction, a high voltage is applied to the electrode and it induces opposite charge to the pollen grains. The studies shown that the principle behind this induction is dielectrophoresis.

a. *Dielectrophoresis*

Electrophoresis is the separation of charged molecules and it is performed under uniform or non- uniform electrical field. When a polarized cell is exhibited to a non-electric field, the algebraic sum of the translational forces acting on the positive and negative elements of the induced dipole moment is called dielectrophoretic force.

Pohl (1978) explained two cases of pollen charging. If the pollen grains are sitting on the conducting electrode, the pollen grains acquire the negative charge by sharing and it tends to move by the principle of electrophoresis. And when a positively charged electrode approaches a pollen grain, it induces a dipolar charge distribution in the pollen grains and grains then tend to move by dielectrophoresis.

In pollen grains, dipole moment produces due to the presence of water in it. Water has a polar molecular structure. Due to the hydrogen covalent bonds, water molecule has a large dipole moment in it. Water molecule has two hydrogen atoms and one oxygen atom. Hydrogen atoms have one electron each and oxygen atom has eight electrons. Hydrogen atom shares its electrons with the oxygen atom and these electrons stay closer to the oxygen atom and form a negative charge on the oxygen atom. And the outside of the hydrogen atom tends to be positively charged. These opposite charges form dipole moment in water molecule. (Fig. 2.1).

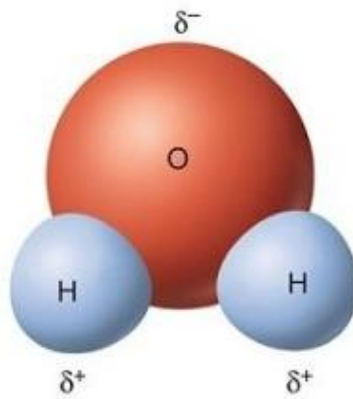


Fig. 2.1. Polar structure of water molecule

Law *et al.* (2000) explained that electrophoretic and dielectrophoretic processes behind the pollen transfer depends upon the physical and environmental variables such as shape and conductivity of stigma and electrode, air- ion concentration and variations in the earth's ambient electrical field.

2.3.3 Electrostatics on natural systems

Rines and Beach (1904) mentioned that electric field exist in the earth's surface and in fair, weather condition, it is of the order of 100 V with every 1 m increase in the altitude. This continues up to an altitude of 30- 50 km, reaches around 300 kV and after that, its value decreases.

Es' kov and Sapozhnikov (1976) studied about the mechanism of generation and perception of electric fields by honey bees. They suggested that bees during flight can acquire up to hundreds of volts. The greatest charge that measured from the body of a foraging bee was recorded as 45 pC. It was observed that generation of electric field is not due to the presence of specialized organs and it is concerned with the characteristics of the integuments to carry a high amount of electrostatic charge. Transport of charged bodies was percieted by bees with the help of antennae subjected to the action of couloun powers. Perception of alternating artificially generated electric fields is mainly concerned with physiological influence of induced currents flowing in the site of contact of bees to one another or to current-conducting bodies.

Warnke (1977) observed that foraging bees generally acquires electrically positive charges in its surface. When the bee flies through the air, it is confronted with the positive ions in the atmosphere and it will get electrostatically charged with frictional electricity. This formation of opposite charges between bees and plants enhance the pollen detachment from the anther to the body of bees and deposition of pollens by insect. He also added that under rainy or cloudy conditions, the polarity of the electric fields will get reversed and the charges in the plant surface will become positive.

Buchmann and Hurley (1978) developed a biophysical model to investigate about buzz pollination or vibrational pollination in angiosperms. A model was created which resembles a *Solanum* flower and it could expel pollen upon the vibration by bees

or by artificial means. They mentioned that the attachment of small, light, dry pollen grains to the bees are mainly electrostatic in nature.

Corbet *et al.* (1982) described the role of electrostatic forces in pollen transfer. Pollen grains of oilseed rape, *Brassica napus* L. were used for the experiment. The attraction of falling rape pollen towards a living bumble bee which was held down with silken threads to a cork on a wax block was observed under a dissecting microscope. It was found that falling pollens were drifted rapidly towards the bee and adhered into it. A freshly killed honey bee transfixed on a pin, connected to a potential of 750 V and moved towards an anther which was connected to a grounded pin. It was observed that pollens in the anther jumped to the bee at a distance of 630 μm and at 230 μm for 500 V. When this pollen carrying charged bee at 750 V moved towards a rape stigma in grounded state, pollen jumped towards the stigma from the bee at a distance of 376 μm . Compared to other exposed surfaces of the flower, stigma is uninsulated and provides a low impedance path to earth. These experiments inferred that pollens can jump from anther to bee and from bee to stigma at potentials of a few hundred volts over distances of about 0.5 mm. The study also proved the hypothesis that electric fields can facilitate the pollen transfer in bee- mediated pollination.

Erickson and Buchmann (1983) reported that electrostatic forces have a role in the buzz- released pollen collection by bees. Flying bees acquire strong positive charges by the interaction with the moving air using their setae and cuticles. When the positively charged bee approaches a grounded flower, it induces negative charge on the pollen grains. So the study hypothesized that these opposite attracting physical forces would be act as an aid in the pollen separation from the flowers. When the bees carry pollen grains through the air, it would be positively charged and then it would attract to the terminals of the negatively charged female flowers. Thus along with air flow, electrostatic attraction could form an efficient mechanism for pollen collection by anemophiles.

Dai and Law (1984) developed a 3-D finite element model for the analysis of transient electrical field when a charged cloud of pollen grain approaches a flower. The electric field above the stigma was nearly three times that of the field at the petals. With the increase in the opening angle between petals and style, electric field at the stigma gets maximized and this causes the maximum deposition of the pollen on the stigma. The pollen transfer between the bee and flower depends upon the physical variables such as the dielectric properties of the media, magnitude and spacing of the charge source and the geometry of the flower. The study also gave information about the relaxation time of the style and petals. Relaxation time is the time taken for charges to flow from the ground to the flower. Relaxation time was measured as 03 ns for the style and 25 ns for the petals.

Buchmann (1985) observed that the use of electrostatic forces in pollen collection by bees would be significant in poricidal flowers like *Solanum* and *Cassia*. For these flowers, pollen must be small and relatively free from oil surface to be discharged successfully through pores upon bee vibration.

Gan-Mor *et al.* (1995) conducted experiments to determine the charge on the bee and to measure the force required to detach pollen from the flower. Passive passage and active passage tests were done to determine the charge on the bee. An electrostatic field meter (Model 245, Monroe Electronics inc, Lynodnville, NY) was used to measure the electrostatic field strength. There was no indication of electrostatic field in the case of bees after passive passage. But in the case of bees after active flight, the electrostatic field values reached up to 15 V mm^{-1} and the maximum charge was 93 pC. The forces required to detach pollen grains from avocado, lizianthus and eucalyptus were measured by applying a series of known electrostatic forces. The forces were calculated as $4 \times 10^{-10} \text{ N}$, $3 \times 10^{-10} \text{ N}$ and $39 \times 10^{-10} \text{ N}$ for avocado, eucalyptus and lizianthus respectively.

Schroeder (1995) conducted studies to determine the effect of electrostatic forces on the pollen collection from the avocado flowers by bees. It was concluded that not only pollen stickiness but also electrostatic forces were responsible for pollen collection by the bees. Endress (1997) conducted experiments on pollination of *Dillenia* and reported that pointed ends of flowers like stigma, anther etc. improve the strength of electrostatic forces which causes the transfer of pollens from anther to bees and from bees to stigma.

Vaknin *et al.* (2000) described the detailed mechanism of electrostatics in the pollen transfer between bee and flower (Fig. 2.2). When a charged bee approaches a flower, it induces an opposite charge on the flower and it leads to the creation of a temporary force of attraction between the bee and the flower. This initiates the pollen transfer towards the bee from the anther. These same forces in turn can initiate the deposition of the pollen on to the stigma. From the studies on the two theoretical models of the charged cloud approaching a flower, they observed that style length and flower opening are the two important features of the flower which are adapted to electrostatic pollination.

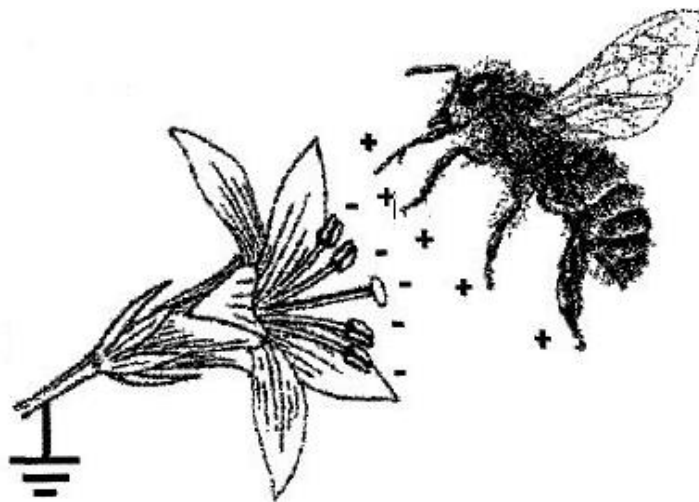


Fig. 2.2. A positively charged bee with pollen grains approaching a flower and inducing negative charge on the terminals of flower

Armbruster (2001) observed that when a pollinator having charge approaches a flower, the pollen might get detached and attracted to the pollinator due to the development of opposite charge in the flower. These same forces can enhance the deposition of pollen on the stigma. Some plants are morphologically adapted to take the advantages of electrostatic pollination. For instance, plants develop flat corollas and long stigmas for electrostatic enhancement of pollination.

Bowker and Crenshaw (2006) studied about the electrostatic forces in wind pollination by modeling the electric fields around the grounded plants and estimated the forces and simulated the trajectories of charged pollen grains. The electric fields present near the plants enhance the capturing of wind-dispersed pollen grains. Atmosphere consists of positively charged ions. Plants get into contact with this and carry a net negative charge equal to that cumulative positive charge in atmosphere. Thus, positively charged pollens attracted into the flower and negatively charged pollens get repelled. In fair weather conditions, an electric field of 100 V m^{-1} exists between the plants and atmosphere. This can reach up to a magnitude of $3 \times 10^6 \text{ V m}^{-1}$. The magnitude of electric field depends upon the size, shape and height of the plant and maximum near the sharp ends. Using electrostatic forces, pollens with less size ($5 \mu\text{m}$) are more capturable than pollens with larger size ($20 \mu\text{m}$) and pollen grains can be attracted to flowers from millimeters or even centimeters.

Bowker and Crenshaw (2007) conducted experiments to determine the electrostatic charge on wind-dispersed pollen grains by giving a modification in the Milikan oil drop experiment. Study was carried out on seven anemophilous plants. Charge was calculated by equating the gravitational forces and electrostatic forces for a spherical pollen in a known electric field. Velocity of the pollens was measured by videotaping the pollen as they moved because of gravity and electric field. Pollens were released from an electrically grounded plant and allowed to fall into the region between two parallel plates which consist of an electric field of $6.34 \times 10^4 \text{ V m}^{-1}$. Nearly all pollen grains were found to be electrostatically charged and the magnitude

of charge carried by a typical pollen grain was reported as 0.84 fC. Some pollen grains carried charges up to 40 fC.

Clarke *et al.* (2013) investigated about the detection and learning of electrical fields by bumble bees. Floral electric potential could be affected by pollination and pollinators. Bees carry positive charges with it. The force between the bee and flower increases as the bee approaches a flower. Bumble bees can discriminate rewarding and non-rewarding flowers based on the floral electric field. It was observed that bumble bees could detect these electric fields and it helped to achieve rapid and dynamic communication between pollinator and flower. The study suggested that a 30 cm grounded plant in 100 V m^{-1} can develop a patterned electric field having a potential difference of 30 V between flower and surrounding air. This study also revealed that the most important factor that determines electric field is the floral morphology.

Greggers *et al.* (2013) assessed electric field in honey bees by measuring the static and modulated electric fields of flying, landing and dancing honey bees. The electric charge of the bee entering the hive was measured by using an electrometer consisting of eight electrodes. A transparent flat sensor consisting of two round Plexiglass plates was used to measure the electric field of dancing honey bees. Potential of 0 - 450 V was observed in the electrometer when the bee entered the hive. By vibrating the thorax and wings, dancing honey bees emit air borne signals as well as electric fields. These signals induce antennae flagellar vibrations. The amplitudes of these vibrations exceed those caused by air flows of the beating wing. The study inferred that the mechanosensory organs associated with both joints of the antennae are the most responsible parts for the bees to sense electrical field.

Badger *et al.* (2015) carried out an experiment to determine the electrostatic charge on wild hovering Anna's humming birds using charge sensor and Faraday's cage ($35 \text{ cm} \times 35 \text{ cm} \times 35 \text{ cm}$) and it was found that charge of free flying humming birds varied from -250 pC TO + 800 pC. Stamen attraction of four plant species such as nicotiana, penstemon, aloe and hemerocallis towards the charged model of the

humming bird was also studied and results indicated that all stamens were attracted between 10 to 100 μm . As the charge of the model increases, distance of separation and speed of attraction also increased. To determine the attraction of falling pollen grain towards the charged hummingbird model, model was charged at 490 pC and at 840 pC. It was observed that lycopodium spores were attracted laterally towards the model at horizontal speeds of $13 \pm 4 \text{ mm s}^{-1}$ and $13 \pm 3 \text{ mm s}^{-1}$ respectively for model charges of 490 pC and 840 pC. It was also found that triboelectric charges were developed as a result of rubbing of a wing of hummingbird with leaves.

Clarke *et al.* (2017) developed a finite element model to investigate about flower - bee interactions. Three forces such as gravitational forces, viscous drag forces and electric forces were included in this model. Results indicated that pollen grains were readily moved under electrical forces against gravity towards bee. Viscous drag forces and electric forces have a significant role in the trajectory of pollen grains. The study also gave information about the influence of environmental factors such as humidity and wind on the electric field. High humidity forms a moist film and prevents the formation of electric field. In high wind areas too, wind forces control the trajectory of pollen grains.

2.3.4 Electrostatic pollination in agricultural systems

Philippe and Baldet (1996) developed an electrostatic dusting technique for pollen application in larch trees. The pollinator was carried in a tractor and it comprised of a tank which encompass the tree, electrostatic dusting device and an energy unit. Pollen was dusted by using a nozzle situated at the tip of the gun and pollens were charged (15 to 80 kV) by four electrodes present inside the nozzle. Compared to conventional pollen application methods, pollen deposition was increased by three times, improved fruit set efficiency and reduced pollen consumption.

Bechar *et al.* (1999) conducted modeling and experimental analysis of electrostatic date pollination. Modeling study using a 3-D finite element model of a

charged cloud of pollen approaching a date flower showed that electric field was maximum at the pistil top and the electric field decreases as the distance from the pistil increases. An electrostatic pollination system based on corona charging which charges the electrode up to 80 kV was developed. In laboratory experiments using this system, it was observed that pollen grain density on the pistil top was 60 times higher than that of the pistil envelope for 80 kV treatments. In the field, the system was evaluated based on the fruit set efficiency and the yield. Results indicated that percentage of fruits developed from flowers subjected to electrostatic pollination was twice than that of non-electrostatic pollination.

Law *et al.* (2000) designed an efficient pollen deposition equipment by using pneumatic atomization and electrostatic induction techniques for high quality fruit production. Knowledge of various electrostatic techniques in the industrial field to handle the motion of 30 - 150 μm particles enabled them to develop this system. Using this equipment, osmotically balanced carrier - liquid suspensions of almond pollen were atomized at air pressure between 138 kPa to 276 kPa and induction charged at 0, 500 and 1000 V. They observed that charged pollen deposition significantly increased 5.6 times in different target orientations than that of uncharged pollen deposition without much reduction in pollen germination ratios.

Vaknin *et al.* (2001) developed an electrostatic pollination device which charges and delivers the pollen by corona charging system. Field experiments were conducted in pistachio and studied the effect of electrostatic pollen supplementation in nut quality and yield. The experiment consisted of two treatments such as open pollination and 0.2 g of pollen grains were applied per tree by electrostatic application device. They concluded that electrostatic pollen supplementation produced 16.2% more fruitlets per cluster, 11.3% higher yield, 18.6% higher split percentages and 60% lower blank percentages than that of open wind pollination. They suggested that natural pollination can be replaced with electrostatic pollination technique without can replace by yielding fruits with high quality.

Gan-Mor *et al.* (2003) developed an electrostatic pollinator with corona charging system and evaluated it in almond, kiwi, date and pistachio. The main components of the electrostatic pollinator were feeder motor, air - pollen mixer, feed - rate regulator, air blower, delivery hose, high voltage DC converter and corona charging system. Near to 80 kV potential were developed through corona charging system and air velocity was adjusted between 4 to 8 ms⁻¹ depending on the tree or flower geometry. Treatments such as charged pollen supplementation, uncharged pollen supplementation and open wind pollination were applied in almond, kiwi, date and pistachio trees. The application of charged pollen lead to 13% increase in the total yield of almond, 28% increase in the percentage of split fruits of pistachio, double the fruit set percentage in date trees and increased the no of seeds in the kiwi fruit.

Gan- Mor *et al.* (2009) conducted studies on date pollination by developing an electrostatic pollen applicator. It consists of pollen feed system, air and pollen hose, adjustable blower and electrostatic charging system. The air outlet geometry was designed in such a way that which it can diverge the air stream to a height of 2 m at a distance of 2.5 m from the outlet. Four corona electrodes were placed at the outlet and charged by using high voltage power supply. To enhance the charging, a rectangular frame of steel wire is positioned at 125 mm away from the corona electrode. Air velocity was 22 ms⁻¹ to use the applicator under wind conditions. To provide good insulation, outlet housing was fabricated by using acrylic. Focused pollen application and less labour requirement are the major advantages of this device. It was found that to obtain same fruit set efficiency the device requires twice the amount of uncharged pollen than that of charged pollen.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

As pollination is the key factor in reproduction system of the plant, it has direct impact on crop yield, quality of produce in terms of bio-morphological characters and sensory evaluation which in turn influences the product market value. The crops growing under greenhouses have the structural obstacles to natural pollination and demands artificial pollination. Most of the mechanical pollination devices are contact in type and may cause mechanical injury to the flower. Hence an electrostatic non-contact type mechanical pollinator has gaining more importance. In this chapter, for the development of an electrostatic artificial pollinator the crops selected for the study and its floral characteristics are explained. The methodology adopted for the development of electrostatic pollinator and the evaluation of the prototype in crops under protected cultivation are also explained in detail.

3.1. LOCATION OF EXPERIMENTAL SITE

The experiments were conducted at Department of Farm Machinery and Power Engineering, Kelappaji College of Agricultural Engineering and Technology, Tavanur, Malappuram, Kerala, located at 10°52'30" N latitude and 76 °E longitude and is 13 m above mean sea level. The area receives an average rainfall of 2952 mm and experiences humid tropical climate with average annual temperature of 32 °C.

3.2. CROP SELECTED

The vegetable cultivation in Kerala under protected condition demands mechanical pollination for better yield even if it is a self-pollinated plant. This is because of protrusion of stigma due to the high temperature inside the greenhouse. This irregular position of stigma restricts pollination in a self-pollinated plant. The only solution for this problem is artificial pollination. Hence the study was undertaken for the crop tomato (*Solanum lycopersicum*), a self-pollinated crop which exhibits stigma

exsertion under high temperature. A cross- pollinated crop bitter gourd (*Momordica charantia*) also were selected for the pollination studies. Anagha variety of tomato and Preethi variety of bitter gourd were grown under protected condition. Irrigation was done through drip system.

3.3. FLORAL CHARACTERISTICS

The success of pollination depends up on the floral biology and hence the design of electrostatic pollinator was evolved through the basic floral characteristics. The floral features of tomato and bitter gourd flowers at its proper development and pollination stage were studied.

3.3.1 Tomato flower (*Solanum lycopersicum*)

The tomato flower is regular, pendant, bisexual, hypogynous and six – merous (Fig. 3.1). Generally the length of a tomato flower with fully expanded corolla limb and petals is approximately 10 mm (Brukhin *et al*, 2003). Stamen, the male reproductive part of the flower, consists of an anther and a filament. In this, anther is the most important part as it produces the male gametophyte known as pollen. Tomato has usually four to six bi - lobed anthers and they fused together to form like a cone (Cooper, 1927).

The stamens are attached to the throat of the corolla tube. Pistil occupies the central portion of the flower. The pistil consists of an ovary at the basal portion and a stigma. For self- pollination, stigma should be recessed within the anther cone. In protected cultivated condition, due to high temperature, the stigma exserted beyond the anther cone. The following characteristics of tomato flowers were found out.

- Length of flower
- Pedicel girth
- Diameter of flower
- Diameter of anther cone

- Number of anthers
- Length of anther
- Thickness of anther

Length and pedicel girth of flowers, diameter of anther cone were measured using vernier caliper. Diameter of flower was also measured. Number of anthers present in the each flower was counted manually. Length and thickness of anthers were measured by using Vernier caliper.

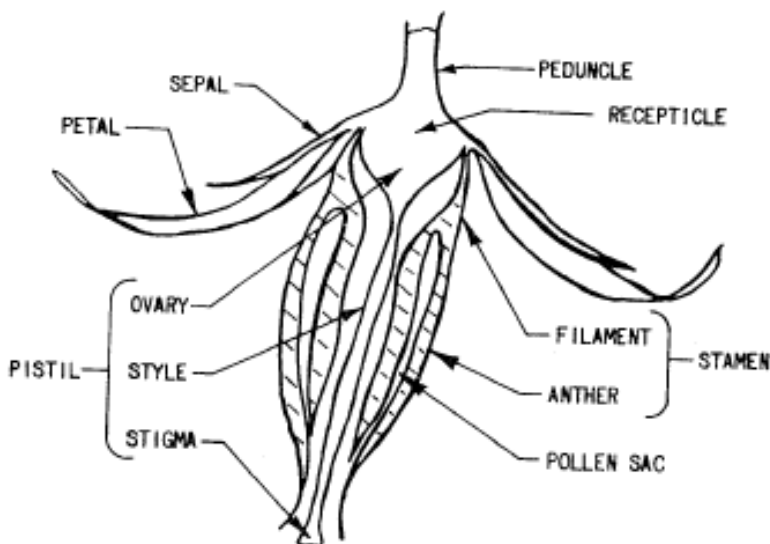


Fig. 3.1. Sectional view of tomato flower

a. *Stigma exsertion*

Stigma exsertion that eliminates the self-pollination in tomato flowers inside the green houses (Fig. 3.2). Exsertion of stigma was measured for ten randomly selected tomato flowers. It was calculated by using following formula:

$$\text{Stigma Exsertion (\%)} = \frac{\text{Length of stigma exerted above anther cone}}{\text{Total length of stigma}} \times 100$$



Fig. 3.2. Stigma exertion of tomato flower



Fig. 3.3. Male and female flowers of bitter gourd

3.3.2 Bitter gourd flower (*Momordica charantia* L.)

Flowers are usually classified based on the presence of their sexual parts. Bisexual flowers contain both the stamen and pistil in the same flower. Unisexual flowers contain stamen and pistil in different flowers. Bitter gourd flowers are unisexual. Male flowers appear first and female flowers appear after that. Generally the number of male flowers is more than that of female flowers (Fig. 3.3).

The male flowers are imperfect, incomplete, regular, actinomorphic and whorled flowers. Staminate flower of bitter gourd normally consists of five stamens and it is appeared as three. The two sets have two fused anthers forms two compound stamen and a single stamen. The anthers of bitter gourd flowers are dorsifixed. Female flowers are easy to recognize due to their ovaries at the bottom portion. The pistil has six stigmas which are attached to a single style. The receptive stigma is moist and green in color.

Bitter gourd male flower has a mean diameter of nearly 3.2 cm and female flower has a mean diameter of nearly 2.5 cm. The mean length of the flower up to the axil is 9.2 cm (Deyto and Cervancia, 2018). The following characteristics of the bitter gourd (Preethi) flowers cultivated under protected condition were found out.

- Length of flower
- Pedicel girth
- Number of anthers
- Length of anther
- Thickness of anther
- Number of stamens
- Diameter of male flower
- Diameter of female flower

The length and pedicel girth of the twenty randomly selected flowers were measured by digital Vernier caliper and the mean value was calculated. The length and thickness of the anther was measured using digital Vernier caliper. The number of anthers and stamens were counted manually. The diameter of male and female flowers was measured.

3.3.3 Time of pollination

Anthesis, anther dehiscence and stigma receptivity are the important events that determine the success of pollination (Aleemullah *et al.*, 2000). These timings vary with respect to plant species. The proper co-ordination of these timings governs the fruit quality and yield. Hence the time of pollen collection and deposition be contingent upon these timings.

a. *Anthesis time*

It is the time at which flower blooms fully. In natural pollination, the foraging activity of bees are synchronized with anthesis time (Free, 1962). In tomato flower, the corolla begins to open during anthesis, stamens are in pale yellow color and spore chamber is closed.

In bitter gourd flower, on the day before anthesis, corolla gets elongated above the calyx. On the day of anthesis, the corolla begins to loosen up and the apexes of the petals get separated forming a bell shaped corolla. Stamens become visible with the opening of petals. The anthesis time was identified by continuous and close observation.

b. *Anther dehiscence time*

Anther dehiscence is a multistage process that involves localized cellular differentiation and degeneration, combined with changes in the structure and water status of the anther to facilitate complete anther opening and pollen release (Wilson *et al.*, 2011). The anther dehiscence time varies with respect to plant species. In tomato

flower, the color of the stamens gets changed into bright yellow and dehiscence begins. It is accomplished by longitudinal splitting of anthers along its weakest part. In bitter gourd also pollen grains are shed by longitudinal splitting of anthers.

c. *Stigma receptivity time*

Stigma receptivity is the ability of stigma to support the viable and compatible pollen. Stigma becomes receptive in tomato flower 12 hrs prior to anthesis and it continues up to withering stage of the flower. The stigma becomes fully receptive after anthesis of the flower.

The receptive stigma in bitter gourd becomes moist and bright green. Stigma continues its receptive state for duration of 24 hrs after anthesis. After anther dehiscence, pollens were applied into stigma of the same flower for tomato and pistillate flower in the case of bitter gourd.

3.3.4 Size of pollen

Size of pollen significantly influences the electrostatic potential required to detach the pollen from the anther. It also influences the quantity of pollen detachment (Bowker and Crenshaw, 2006). The pollen size was measured by collecting pollens just after anthesis, using a dissecting needle and placed it on a glass slide. Two drops of glycerin were added into the collected pollen sample. It was covered by a cover slide and placed under the Magnus compound microscope with micrometer. The size of pollens was measured with 40 X resolutions.

3.3.5 Number of pollen

It defines the maximum amount of pollen that can be collected by using the pollen collection unit and also the amount of pollen required for pollination. To quantify the number of detachable pollen, tomato and bitter gourd flowers after anthesis and before anther dehiscence stage were collected. Pollens from the flowers were dispersed into petri dish with grid (Deyto and Cervancia, 2018). Then two drops of

glycerine was added to it. The pollen count was measured by counting the pollens in each grid with the Magnus compound microscope.

3.4. ENVIRONMENTAL FACTORS AFFECTING POLLINATION

Environmental factors such as temperature, relative humidity and light intensity have a great effect on pollination. Variations in temperature and light intensity can influence the position of stigma in tomato flower which determines the type of pollination needed for successful yield. Favorable temperature for bitter gourd species is 25 to 28 °C (Behera *et al.*, 2010). The temperature, relative humidity and light intensity at the time of anther dehiscence and stigma receptivity of tomato and bitter gourd flowers were observed by using temperature and humidity meter and digital illuminance meter.

3.4.1 Temperature and Humidity meter

It consists of a single chip Relative Humidity and Temperature sensor to measure the humidity and temperature (Fig. 3.4). Specifications of temperature and humidity meter are given below:

Make	: Equinox
Model	: EQ-321S
Measuring range	: Temperature : - 30 °C – 100 °C
	: Relative humidity : 0 to 100 % RH
Accuracy	: ±0.5 °C; ±2 % RH
Resolution	: 0.01 °C; 0.01 % RH

3.4.2 Digital illuminance meter

The digital illuminance meter is an instrument used to measure the luminous flux inside the poly house (Fig. 3.5).



Fig. 3.4. Temperature and Humidity meter



Fig. 3.5. Digital illuminance meter with photon detector

It consists of a digital meter which shows the value of illuminance in Lux or fc and a photon detector. Photon detector is made up of a silicon diode with filter. The illuminance can be recorded by exposing the photon detector to the light source. Specifications of the Digital illuminance meter are given below.

Make	: Equinox
Model	: EQ-802
Measuring range	: 0.1 Lux to 2, 00,000 Lux

3.5 POLLINATION

For the process of pollination to be successful, a pollen grain produced by the anther must be transferred to stigma of the flower, of same plant or the same species. As the pollens are immobile, it requires an artificial agent for its transfer to stigma, which can be bees, birds, butterflies, insects, water, wind etc. Pollination phenomena can be resolved into two major groups viz. self-pollination and cross pollination. When the pollen grains deposit on the receptive stigma of same flower or the pollens of one flower deposit on receptive stigma of another flower of same plant known as self-pollination. On other hand, when pollens from flower of one plant deposit on stigmas of neighbouring plants of same species, called as cross pollination. While, hybridization is the process of pollination in which pollens of flower from one plant deposit on the stigmas of another plant of same family but different species.

Natural pollination does not occur inside the greenhouses as it obstructs the free circulation of air and also the entry and survival of biological pollinators. Hence artificial pollination is inevitable in poly houses for better yield even for self - pollinated crops.

Tomato is a self - pollinated flower. During anther dehiscence stage, pollens fall directly onto the stigma. But in poly houses, due to high temperature, stigma exertion in the tomato flower occurs and it eliminates the possibility of self –

pollination, hence it demands artificial pollination. Bitter gourd is a cross - pollinated flower as the staminate and pistillate flowers are separate. Hence for bitter gourd growing under protected condition requires artificial pollination.

3.5.1 Artificial pollination

Artificial pollination occurs when human intervene with the natural pollination process. It can be either manual or mechanical.

a. *Manual pollination*

It is the process of collection, transfer and deposition of pollen grains by hand. Bitter gourd flowers are usually pollinating by manually rubbing the male flower with the female flower. Tomato flowers are pollinating by vibrating the branches by hand. This method is effective only for small garden or green houses. As this method involves human interaction with delicate parts of flower, it can cause damage to the pollens and other flower parts. This method requires skilled and trained person as the degree of pollination depends upon the pollen viability and targeted deposition of pollens onto the stigma. The targeted deposition of pollens on to the stigma requires more care and time.

b. *Mechanical pollination*

Artificial pollination done with the help of mechanical devices is known as mechanical pollination. Different types of vibrators, brushes, tuning forks etc. are available for the pollination of tomato. Mechanical pollinating devices such as hand operated pressure sprayer, turbo bees, puffer gun, robotic bees etc. are also available. Majority of these methods achieve the objective by coming into contact with the flowers which may affect the quality of the products. The targeted deposition of pollens by these methods is also difficult. The pollen requirement is also more due to the pollen wastage during deposition. So, for the targeted deposition and non- contact

pollen detachment and deposition, a technology employing electrostatic forces has been adopted.

3.5.2 Electrostatics in pollination

The concept of electrostatics in natural bee pollination has been used for the development of electrostatic pollinator. When a bee flies through the air, it gets confronted with the frictional electricity and gets positively charged. The approach of a positively charged bee induces a negative charge to the flower since the plants are in grounded state. These opposite charges cause a creation of temporary attractive forces between the bee and the flower. These forces are responsible for the detachment of pollen grains towards the bees. When these charged pollen grains attached to the body of the bees, its charge get inverted to positive. When the bee approaches another flower, these positively charged pollen grains get attracted to the negatively charged flower. In flowers, the charge creation is more intense at sharp points such as stigma. So, the pollen grains get deposited towards the stigma more than other parts of the flower.

The charge induction in the pollen grains is due to the water content present in it. Water molecule has a large dipole moment due to its polar molecular structure. Water molecules can be electrostatically charged which implies that pollen grains can also be electrostatically charged.

3.6 DESIGN REQUIREMENTS OF ELECTROSTATIC POLLINATOR

The essential components of a pollinator are the pollen collection unit to collect pollen from the anthers and the pollen deposition unit to deposit the collected pollens into the stigma of the flower. The functional requirements considered in the development of electrostatic pollinator are listed below:

- The equipment should be hand held and light in weight.
- Operation of the equipment should be easier.

- It should enable artificial pollination in the crops cultivated under protected cultivation.
- Pollen grains from the anther should be collected without contacting the flower.
- The viability of the pollen grains should not be affected.
- The equipment should not cause harm to the operator.
- Proper insulation should be provided around the electrical circuit.
- The collected pollens could be deposited into the flower without damaging the stigma.
- It should be economic.

3.7. POLLEN COLLECTOR

A pollen collector was designed based on the functional requirements. The system consists of an electrode and a high voltage generating circuit. When a high voltage DC potential is applied to the charging electrode, an electrostatic field will be created around the electrode. Since the plants are in grounded state, this field induces an equal and opposite charge on the flower. Thus the approach of a positively charged electrode induces a negative charge on the flower. These opposite charges create a temporary force of attraction between the electrode and pollen of flower which initiates the detachment of pollen grains towards the high voltage DC electrode (Fig. 3.6).

The detaching force acting on the pollen located at the tip of the flower varies according to the potential in the pollen collection unit ie., the high voltage direct current (HVDC) electrode. Distance between the pollen and the electrode is another important factor affecting the pollen detachment.

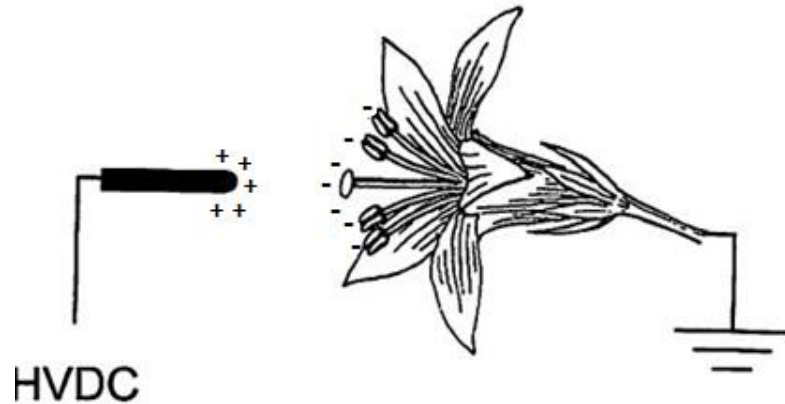


Fig. 3.6. Charge induction in flower due to the presence of HVDC electrode

Charge on the electrode for a known potential was calculated using the following equation.

$$Q_b = V.C$$

Where,

Q_b = Charge on the electrode (C)

V = Potential on the surface for a uniformly distributed charge (V)

C = Capacitance of the electrode (F)

The capacitance of the electrode depends upon the radius of electrode. It defines the charge storage ability of the electrode (Moore, 1973). The capacitance of the electrode was calculated as

$$C = 4 \pi \epsilon_0 r_s$$

Where,

ϵ_0 = Dielectric constant in vacuum (approximately that of dry air) ($F m^{-1}$)

r_s = Radius of electrode (mm)

Electrostatic field strength created by the electrode was determined in $V\text{ mm}^{-1}$ by the equation,

$$E = \frac{Q_b}{4\pi\epsilon_0 r^2}$$

Where,

r = Distance between the tip of the flower and center of sphere (mm)

The charge induced on the pollen grain, Q_p by the electrode was calculated using the electrostatic field strength, E (Cho, 1964).

$$Q_p = 1.65 (4\pi \epsilon_0 r_p^2 E)$$

Where,

Q_p = Charge induced on the pollen grain (C)

r_p = Radius of pollen grain (mm)

The electrostatic force or detaching force acting on the pollen grain is the product of electrostatic charge of pollen grain and the electrostatic field strength by the HVDC electrode. Hence detaching force on the pollen grain, F in N was calculated with the following equation.

$$F = E \cdot Q_p$$

3.7.1 HVDC Electrode

Suitable metal electrode is an essential component of HVDC system which used to induce high voltage potential to the flower surface. The material of electrode was selected by considering its high electrical conductivity. The diameter of electrode was fixed in correspondence to the pedicel girth of the flower. The surface of the electrode was smoothly polished and to eradicate any mode of mechanical damage to

the pollen during transport and collection. The sharp edges of electrode also smoothed to completely eliminate the possibility of charge accumulation. The presences of sharp edges may also cause the formation of corona discharge. Thus, spherical shaped electrodes were considered for the uniform distribution of charges.

3.7.2 High Voltage Amplification Circuit

A high voltage generation was essential for inducing charge on pollen grains. The voltage amplification for pollen collection unit was mainly achieved by developing a unit consists of a diode split flyback transformer and a MOSFET.

a. *Flyback transformer*

Flyback transformer, also called line output transformer (LOPT), is a coupled inductor with a gapped core. Isolation between primary and secondary, choice of positive or negative voltage for the output and ability to provide multiple outlets are the main advantages for fly back transformer (Fig. 3.7). Gapped core construction facilitates high energy storage which avoids immediate energy transfer from primary to secondary. In this transformer, energy was stored in the magnetic field during first half of the switching cycle and then released to the secondary winding connected to the load in the second half of cycle.

The primary winding of the fly back transformer was driven by a switch from a DC supply. MOSFET (Metal Oxide Semiconductor Field Effect Transistor) was used as the switch.

b. *MOSFET*

MOSFET was Metal Oxide Semi-Conductor Field Effect Transistor. It was a semi-conductor device and worked like a circuit switch. It was a core of integrated circuit, designed and fabricated in the form of a single chip which consists of three terminals, viz. Source, Gate and Drain (Fig. 3.8).



Fig. 3.7. Flyback transformer

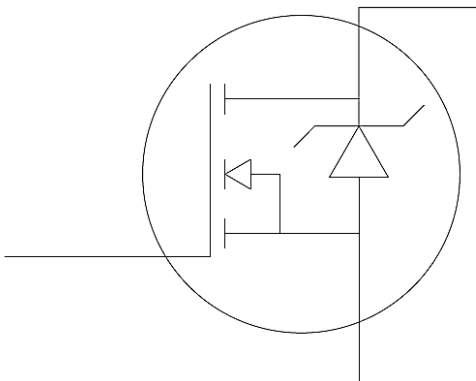


Fig. 3.8. MOSFET Circuit diagram

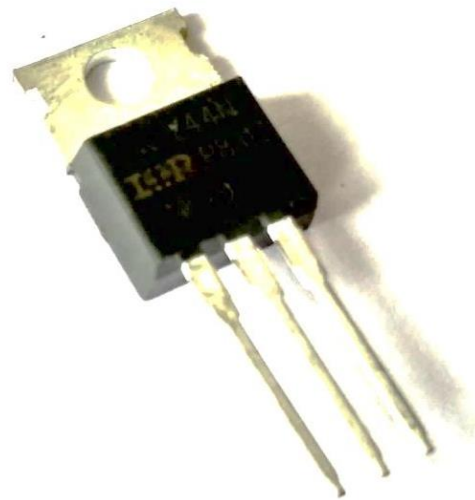


Fig. 3.9. MOSFET

The width of the channel was controlled by the voltage on an electrode called 'gate' which located between 'source' and 'drain'. The MOS capacitor was the main part of the device (Fig. 3.9). A semi-conductor surface was present below the oxide layer which located between source and drain terminal. It could be converted from p-type to n-type by applying positive or negative voltages respectively.

For the generation of high voltage amplification circuit, negative potential of the battery was connected to the gate which generates a channel of holes in it. When the MOSFET switch was closed (ON), current was conducted through the primary of the transformer. Then a magnetic field was created in which the energy was stored. The diode connected in series with the secondary winding prevents the formation of secondary output electric current. Thus, electric current in the primary will ramp up over time to store energy.

When the switch was in open position (OFF) the magnetic field collapses and energy transfers to the secondary winding and ultimately to the load. At closed position of the switch, electric current in the secondary was at its peak and ramps downward as the stored energy was transferred to the load.

c. *Voltage regulator*

To obtain different potential at the charging electrode, input voltage has to be regulated. This regulation of input voltage was achieved by using an adjustable voltage regulator step-down power supply module with LED meter (Fig. 3.10). It was a versatile DC board which can be adjusted continuously by using a knob. It consists of a double potentiometer which enables it to regulate the voltage.

The general specifications of voltage regulator used were

Model : LM-2596

Input voltage : DC: 0 to 30 V

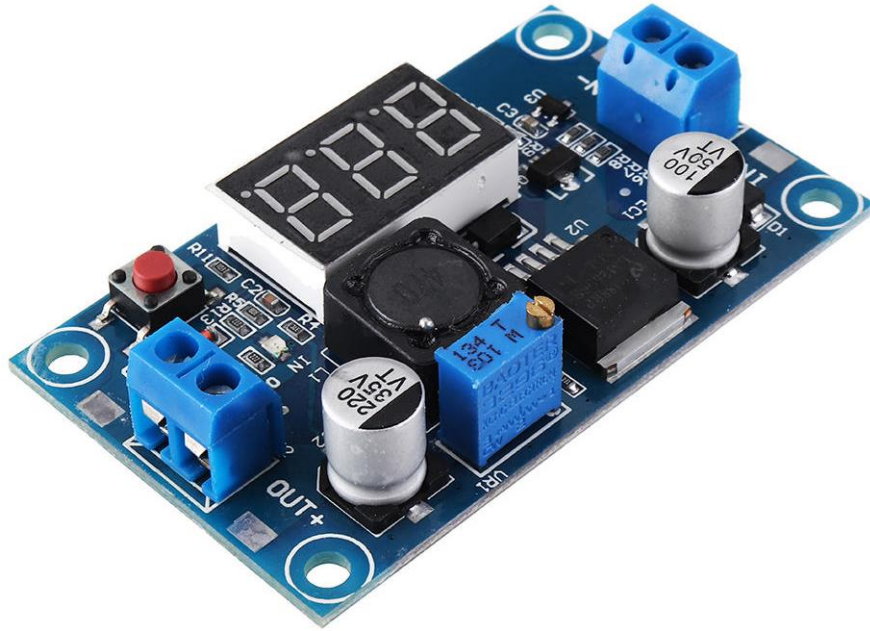


Fig. 3.10. Voltage regulator

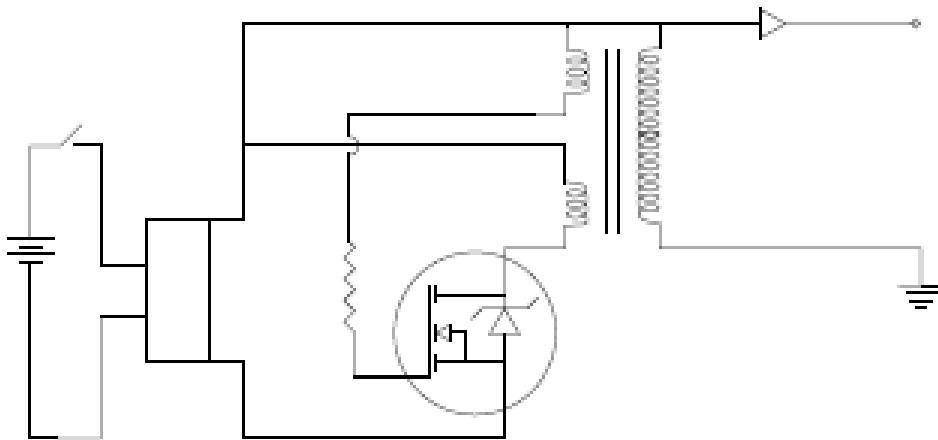


Fig. 3.11 High voltage generating circuit

Output voltage : 1.16 to 28 V

Output current : 2 A (Max)

d. *Development of high voltage generating circuit*

To convert low voltage DC to high potential, a high voltage DC generating circuit was fabricated (Fig. 3.11). It consists of an input power source, diode-split fly back transformer, MOSFET, and an adjustable voltage regulator step-down power supply module.

The input power supply was provided by three rechargeable lithium ion batteries each with 3.7 V capacities. This input supply was connected to the voltage regulator which reduces the input potential from 11.2 V to 10.4 V. LED display in connection with the voltage regulator displays the input voltage to the fly back transformer. The knob in the voltage regulator was adjusted at different positions to obtain an output voltage of 3000, 4000, 5000, 6000 and 7000 V. The input voltage and its corresponding output voltage are shown in the table 3.1.

Table. 3.1. Input voltage and corresponding output voltage from voltage regulator

Input voltage (V)	Output voltage (V)
2.70	3000
2.80	4000
2.90	5000
3.10	6000
3.40	7000

The high voltage generating circuit was placed in an insulated box to avoid direct exposure to the operator.

3.7.3 High voltage measurement

Measurement of high voltage generated by the high voltage DC generating circuit was an inevitable factor, as this high voltage determines the charge induction of pollen grains. A high voltage probe in combination with digital multi-meter unit was used for the high voltage measurement.

a. *Digital multi-meter unit*

The digital multi-meter Model No. KM-5040T/BM-812a of make KUSAM-MECO Brymen, Ind. Ltd. was used for the measurement of voltage generated by the high voltage DC generating circuit (Fig. 3.12). It was equipped with 5000 counts and analog bar graph screen which show the high voltage generated in the circuit. The specifications of the digital multi-meter used were given below:

Make : Kusam-Meco Brymen Industries Ltd.

Model : KM-5040T/BM-812a

Capacity : 1000 V, 10 A max.

Sensitivity : 0.1 μ A, 10 μ V

Accuracy : ± 0.5 % (3 Digits)

b. *High Voltage Probe*

A High Voltage Probe Model No. PD-28 of make Kusam-Meco Brymen Industries Ltd. was used to measure the high voltage at the charging electrode and it was connected to the Digital Multimeter (Fig. 3.12). Basically, it was voltage divider networks which consist of high resistances in series form.



Fig. 3.12. High voltage probe and digital multi-meter

When the high voltage was connected to the voltage divider network, Digital Multimeter measures the corresponding electric current flow through the resistances. The instrument has to be calibrated to get the corresponding high voltage. It has an insulated hollow body with slender shape in which voltage divider network has been provided. The measuring contact surface of the probe is a pointed tip which made of brass. An insulated handle with the surge protector shield has been provided at the base for the operator's safety during measurement.

Make	: Kusam-Meco Brymen Industries Ltd.
Model	: PD-28
Capacity	: 40 kV DC or 28 kV AC max.
Input impedance	: 0.1 μ A, 10 μ V
Attenuation ratio	: 1000 M Ω
Accuracy	: \pm 1 %

As per the following procedures, the prototype of the pollen collection unit was evaluated to determine the maximum amount of pollen collected from the flowers. Experiments were conducted at anther dehiscence stage of the flower. As trial and error method, different potentials starting from 1000 V were applied to the electrode to determine the potential at which pollen detachment takes place. As a result of this, charging potential above 3000 V was selected for the experiment.

3.7.4 Experimental procedures

The experimental procedures followed for the evaluation of pollen collector with the high voltage DC generator and high voltage electrode are detailed below. The number of pollens detached was recorded by grid counting method.

Experiment I:

Electrostatic pollen collection on Tomato

Diameter of electrode : E1 = 10 mm, E2 = 7.5 mm

Voltage potential : 3 kV, 4 kV, 5 kV, 6 kV and 7 kV

Electrode position : 5 mm, 10 mm and 15 mm distant from the tip of anther of the flower.

Experiment II:

Electrostatic pollen collection on Bitter gourd

Diameter of electrode : E1 = 10 mm, E2 = 7.5 mm

Voltage potential : 3 kV, 4 kV, 5 kV, 6 kV and 7 kV

Electrode position : 5 mm, 10 mm and 15 mm distant from the tip of anther of the flower.

As the pollens were collected after inducing high electric potential through electrode, the testing of pollen viability become very important. Pollen viability determines the fruit set efficiency of a flower. Pollen viability was tested by using acetocarmine staining (Prakash, 1953). The collected pollens were placed on a glass slide. It was stained with 1:1 acetocarmine to glycerin. After 5 minutes, this slide was viewed under 40 X magnifications using Magnus compound microscope. Stained pollen grains were counted as viable, whereas unstained, flaccid pollen grains were taken as non - viable. Pollen viability percentage was calculated using the following formula.

$$\text{Pollen viability} = \frac{\text{Number of stained}}{\text{Total number of pollen}}$$

3.8 POLLEN DEPOSITION UNIT

The pollen deposition unit was developed based on the principle that stigma has a low value of impedance and also the stigma has a conducting path to the earth (Corbet *et al.*, 1982). The concentration of electric field in a flower resulting from the proximity of HVDC electrode was more at stigma than the other floral parts. As a result, when high voltage electrode carrying pollen approaches a flower, pollen grains tend to move from the electrode to the stigma.

There are mainly three methods for pollen charging such as corona charging, tribo - electrification and electrostatic induction charging. The method of electrostatic induction charging system was adopted for charging the pollen grains, because of its advantages such as less hazardous to life, high charge transferability and simplicity in construction.

The electrostatic forces acting on the electrode and flower are responsible for distribution of pollen grains towards the stigma. The high voltage DC electrode transfers its positive charge to the pollen grains attached on it. The approach of positively charged electrode induces negative charge on the flower. These opposite charges cause the formation of temporary force of attraction between the electrode and stigma. Then pollen grains on the electrode tend to move under these forces towards the stigma. The timing of pollen deposition depends upon the stigma receptivity time of flowers. The voltage potential such as 1000 V, 3000 V and 6000 V were selected for the pollen deposition. Hence a 1000 V generating circuit was also fabricated.

3.8.1 High voltage amplification unit for pollen deposition

A circuit which can generate 1000 V has been developed for the pollen deposition unit (Fig. 3.13). It consists of input power supply, oscillator stage and a voltage doubler. The input power supply was from a 4 V DC battery. The oscillator stage comprises of 2N5609 transistor and a center tapped high voltage transformer.

CBB22 474J/400V 0.47 μ F 15mm Polypropylene capacitor along with the transistor determines the frequency of oscillation.

Transformer consists of 2E19 type ferrite cores. Primary coil was made up of enameled copper wire of diameter 0.22 mm with 22 turns. The feedback coil was made of enameled copper wire of diameter 0.22 mm with 8 turns. Secondary winding constitutes of 0.08 mm enameled copper wire and has 1400 turns. When the switch was in ON position, the transformer and transistor get energized using the DC supply generating a high frequency alternating current of about 18 kHz, boosted by transformer to 500 V.

Transformer was connected to the voltage doubler which comprises of two capacitors and two diodes. The output of the voltage doubler was DC and it was twice the peak value of the input.

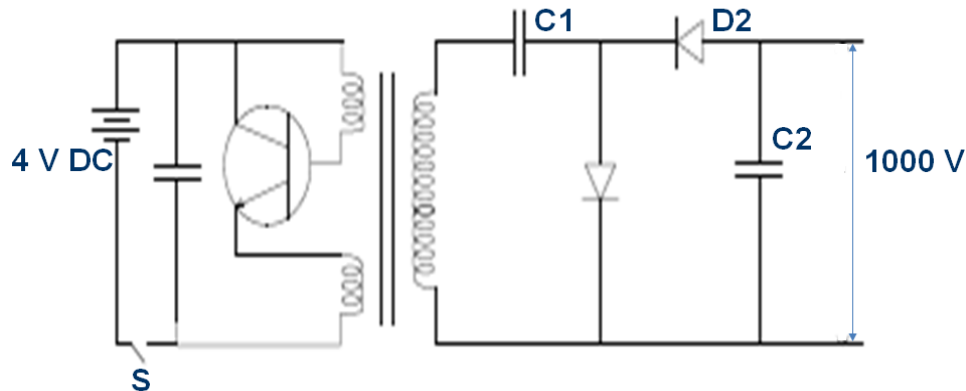


Fig. 3.13. 1000 V Generating circuit

During the positive half cycle of AC, diode conducts electricity due to its forward biased state. And it charges the capacitor C1 equal to the peak value of AC secondary voltage of transformer (V_{max}). During the negative half cycle of AC, the second diode conducts electricity and first diode D1 was in reverse biased state. D1 blocks the discharge of the connected capacitor C1 and then C2 gets charged. As there was a voltage across the capacitor C1 already equal to the peak input voltage, capacitor

C2 charges to twice the peak input voltage. Then the voltage across the capacitor C2 became $2 \times V_{\max}$. Therefore, the transformer voltage gets double and became 1000 V.

3.8.2 Experimental procedures

After collecting the pollens by the developed unit, electrode was placed near to the stigma of the female flower in the case of bitter gourd and same flower in the case of tomato for pollen deposition experiments. The pollen deposition experiment was repeated for three voltages viz., 1000 V, 3000 V and 6000 V with two electrode diameters. Time taken for the pollen deposition was recorded.

Experiment I: Electrostatic pollen deposition on tomato

Diameter of electrode : 7.5 mm, 10 mm
 Voltage potential : 1000 V, 3000 V and 6000 V
 Electrode position : 5 mm distant from the tip of stigma of the flower

Experiment II: Electrostatic pollen deposition on bitter gourd

Diameter of electrode : 7.5 mm, 10 mm
 Voltage potential : 1000 V, 3000 V and 6000 V
 Electrode position : 5 mm away from the tip of the flower

The success of pollination could be evaluated from fruit set efficiency.

3.9 FRUIT SET EFFICIENCY

Fruit set efficiency is the proportion of flowers that reached the stage of anthesis and flowers that developed into a fruit (Picken, 1984). The formula for calculating fruit set efficiency is given below.

$$\text{Fruit set efficiency (\%)} = \frac{\text{Number of fruits}}{\text{Number of flowers pollinated}}$$

To evaluate the fruit set efficiency of the developed unit in tomato, pollen grains were collected and deposited in the same flower with the unit. In bitter gourd, pollens were collected from the anthers of the male flower and deposited into the stigma of the female flower. This fruit set efficiency was compared with the fruit set efficiency obtained by artificial manual pollination.

Artificial manual pollination in tomato was carried out by gently shaking the flowers (McGregor, 1976). In bitter gourd, hand pollination was done by directly rubbing the anthers of the male flower with the lobes of the receptive stigma of the female flower (Deyto and Cervancia, 2018).

3.10 STATISTICAL ANALYSIS

The Completely Randomized Design with two-way ANOVA was used to determine whether the differences in number of pollens collected between the given conditions were significant. The voltage potential, electrode position and diameter of the electrode were taken as the independent variables. The number of pollen grains obtained was considered as the dependent variable. Three replications were taken under each condition. All the analyses were done by using R software (Base and Agricola Packages).

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

An electrostatic pollinator for pollinating vegetable crops under protected cultivation are designed, fabricated and evaluated. The results of the studies of floral characteristics, design and development of the equipment and its evaluation are explained in this chapter.

4.1 FLORAL CHARACTERISTICS

Floral characteristics of tomato and bitter gourd flowers grown under protected cultivation were recorded and the mean values of the observations are presented in table 4.1.

Table 4.1. Floral characteristics of tomato and bitter gourd flowers

Sl. No.	Parameters	Crop	
		Tomato	Bitter gourd
1	Type of flower	Bisexual	Unisexual
2	Length of flower	15 mm	7 mm
3	Diameter of flower	13 mm	27 mm
4	Pedicel girth	3 mm	2 mm
5	Length of anther	8 mm	3 mm
6	Thickness of anther	0.48 mm	1 mm
7	Number of anthers	6	5
8	Number of stigma	1	6
9	Stigma exsertion	20 %	NIL

4.1.1 Time of pollination

Anthesis time, anther dehiscence time and stigma receptivity time of bitter gourd and tomato flowers were observed and the time period recorded are presented in the table 4.2.

Table 4.2. Anthesis, anther dehiscence time and stigma receptivity time

Sl. No.	Crop	Anthesis time	Anther dehiscence time	Stigma receptivity time
1	Tomato	0600 to 0800 h	0800 to 1000 h	For 24 hours after anthesis
2	Bitter gourd	0500 to 0700 h	0500 to 0700 h	For 24 hours after anthesis

4.1.2 Pollen size

Size of pollen of tomato and bitter gourd flowers were measured using compound microscope. Mean values of the observations are presented in table 4.3.

Table 4.3. Pollen size of tomato and bitter gourd

Sl. No.	Crop	Length (μm)	Width (μm)
1	Tomato	30	27
2	Bitter gourd	14	6

4.1.3 Number of pollen grains

Number of pollen grains in a single flower was observed by grid counting method. It was found that tomato flower consists of an average number of 500 pollen grains and that of bitter gourd was 3000.

4.2 EFFECT OF ENVIRONMENTAL PARAMETERS ON POLLINATION

The environmental factors such as temperature, relative humidity and light intensity had a great effect on pollination.

4.2.1 Temperature

Temperature between 26 °C to 30 °C was observed as the favorable for pollen production and its viability in tomato. It was 26 °C to 33 °C for bitter melon. The same temperature range was observed to be favorable for pollen detachment from anther and deposition to stigma. But the increase in temperature above 28 °C resulted in stigma exertion in tomato flowers which eliminated the possibility of self-pollination.

4.2.2 Relative humidity

Relative humidity between 68% RH to 80% RH was observed as the favorable for pollen production and its viability in tomato. It was 55% RH to 80% RH for bitter melon. The relative humidity above 80% RH was observed to be negatively influencing the pollen detachment from anther. The pollens were tend to sticky on the anther itself.

4.2.3 Light intensity

Light intensity between 1000 to 7000 Lux was observed as the favorable for pollination process in tomato. It was 800 to 7000 Lux for bitter melon.

4.3 DEVELOPMENT OF ELECTROSTATIC POLLINATOR

The electrostatic pollinator consisted of an electrode and voltage amplification units for pollen collection and pollen deposition. Electrostatic forces between the HVDC electrode and the anther/stigma of flower was created using the principle of electrostatic induction charging.

4.3.1 Pollen collection unit

Copper rod of 0.4 mm diameter with pointed tip was used to make electrode during the preliminary experiments. But corona discharge formation was the result when it was applied with high voltage due to its sharp pointed tip. It also observed that the parts of flowers were injured by this high concentrated electric potential. To eliminate the formation of corona discharge, electrode with spherical tip was adopted for further experiments (Fig. 4.1 and Fig. 4.2). The introduction of spherical tip facilitated uniform distribution of electric charge on its surface, results a wide and uniform potential between the electrode and the flower. When the high voltage positive electrode induce a negative charge on the flower, the pollens get detached from the flower and transferred to the surface of the electrode (Fig. 4.3). Spherical electrodes with two diameters viz. 10 mm (E1) and 7.5 mm (E2) were adopted and evaluated for pollen detachment capacity. The pollen collection unit was evaluated based on the number of pollen grains detached from the anther respectively for the voltage potentials 3 kV, 4 kV, 5 kV and 6 kV at distance of 5 mm, 10 mm and 15 mm from the anther.

a. *Detaching forces acting on the pollen grains*

The detaching forces induced on the pollen grains were proportional to the electrostatic potential developed by the HVDC electrode. The capacitance of the electrode E1 was 5.56×10^{-13} F and that of electrode E2 was 2.78×10^{-13} F. The electrostatic induction charge of the electrode varies proportional to the voltage applied on the electrode (Fig. 4.4). Electrostatic field between flower and electrode varied with respect to the electrode potential, size of electrode and distance between the flower and electrode. The highest value (1.2×10^3 V mm⁻¹) of electrostatic field was obtained for E1 with potential of 6 kV placed at 5 mm from the tip of anther.

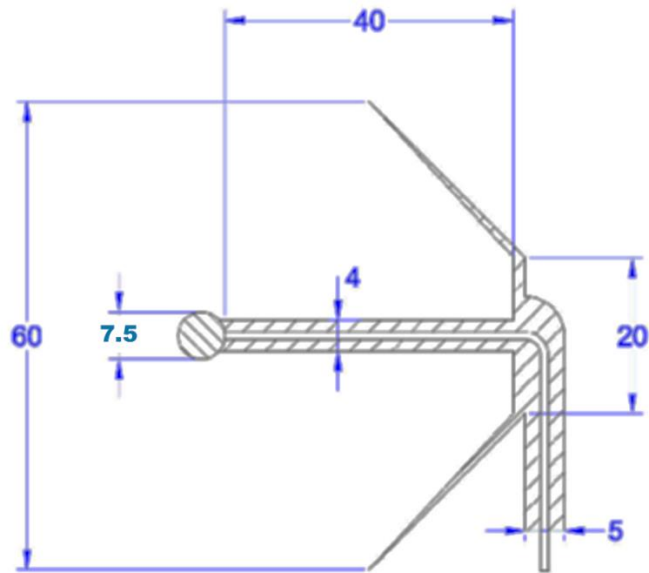


Fig 4.1. Spherical electrode for pollination – Sectional view

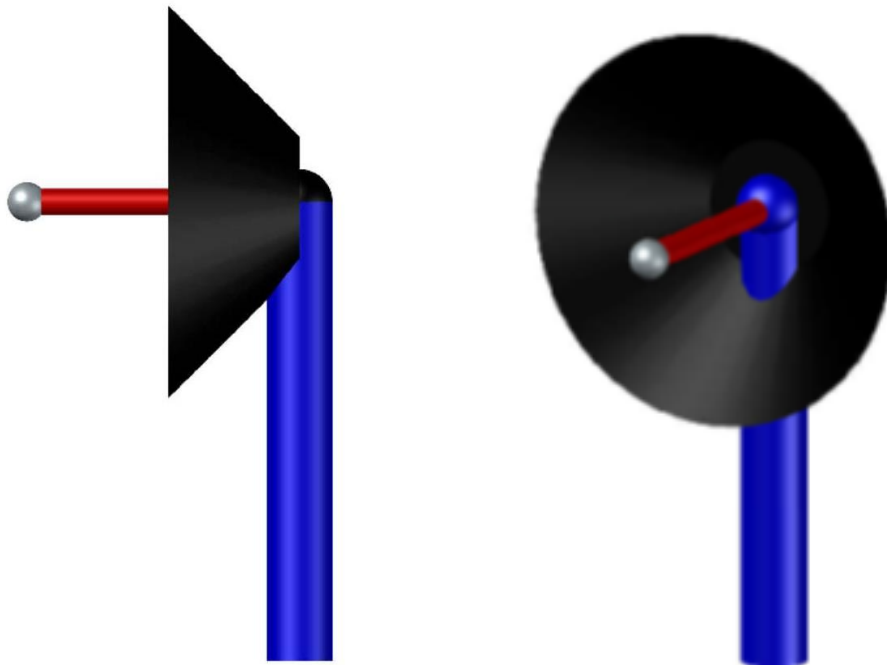


Fig 4.2. Spherical electrode for pollination



Fig 4.3. Electrostatic pollinator

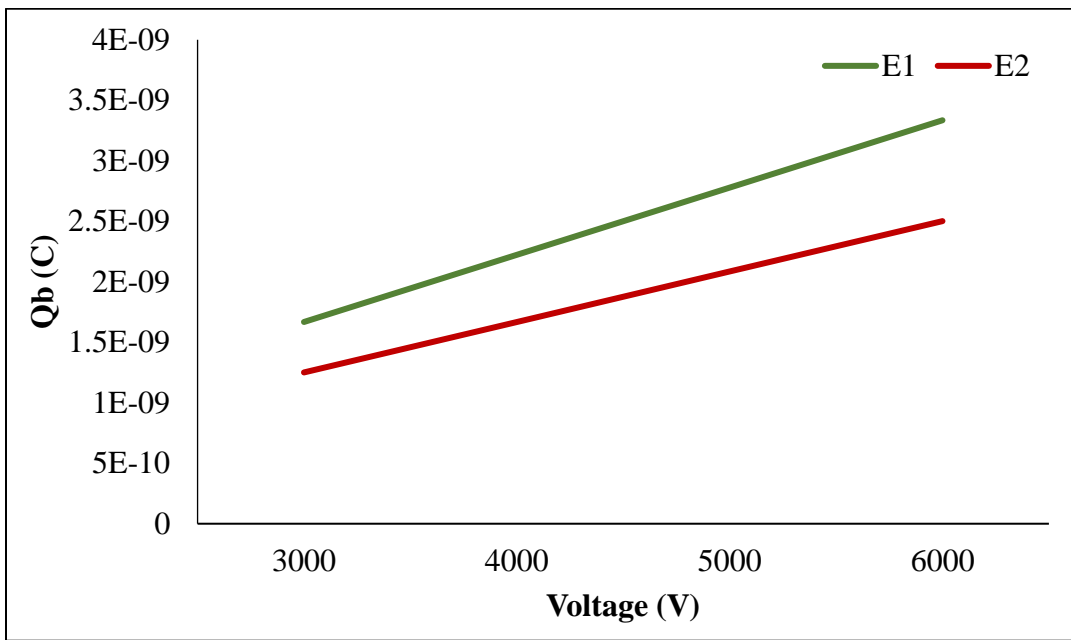


Fig. 4.4. Effect of voltage on electrostatic charge of the electrode (Q_b)

The minimum value ($5 \times 10^1 \text{ V mm}^{-1}$) was observed for electrode E2 with potential of 3 kV at 15 mm distance from the tip of anther. It has an inverse proportionality with the distance between flower and electrode. Electrostatic charge induced on the pollen grain depends on the electrostatic field and the size of pollen grain. As the electrostatic field increases, electrostatic charge in the pollen grain also increases. The size of bitter gourd pollen grain was smaller than the tomato pollen grain and hence the charge inducted was also lesser in bitter gourd pollen.

The detaching force acting on the pollen grain was directly proportional to the electrostatic field strength and the pollen charge (Fig. 4.5 to Fig. 4.10). Detaching force was maximum for electrode E1 with potential of 6 kV when placed at 5 mm distance from anther. This may be due to the high electrostatic field between the flower and electrode under the specified conditions. Detaching force was minimum for electrode E2 with potential of 3 kV at 15 mm distance from the anther.

4.3.2 Pollen collection by electrode E1

The electrostatic induction charging electrode, E1 attracted higher number of pollens from tomato (Fig. 4.11) and bitter gourd (Fig. 4.12) flowers at charging potential of 6 kV at a distance of 5 mm from the anther of flower. The number of pollen grains attracted from both the flowers was observed to be directly proportional with increase in electrode potential (3 kV to 6 kV) and inversely proportional with the increase in distance between the electrode and flower (5 mm to 15 mm). The number of pollen grains collected from the bitter gourd flower varies from 1310 to 2827 and that from tomato flower was 112 to 409. In both cases, the number of pollen grains was minimum at 15 mm distance with a charging potential of 3 kV.

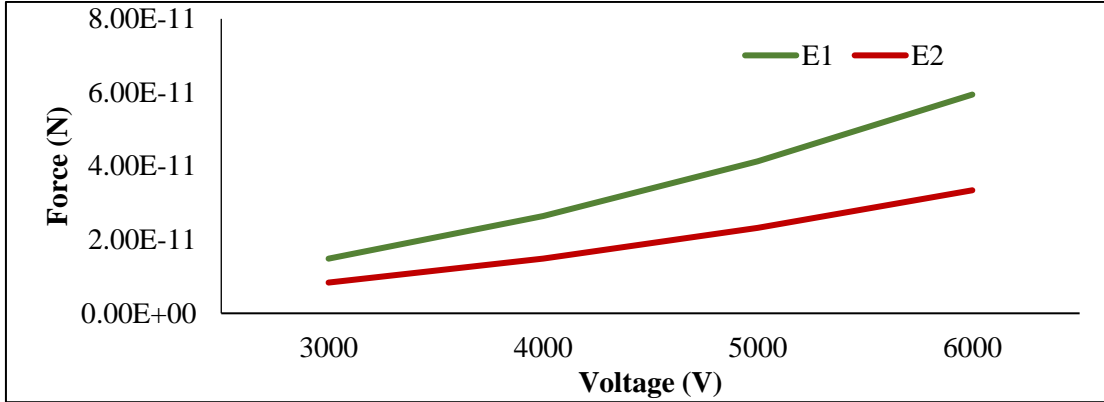


Fig. 4.5. Effect of voltage on detaching force of tomato pollen at 5 mm distance

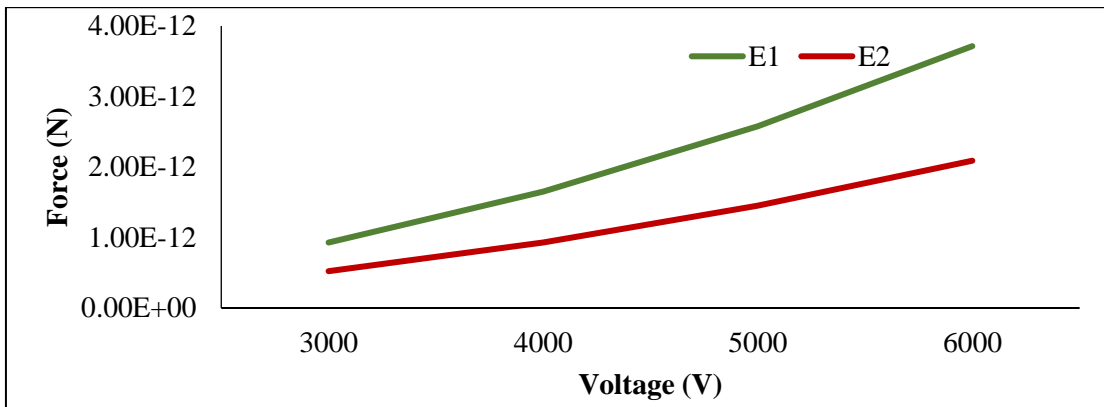


Fig. 4.6. Effect of voltage on detaching force of tomato pollen at 10 mm distance

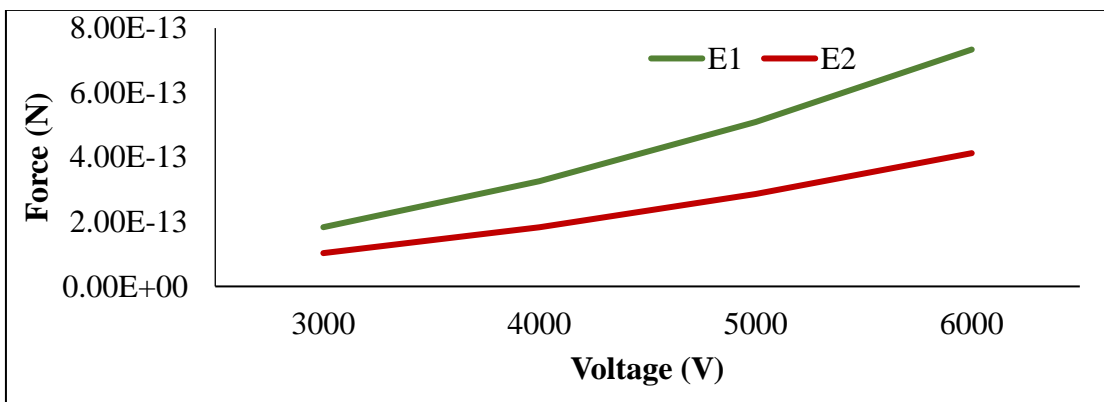


Fig. 4.7. Effect of voltage on detaching force of tomato pollen at 15 mm distance

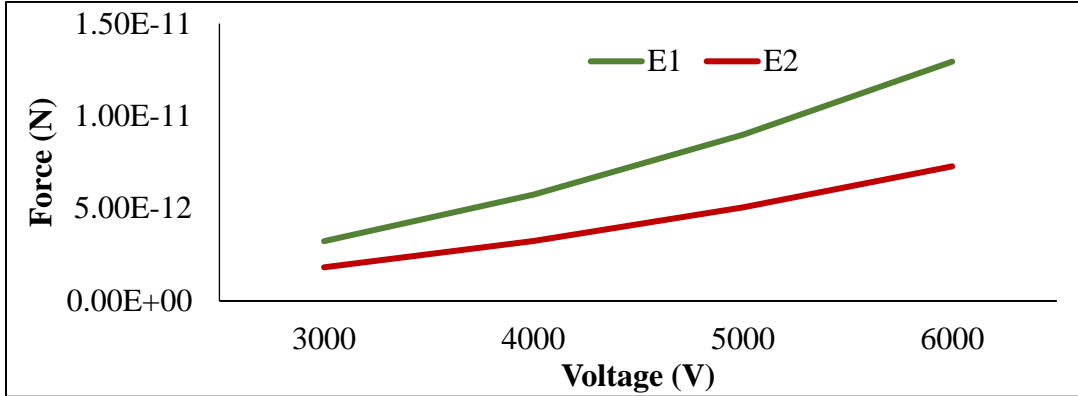


Fig. 4.8. Effect of voltage on detaching force of bitter gourd pollen at 5 mm distance

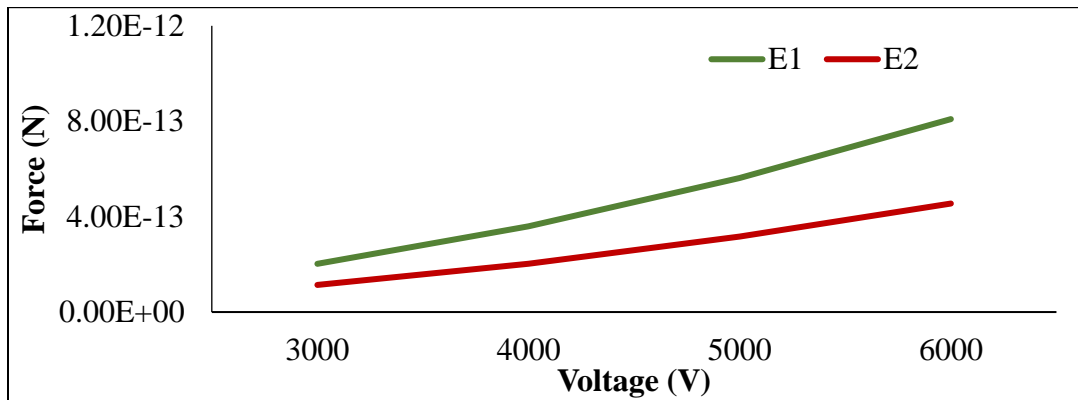


Fig. 4.9. Effect of voltage on detaching force of bitter gourd pollen at 10 mm distance

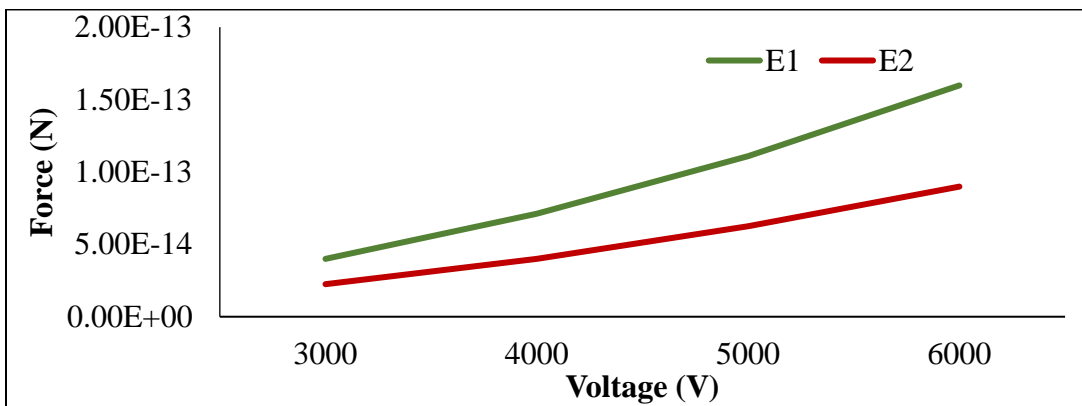


Fig. 4.10. Effect of voltage on detaching force of bitter gourd pollen at 15 mm distance



Fig. 4.11. Pollen collection from tomato flower



Fig. 4.12. Pollen collection from bitter gourd flower

4.3.3 Pollen collection by electrode E2

The electrode E2 attracted maximum number of pollen grains at a charging potential of 6 kV at a distance of 5 mm from the anther tip of the flower. The number of pollen grains was found to be increasing in both tomato and bitter gourd flowers with the increase in voltage and decrease in distance between the electrode and flower. The number of pollen grains collected from the bitter gourd flower varied between 1227 and 2656 and that of tomato flower was 87 to 378. The lowest collection of pollen grains was recorded at 15 mm distance between anther tip and electrode with a charging potential of 3 kV.

The maximum number of pollen grains was attracted by electrode E1 under all combinations of variables, viz. different voltages, distances and diameters. The data was analyzed and analysis of variance of number of pollens collected from tomato (Table 4.4) and bitter gourd flowers (Table 4.5) are represented in the tables.

Table 4.4. Analysis of variance in number of tomato pollen collected

Source of variation	DoF	SS	MS	F value	Pr(>F)
Replications	2	14	7	0.039	0.962 ^{NS}
Voltage	3	491718	163906	946.878	<2e-16 **
Distance	2	103731	51865	299.624	<2e-16 **
Radius	1	26450	26450	152.801	<2e-16 **
Residuals	63	10905	173		

**Significant at 1% level NS: Non-significant

From the ANOVA table, it was inferred that the variations within the replications was not significant. At 1 % level of significance, the voltage, distance and diameter have a significant effect on the number of pollen grains detached from tomato flowers. The distance and voltage have a significant role in the electrostatic field

strength (Bechar *et al.*, 1999) that reflected in pollen detachment from the anther of tomato flower.

Table 4.5. Analysis of variance in number of bitter gourd pollen collected

Source of variation	DoF	SS	MS	F value	Pr(>F)
Replications	2	254	127	0.04	0.961 ^{NS}
Voltage	3	9801945	3267315	1019.51	<2e-16 ***
Distance	2	2464411	1232205	384.49	<2e-16 ***
Radius	1	590966	26450	152.801	<2e-16 ***
Residuals	63	201902	3205		

**Significant at 1% level NS: Non-significant

From the ANOVA table, it was inferred that the variations within the replications was not significant. At 1 % level of significance, the voltage, distance and diameter have a significant effect on the number of pollen grains detached from bitter gourd flowers. The distance and voltage have a significant role in the electrostatic field strength (Bechar *et al.*, 1999) that influenced the pollen detachment from the anther of bitter gourd flower.

4.3.4 Effect of electrode voltage potential on pollen collection

The pollen collection capacity of the developed unit was evaluated at different electrode voltage potentials (3 kV, 4 kV, 5 kV and 6 kV), distance from the anther of flower (5 mm, 10 mm and 15 mm) and by changing diameters of electrode (5 mm and 2.5 mm). The results were analyzed and represented graphically for tomato (Fig. 4.13 to Fig. 4.15) and bitter gourd (Fig. 4.16 to Fig. 4.18).

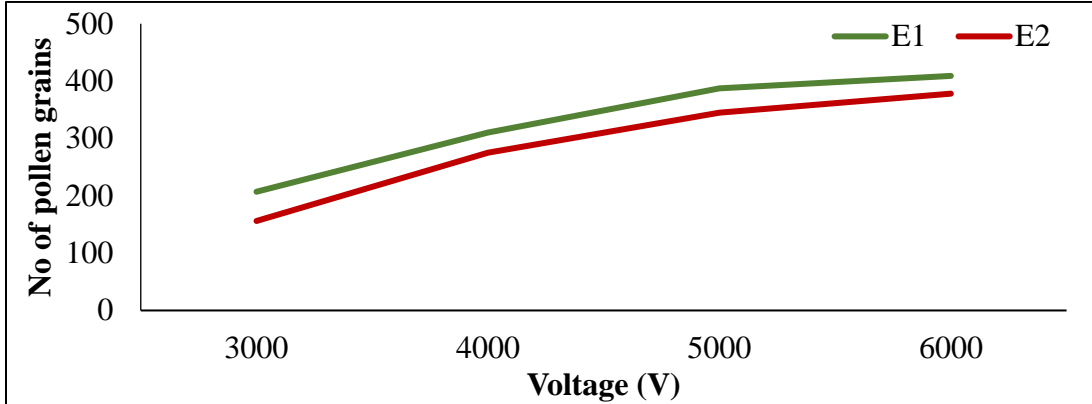


Fig. 4.13. Effect of electrode voltage potential on pollen collection in tomato flower at 5 mm distance

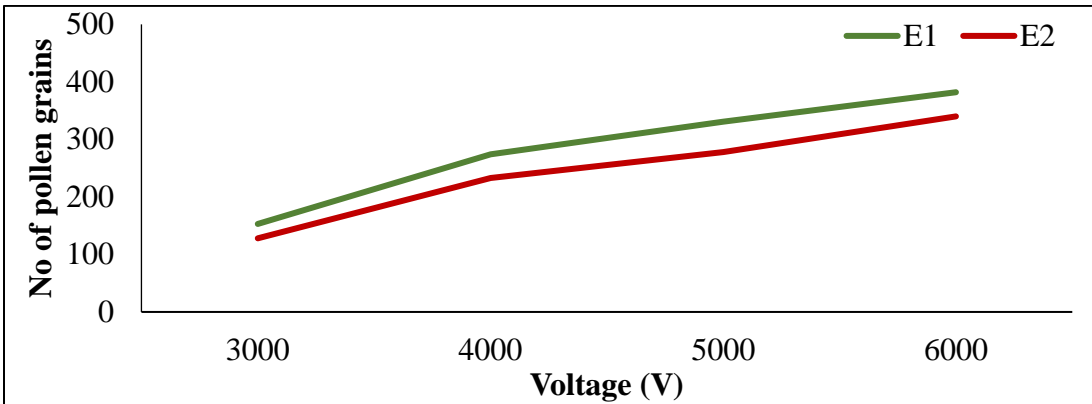


Fig. 4.14. Effect of electrode voltage potential on pollen collection in tomato flower at 10 mm distance

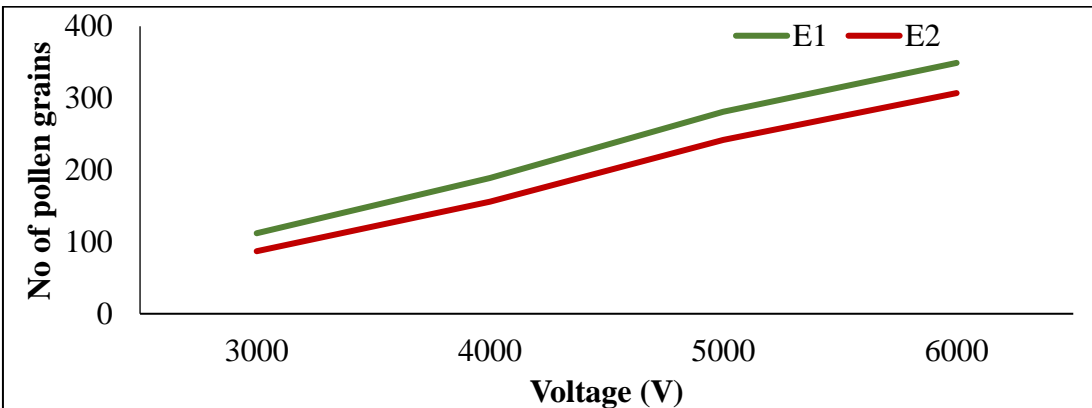


Fig. 4.15. Effect of electrode voltage potential on pollen collection in tomato flower at 15 mm distance

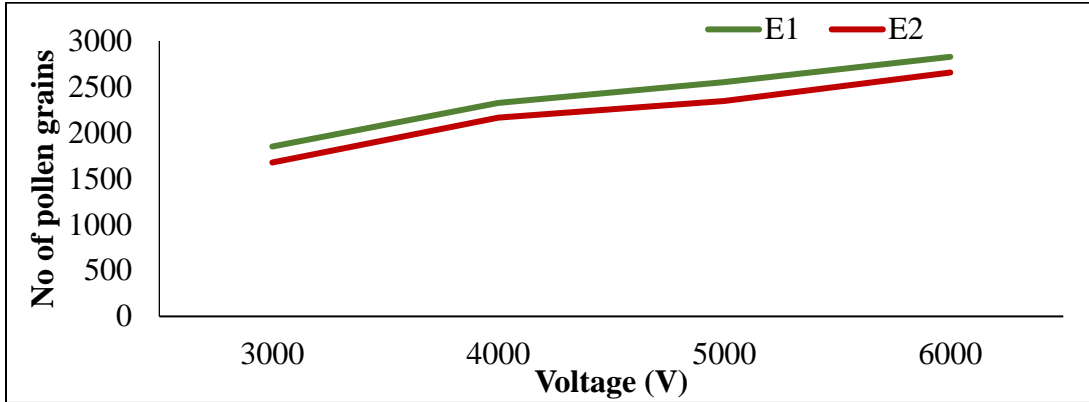


Fig. 4.16. Effect of electrode voltage potential on pollen collection in bitter gourd flower at 5 mm distance

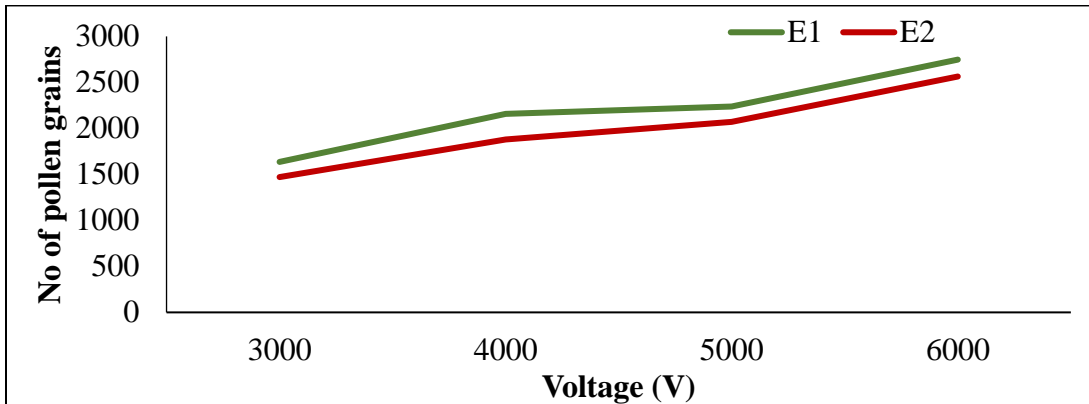


Fig. 4.17. Effect of electrode voltage potential on pollen collection in bitter gourd flower at 10 mm distance

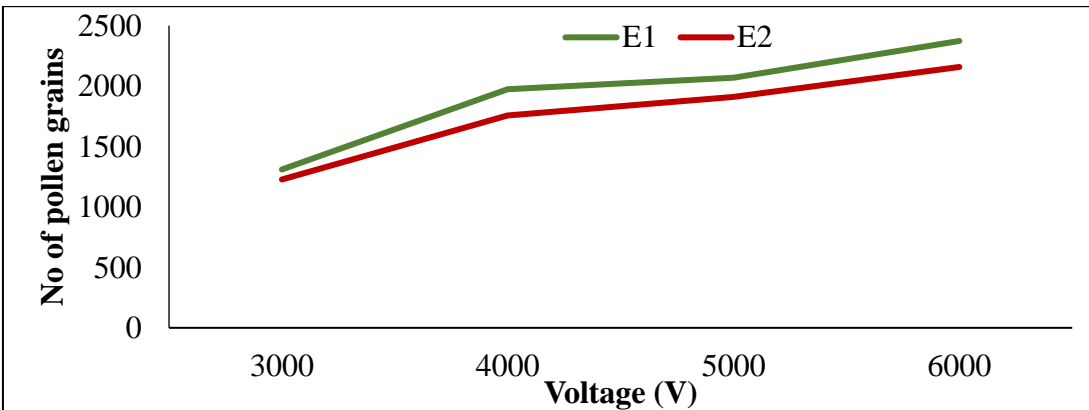


Fig. 4.18. Effect of electrode voltage potential on pollen collection in bitter gourd flower at 15 mm distance

Irrespective of size of electrode, the collection rate of pollens was recorded to be maximum at 6 kV electrode voltage potential at 5 mm distance. This can be justified by the influence of high electrostatic field created by high electrode voltage potential at a shorter distance between the tip of electrode and flower.

Duncan's Multiple Range Test (DMRT) was used to compare the number of pollens collected from tomato (Table 4.6) and bitter gourd (Table 4.7) flowers at different voltages.

Table 4.6. DMRT for comparing tomato pollens collected vs voltage

Voltage (V)	No. of pollen grains
6000	360.9444 ^a
5000	311.0000 ^b
4000	239.8333 ^c
3000	141.0000 ^d

It was concluded that from the table 4.6, variation in voltages have a significant effect on pollen collection in tomato flower with a positive correlation. The maximum number of pollen grains was recorded at 6 kV.

Table 4.7. DMRT for comparing bitter gourd pollens collected vs voltage

Voltage (V)	No. of pollen grains
6000	2554.667 ^a
5000	2198.000 ^b
4000	2043.167 ^c
3000	1528.667 ^d

From the table 4.7, it could be concluded that variation in voltages have a significant effect on pollen collection in bitter gourd flower. The maximum number of pollen grains was recorded at 6 kV with a positive correlation.

4.3.5 Effect of distance of electrode to anther tip of flower on pollen collection

The maximum number of pollen grains was collected at 5 mm distance under all combinations of variables. This could be justified by the higher electrostatic field intensity between the anther tip of flower and electrode when the gap is minimum. Charge induction was observed to be reduced when the distance of electrode increases from anther tip of flower.

When the electrode E1 potential was at 6 kV, the number of tomato pollen grains collected was 409, 382 and 349 at 5 mm, 10 mm and 15 mm distances of electrode from the anther respectively as shown in figure 4.19 to figure 4.22. But in the case of electrode E2 with same electrode potential of 6 kV, the number of tomato pollen grains collected was 378, 340 and 307 at 5 mm, 10 mm and 15 mm distances of electrode from the anther respectively (Fig. 4.19 to Fig. 4.22).

In the case of bitter gourd flowers, at electrode potential of 6 kV, electrode E1 collected 2827, 2748 and 2373 at 5 mm, 10 mm and 15 mm distances of electrode from the anther respectively as shown in figure 4.21 to figure 4.23. But for electrode E2 number of pollen grains collected was 2656, 2564 and 2158 at 5 mm, 10 mm and 15 mm distances of electrode from the anther respectively (Fig. 4.23 to Fig. 4.26).

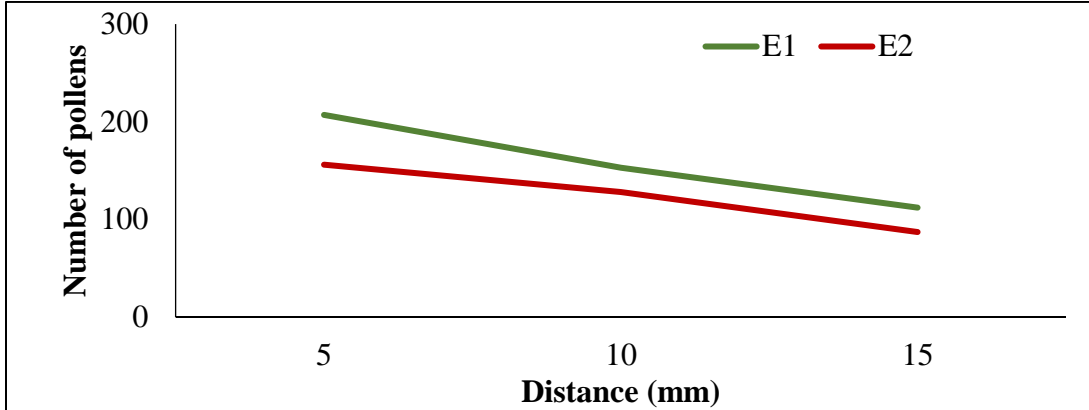


Fig. 4.19. Effect of distance on pollen collection in tomato flower at 3000 V

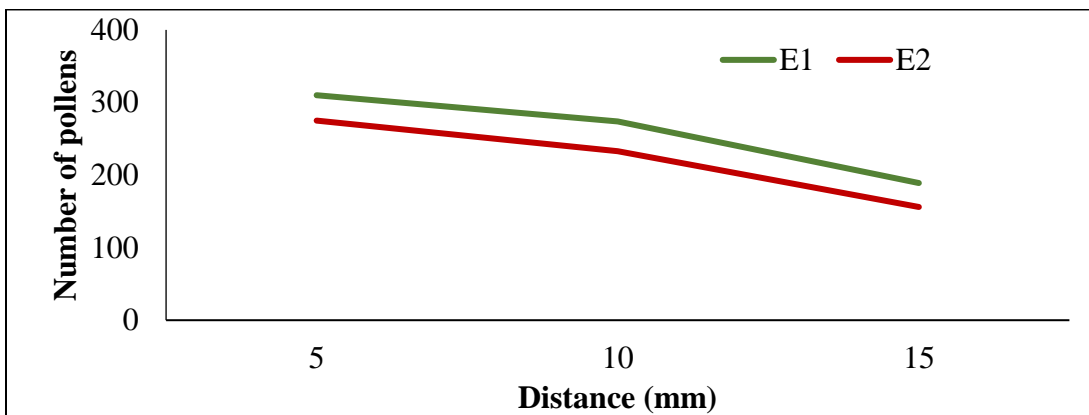


Fig. 4.20. Effect of distance on pollen collection in tomato flower at 4000 V

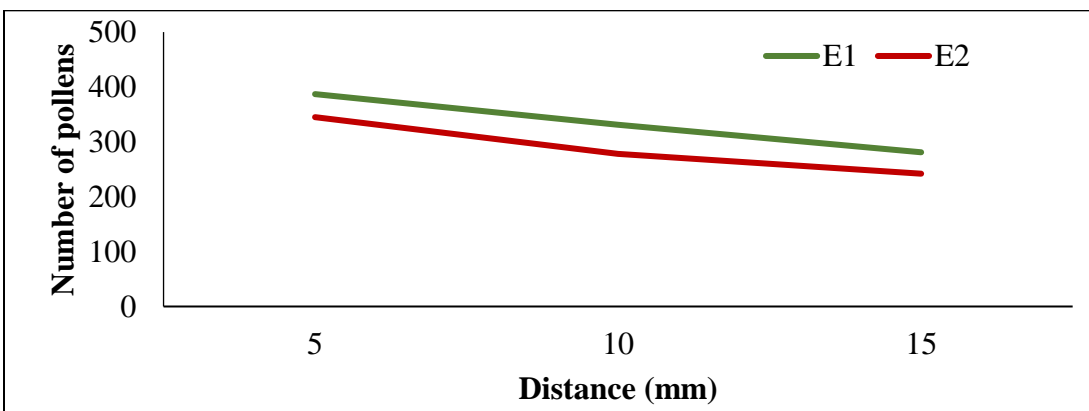


Fig. 4.21. Effect of distance on pollen collection in tomato flower at 5000 V

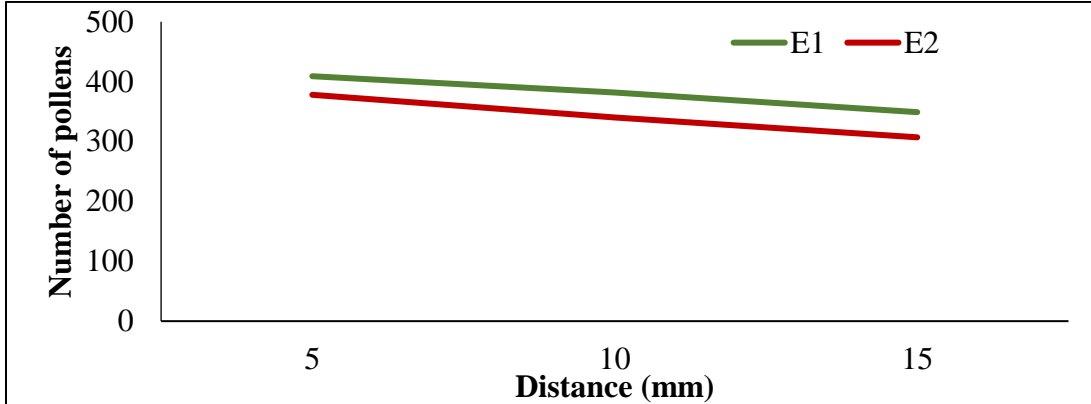


Fig. 4.22. Effect of distance on pollen collection in tomato flower at 6000 V

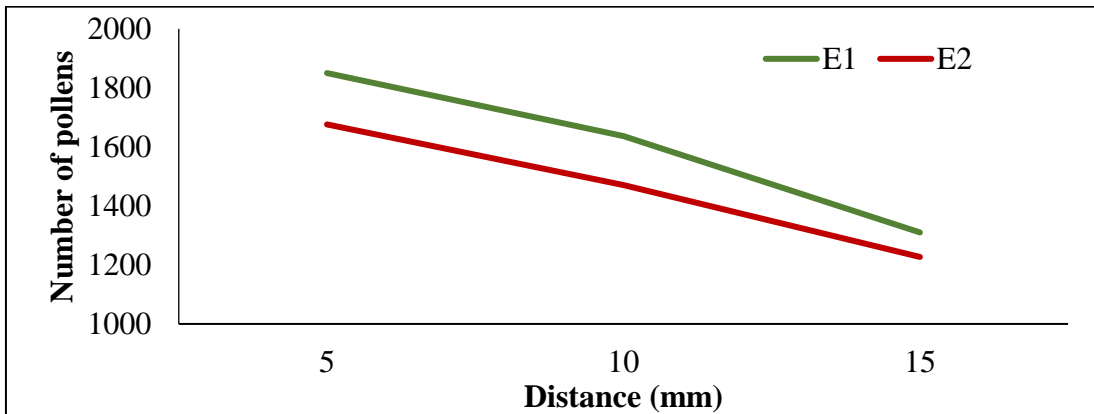


Fig. 4.23. Effect of distance on pollen collection in bitter gourd flower at 3000 V

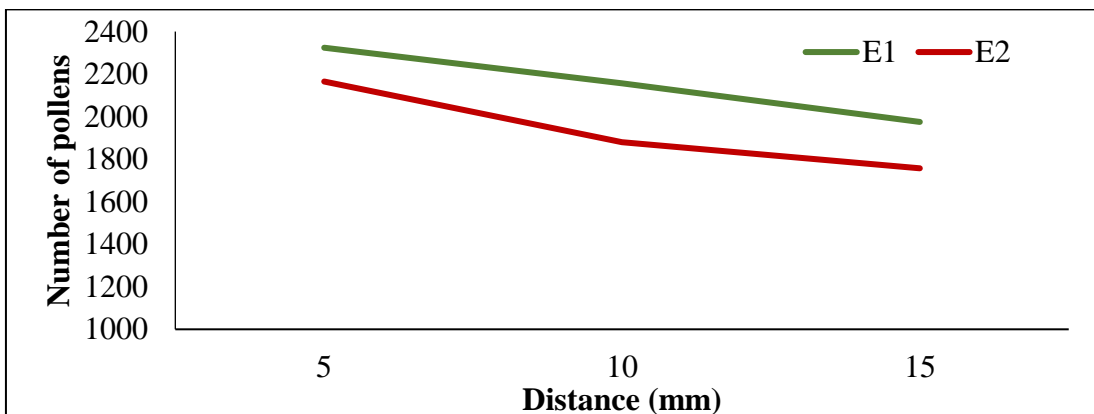


Fig. 4.24. Effect of distance on pollen collection in bitter gourd flower at 4000 V

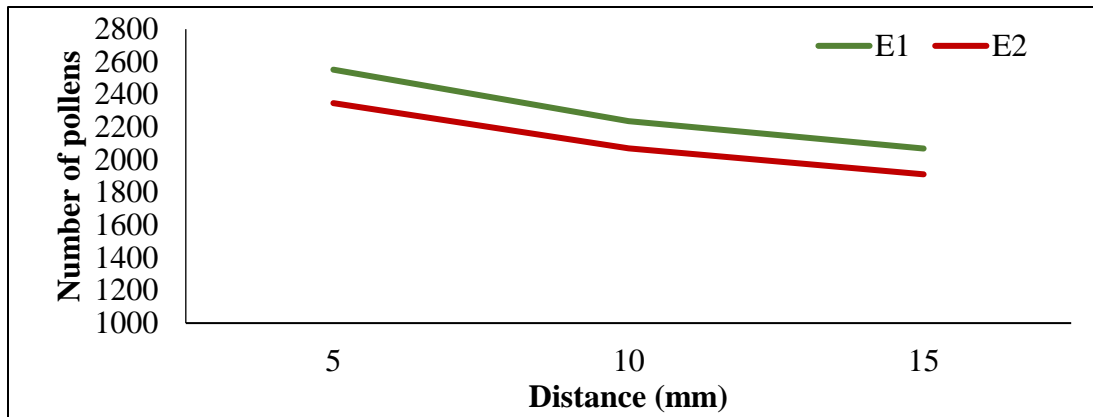


Fig. 4.25. Effect of distance on pollen collection in bitter gourd flower at 5000 V

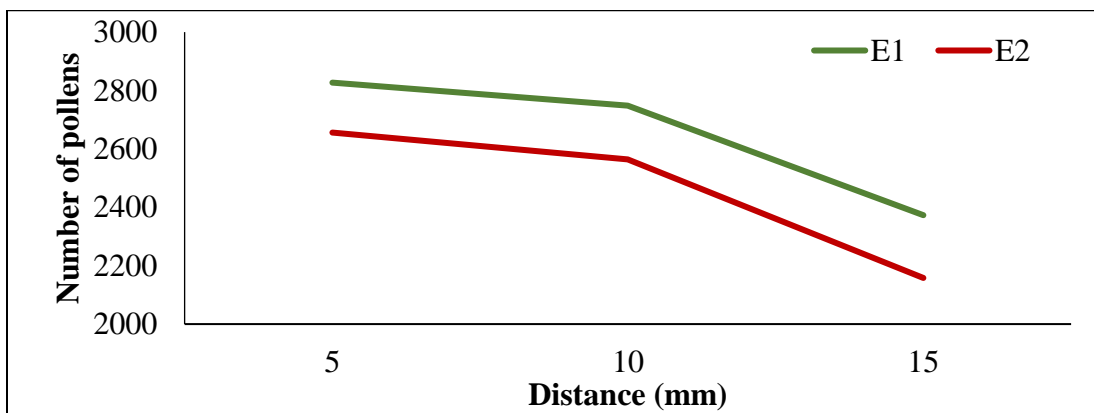


Fig. 4.26. Effect of distance on pollen collection in bitter gourd flower at 6000 V

Duncan's Multiple Range Test (DMRT) was used to compare the number of pollens collected from tomato (Table 4.8) and bitter gourd (Table 4.9) flowers at different distances corresponding to the same voltages.

Table 4.8. DMRT for comparing tomato pollens collected vs distance

Distance (mm)	No. of pollen grains
5	308 ^a
10	265 ^b
15	215 ^c

From the table 4.8, it could be concluded that variation in distance of electrode to the anther tip of flower have a significant effect on pollen collection in tomato flower with negative correlation. The maximum number of pollen grains was collected at 5 mm distance in tomato.

Table 4.9. DMRT for comparing bitter gourd pollens collected vs distance

Distance (mm)	No. of pollen grains
5	2300 ^a
10	2095 ^b
15	1847 ^c

From the table 4.9, it could be concluded that variation in distance of electrode to the anther tip of flower have a significant effect on pollen collection in bitter gourd flower. The maximum number of pollen grains was collected at 5 mm distance in bitter gourd with negative correlation.

4.3.6 Effect of size of electrode on pollen collection

Two different diameters of spherical tipped induction charging electrodes (E1 : Ø 10 mm and E2 : Ø 7.5 mm) were selected based on the pedicel girth of the flowers. It was observed that the number of pollen grains collected at electrode E2 was less compared to electrode E1 under the similar experimental frame work. Electrostatic field induced by electrode E2 was lesser compared to that of electrode E1 due to its smaller peripheral effective surface area which in turn reduce its capacitance. Hence, electrode E1 could induce more detaching forces compared to electrode E2.

Duncan's Multiple Range Test (DMRT) was used to assess the effect of size of charging electrode on the number of pollens collected from tomato (Table 4.10) and bitter gourd (Table 4.11) flowers.

Table 4.10. DMRT for comparing tomato pollens collected vs size of electrode

Diameter (mm)	No. of pollen grains
E1 : 10	282 ^a
E2 : 7.5	244 ^b

From the table 4.10, it was obvious that there was a significant difference between the number of pollen grains collected by the two electrodes E1 and E2 from tomato flowers (autogamy). It authenticates the conclusion that the increase in diameter of the charging electrode increases the induced electrostatic field created for the detachment of pollen grains.

Table 4.11. DMRT for comparing number of bitter gourd pollens collected vs size of electrode

Diameter (mm)	No. of pollen grains
E1 : 10	2172 ^a
E2 : 7.5	1990 ^b

It was evident from the table 4.11 that there was a significant difference between the number of pollen grains collected by the two electrodes E1 and E2 from bitter gourd flowers. It substantiates the conclusion that the increase in diameter of the charging electrode increases the potential electrostatic field created for the detachment of pollen grains from the anther of flower.

4.4 POLLEN DEPOSITION

The collected pollen grains were deposited into the stigma of tomato (Fig. 4.27) and bitter gourd (Fig. 4.28) flowers by the electrostatic induction method. The fruit set efficiency of the developed system with electrode potentials viz. 1 kV, 3 kV and 6 kV were recorded for two different electrodes E1 (Ø 10 mm) and E2 (Ø 7.5 mm) by placing at 5 mm distance from the stigma of the targeted flower. When the charged electrode with the pollen grains approached the stigma, an opposite charge was observed to be induced on it. As a result, the positively charged pollen grains got attracted towards the negatively charged stigma. These pollen grains were deposited exactly into the stigma, lead to fertilization and fruit set.

The focused deposition of the pollens into the stigma was the major advantage observed in the electrostatic pollen deposition than the other conventional methods of artificial pollen deposition. Damage to floral parts by the developed electrostatic pollen grain depositor was observed as zilch, since it was a non - contact type of pollen deposition method.



Fig. 4.27 Pollen deposition in tomato



Fig. 4.28. Pollen deposition in bitter gourd

4.4.1 Pollen repulsion

Pollen grain repulsion phenomenon was observed during the experiments of pollen deposition in tomato flowers. When the electrode potential was at 7 kV, pollen grains got attracted towards the electrode, touches the electrode and immediately repelled back to the terminal ends of the floral parts especially stigma of the flower. This phenomenon was probably due to the corona discharge at the spherical tip of the electrode when it was charged with very high voltage.

The effect of corona discharge on pollen repulsion was advantageous for autogamous flowers (tomato flower) as the collection and deposition of pollen grains happens simultaneously. But for geitonogamous and xenogamous flowers, the electrode with voltage potential of 7 kV was not suitable.

4.5 FRUIT SET EFFICIENCY

Fruit set efficiency in tomato and bitter gourd flowers pollinated with the developed electrostatic pollinator with electrode potentials viz. 1 kV, 3 kV and 6 kV were recorded for two different electrodes E1 ($\text{\O} 10 \text{ mm}$) and E2 ($\text{\O} 7.5 \text{ mm}$) by placing 5 mm distance from the stigma of the targeted flower. Fruit set efficiency of hand pollinated flowers were also recorded, for substantiate the efficiency of developed electrostatic pollinator.

Fruit set efficiency of tomato was maximum (80 %) when flowers were pollinated with electrostatic pollinator while that of hand pollinated flowers was only 40 per cent (Fig. 4.29). This reduction in fruit set efficiency of hand pollinated flowers might be due to the exerted position of stigma. Flower vibrations during the hand pollination might be unable to transfer the detached pollen grains from the anther to the extended tip of stigma. It also observed that a few flowers pollinated with electrostatic pollinator were aborted due to the defects occurred in the flower as a result of high temperature inside the greenhouse.

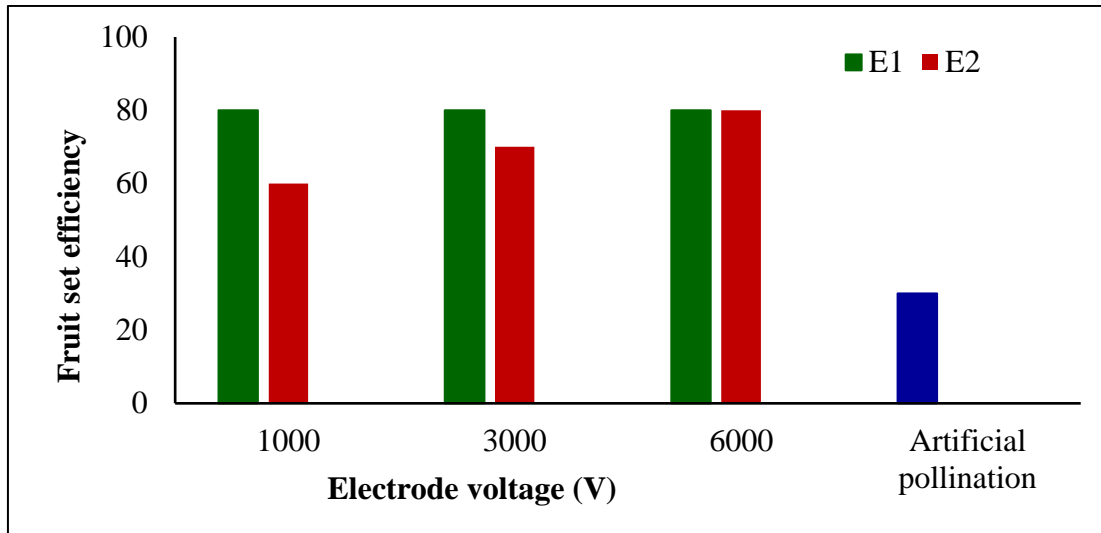


Fig. 4.29. Fruit set efficiency of tomato in protected cultivation under different conditions

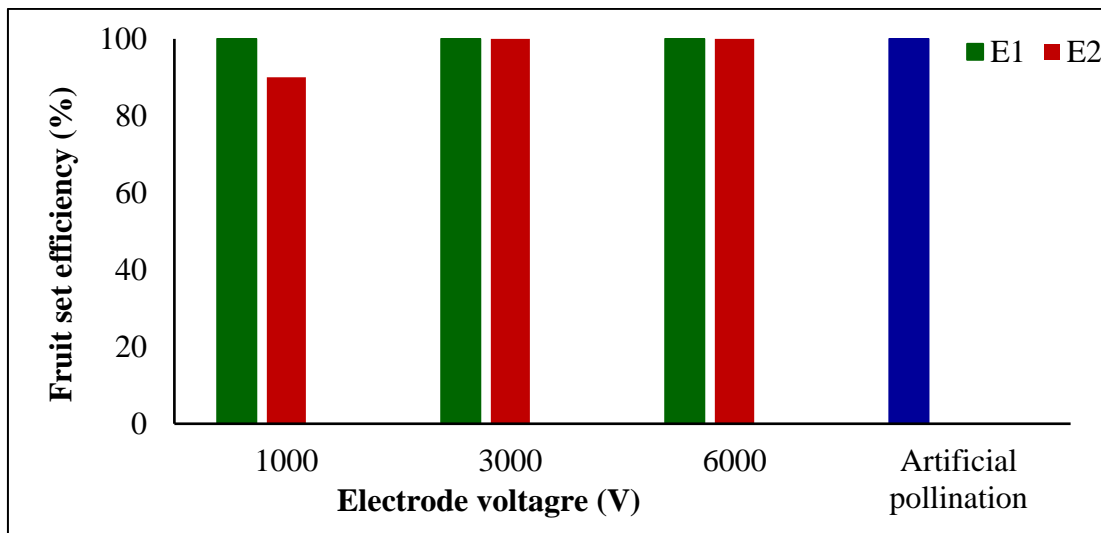


Fig. 4.30. Fruit set efficiency of bitter gourd in protected cultivation under different conditions

The fruit set efficiency of tomato flowers were higher (50 %) compared to the hand pollination only because of the focused deposition of pollen grains into the stigma. It was observed that the fruits produced by the electrostatic pollination were uniform in size and shape than that produced by manual pollination (Fig. 4.31).

The fruit set efficiency in bitter gourd flowers was 100 per cent in both electrostatic pollination and hand pollination (Fig. 4.30). It might be due to the higher number of stigma present in a flower.

The developed electrostatic pollinator could able to collect as well as deposit the pollens with high efficiency and without damaging the floral parts. The viability of the pollens collected by the electrostatic pollinator was found out by acetocarmine staining procedure as 100 per cent for both tomato and bitter gourd.

It was observed that the average size of bitter gourd fruits developed was higher and uniformly shaped from electrostatically pollinated flowers (Fig. 4.32) than that of hand pollinated ones. It was also recorded that the number of seeds present in the fruits produced from electrostatic pollinator were also higher. This was due to the higher rate of targeted deposition of pollen grains in cluster of stigma without any damage. In hand pollination, the some of the fruits developed was lesser in weight and irregular in shape. The sound seeds inside such fruits also lesser in number, due to the lack of successful fertilization. It was inferred that damage of stigma during hand pollination was the major reason of the reduction in weight and number of sound seeds.



Fig. 4.31. Tomato produced by electrostatic pollination



Electrostatically pollinated



Manually pollinated

Fig. 4.32 Bitter gourd produced by artificial pollination

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

For the process of pollination to be successful, a pollen grain produced by the anther, the male part of a flower, must be transferred to a stigma, the female part of the flower, of a plant of the same species. Successful pollination results in fruit and seed formation. Greenhouses create barriers that protect plants from adverse growing conditions but it form structural obstacles to natural pollination. The vegetable cultivation in Kerala under protected condition demands artificial-mechanical pollination for better yield even if it is a self - pollinated crop. Most of the mechanical pollination methods are contact in type, hence they may cause mechanical injury to the flower and leads to the decrease in fruit set efficiency. In this juncture, development of a non - contact type electrostatic pollinator has gaining more importance.

The objectives of this research work were to study the physical characteristics of the flower, design and development of electrostatic pollinator and evaluation of the developed unit under protected condition.

Tomato, a self - pollinated plant and bitter gourd, a cross - pollinated plant were selected for the study. The exserted position of stigma in tomato flower due to the high temperature inside the protected conditions eliminates the possibility of self-pollination. As preliminary studies, various factors affecting the pollination such as morphological characteristics of the flower, environmental conditions were observed. Tomato flowers are bisexual having an average length of 15 mm with six anthers. Bitter gourd flowers are unisexual with an average length of 7 mm with five stamens. Anthesis time, anther dehiscence time and stigma receptivity time of the tomato and bitter gourd flowers were observed as they have a profound role in the success of pollination. Tomato flower consisted of an average 500 pollen grains and that of bitter gourd flower was 3000 pollen grains. Tomato pollen has bigger size compared to bitter gourd pollen.

Electrostatic pollen collection unit comprised of an electrode and a high voltage amplification unit. The high voltage amplification circuit was made by using fly back transformer and a MOSFET. Input voltage source was given from three 3.7 V lithium ion rechargeable battery (11.1 V). Spherical shaped electrode was selected after preliminary laboratory trials as it could perform uniform distribution of charges over its periphery. Diameter of electrode was fixed in accordance with the morphological features of the flower. The pollen collection capacity of two electrodes E1 and E2 having diameter 10 mm and 7.5 mm respectively were evaluated at 3 kV, 4 kV, 5 kV and 6 kV at 5 mm, 10 mm, 15 mm distance from the anther tip of the flower. Corresponding to all the combinations of variables, the charge induced on the electrode, electric field intensity, charge induction on the pollen grain and detaching forces were calculated. The charge induced on the electrode increased with increase in the potential. The electric field intensity was maximum at 6 kV potential at 5 mm gap between the electrode and the tip of the anther of flower. Tomato pollen grains acquired more charge compared to bitter gourd pollen due to its increased size. Detaching force acting on the pollen grain was maximum at 5 mm distance with 6 kV potential.

- The maximum number of tomato pollens (409) were collected by electrode E1 at 5 mm distance from the anther tip of the flower with 6 kV electrode potential.
- The maximum number of bitter gourd pollens (2827) were collected by electrode E1 at 5 mm distance a from the anther tip of the flower with 6 kV electrode potential.
- The minimum number of tomato pollens (87) was collected by electrode E2 at 15 mm distance from the anther tip of the flower with 3 kV electrode potential.

- The minimum number of bitter gourd pollens (1227) was collected by electrode E1 at 15 mm distance from the anther tip of the flower with 3 kV electrode potential.
- The voltage, distance and diameter have a significant effect (at 1 % of level of significance) on the number of pollen grains detached from the tomato and bitter gourd flowers.
- Among the two electrodes E1 and E2 , the number of pollen grains collected at all electrode voltage potentials corresponding to each distance was significantly higher for E1 compared to E2.
- The variation in number of pollens collected could be explained with the high detaching forces and high electrostatic field intensity due to high electrode potential and less distance between the flower and electrode.

The pollens were transferred from the anther and attached to the electrode due to the strong electrostatic forces. Further, the pollens were deposited back into the stigma of the flower by placing the electrode near to it with a different electrode potential. As the stigma has low impedance compared to any other parts of the flower (Corbet et al., 1982), pollens in the electrode were attracted towards the stigma. The electrodes E1 and E2 were charged with 1 kV, 3 kV and 6 kV for pollen deposition. It was observed that pollens in the electrode were deposited to the flower especially to the stigma at 5 mm distance.

Fruit set efficiency is the important parameter for the evaluation of a pollinator. Fruit set efficiency of the flowers after electrostatic and artificial pollination was assessed separately.

- The fruit set efficiency of tomato was 80% after electrostatic pollination whereas it was 40% after hand pollination.

- The fruit set efficiency of bitter gourd was 100% in both electrostatic pollination and hand pollination. But the fruit size was bigger, uniformly shaped and number of fertile sound seed was more in electrostatic pollination.
- It was inferred that damage of stigma during hand pollination was the major reason of the reduction in weight and number of sound seeds.

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**INVESTIGATIONS FOR THE DEVELOPMENT OF ELECTROSTATIC
POLLINATOR**

By

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ABSTRACT OF THESIS

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ABSTRACT

The problems in fruit setting with artificial pollination (contact type) can be eradicated by the application of electrostatic forces (non - contact pollen collection and deposition), hence the study was undertaken to develop an electrostatic pollinator. Anagha variety of tomato and Preethi variety of bitter melon were selected. Morphological characteristics of these flowers were studied for design of pollinator. A high voltage amplification unit with flyback transformer and MOSFET, a spherical shaped electrode and a DC input source were the major components of the electrostatic pollinator. The pollen collection capacity of two electrodes E1 (10 mm) and E2 (7.5 mm) were evaluated at voltage potentials of 3 kV, 4 kV, 5 kV and 6 kV at 5 mm, 10 mm and 15 mm distance from the anther tip of flower. The maximum number of pollens (409 for tomato and 2827 for bitter melon) was collected by electrode E1 with a charging potential of 6 kV at 5 mm distance, both in case of tomato and bitter melon. This high pollen collection rate was due to high detaching forces acting on the pollen grains at shorter distance between the anther tip and electrode. The pollen collection capacity was minimum (87 for tomato and 1227 for bitter melon) for electrode E2 with an electrode potential of 3 kV at 15 mm distance. Pollens were deposited into the flower using the two electrodes (E1 and E2) at voltage potential of 1 kV, 3 kV and 6 kV at 5 mm distance from the tip of stigma. The fruit set efficiency of electrostatic pollination in tomato was 80% and artificial manual pollination was 40%. In bitter melon, fruit set efficiency of electrostatic pollination and artificial pollination was 100%. But damage of stigma during hand pollination caused reduction in size, weight and number of sound seeds.