AN ASSESSMENT OF THE MORPHOLOGICAL VARIATIONS ON THE POLLEN GRAINS OF *ABELMOSCHUS ESCULENTUS* L. CAUSED BY CHEMICAL AND BIOLOGICAL PESTICIDES

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DECLARATION

I hereby declare that the dissertation entitled **"An assessment of the morphological variations on the pollen grains of *Abelmoschus esculentus* L. caused by chemical and biological pesticides"** is an authentic record of the research work carried out by me under the supervision of **Dr. Alphonsa Vijaya Joseph**, Associate Professor, Department of Botany, St. Teresa's College (Autonomous), Ernakulam, Kochi-682011.

Date: ETNA BIVERA

Place: Ernakulam (Reg. No. SMP20BOT004)

CERTIFICATE

This is to certify that the investigatory project entitled **"An assessment of the morphological variations on the pollen grains of *Abelmoschus esculentus* L. caused by chemical and biological pesticides"** submitted in partial fulfillment of the requirements for the award of Degree of Master of Philosophy in Botany, is an authentic record of the research work carried out by **ETNA BIVERA (SMP20BOT004)** under the supervision and guidance of **DR. ALPHONSA VIJAYA JOSEPH**, Associate Professor, Department of Botany, St. Teresa's College (Autonomous), Ernakulam. I further certify that no part of the work embodied in the project has been submitted for the award of any other degree or diploma.

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CONTENTS

List Of Tables

List Of Figures

ABSTRACT

The use of chemical pesticides has caused an imbalance in all forms of life and its associations. In this study we focus on the variations that are brought before and after the use of chemical and biological pesticides on the pollen grains of *Abelmoschus esculentus* L. The use of chemical pesticides on plants effects not only the plant pollen grains and its environment but effects the underground water, aquatic environment through leaching, biomagnification of chemicals in humans and thus leading to many health issues.

The morphological features of A. *esculentus* L. were studies using light microscope and the pollen grains were porolate, spheroidal, pantoporate and isopolar in nature with spines, ranges a size of 120-140 µm. Pore number varies from 22-45, pores were large, 6-9 µm in diameter, annulate. The exine showed ornamentations. The germination studies exhibited the growth of pollen tubes and maximum growth was exhibited in 15% sucrose solution and on 3rd day of incuabation in agar media. The viability remained 48 hours in *in vivo*. In the pollen grains treated with chemical and biological pesticides, maximum growth was exhibited on pollen grains treated with biological pesticides. There were variations on the viability, on the application of these pesticides, but retained till 48 hours.

The pollen grains under SEM analysis exhibit drastic change in their structure. Spines were absent on the pollen grains treated with chemical pesticides while, (20.40±2) µm, (18.41±2) µm and (18.35±3) µm length of spines were observed for pollen grains treated with Azadirachtin, *Ocimum tenuiflorum* and control (sucrose solution 15%).

CHAPTER 1

INTRODUCTION

Abelmoschus esculentus L. (Okra) is the only vegetable crop of significance of the family Malvaceae. It is an oligo purpose crop, but usually consumed for its green tender fruits as a vegetable in a variety of ways. These are rich in vitamins, calcium, potassium and other mineral substances. The ripened okra seed is a good source of oil and protein. It is also known to have higher nutritional qualities. They are biologically known as *Hibiscus esculentus* L. and *Abelmoschus esculentus* L.

SCIENTIFIC CLASSIFICATION

Kingdom	**:** Plantae
Division	**:** Magnoliophyta
Class	**:** Magnoliopsida
Order	**:** Malvales
Genus	**:** *Abelmoschus*
Species	**:** *esculentus*
Binomial name	**:** *Abelmoschus esculentus* L.

Abelmoschus esculentus L., also identified as lady's finger is an important vegetable crop, being innate of tropical Africa. It is an elevated, annual dicotyledonous plant associated to cotton. Young pods are gathered in 60-180 days from sowing about 5-10 days after flowering. Successional harvesting of young pods is generally recommended. The pods are harvested, using a slight twist to break the stalk. The fresh and green tender fruits are used as vegetable (Jain *et al.*, 2012).

Abelmoschus esculentus L. (Okra) also has medicinal properties of massive importance with large pharmacological applications. It has medicinal values for curing ulcers and relief from hemorrhoids. The plant was stated to possess diuretic properties (Kumar and Nadendla, 2013). Okra has a medical demand as plasma replacement or blood volume expander. It is also a good source of iodine which is useful in the treatment of simple goiter. It is very useful in genitourinary disorders, spermatorrhoea and chronic dysentery. Tests conducted in China suggest that an alcohol extract of okra leaves can remove oxygen free radicals, alleviate renal tubular interstitial diseases, reduce protein urea and improve renal function. Studies are being developed aiming okra extract as remedy to manage diabetes.

Besides having the above revealed nutritional, medicinal and industrial properties, it has been used as a component of many herbal formulations, which are used for the cure of various ailments, in precise, the regulation of blood pressure, fat, diabetes, chronic dysentery genitourinary disorders, and ulcer (Singh *et al.*, 2014).

The mucilage has medicinal applications, like plasma replacement or blood volume expander. The mucilage of okra not only adheres cholesterol but the bile acid carrying toxins are discarded into it by filtering the liver. It also has industrial applications, when added as size to glaze paper and used in confection (Jain *et al.,* 2012). The bast fibers from the stem are used as a substitute of jute (Kumar and Nadendla, 2013).

1.1 INSECT PEST ON OKRA PLANT

The existence of insect pests is one of the main factors in production of okra. The crop is attacked by numerous insect pests among which shoot and fruit borer, *Earias vittella* (Fabricius) and *Earias insulana* are utmost serious as it takes upper hand by causing direct damage to tender fruits. Yellow vein mosaic virus, cercospora leaf spot, fusarium wilt, powdery mildew, damping off, leaf curl and root-decaying disease are few diseases associated with *A. esculentus* L. (Gupta *et al.,* 2009).

1.2 POLLEN GRAINS

Pollen grains are the male gametophytes otherwise microgametophytes in gymnosperms and angiosperms. It is produced by meiosis and carries half the chromosomal complement of its parent plant (Di-Giovanni and Kevan, 1991). The results of pollen tube competition can have major genetic consequences for plant populations (Snow and Spira, 1991). Although the pollen grain is a relatively simple two or three celled structures, cytogenetic and mutagenesis experiments specify that higher plants make a significant speculation in genetic material devoted to gametophyte production (Bedinger, 1992). The providence of pollen and pollen tubes can have a reflective effect on fruit and seed production (Abdelgadir and Van Staden, 2012).

Pollen physiology, predominantly germination and viability, has received considerable attention for its application in plant breeding, conservation, adaptation, and understanding the physiological behavior of different taxa with varying goals and objectives (Khan and Perveen, 2006).

The viability of pollen grains is an essential requirement for obtaining improved and hybrid genotypes and for a good fixation of the fruit. It is a matter of great importance, particularly for genetic improvement programs, which were used in countless types of controlled pollination (Borem and Miranda, 2007). The release of pollen grains can start from sunrise until noon, depending on the temperature, humidity and genetic structure of the plant (Ferreira *et al*., 2007).

Various procedures were used to assess the viability of pollen grains, the most common being, germination *in vivo* and *in vitro,* besides the chemical dyes test, which was grounded on cytological criteria such as pigmentation (Almeida *et al.*, 2011). The germination *in vitro*, in culture medium, is a technique that emulates the style stigma conditions, persuading germination of the pollen tube. Each species requires a specific formulation of culture medium to get good germination of the pollen grain. Among the elements that comprise the culture medium, sucrose is considered vital, while boron as boric acid, and calcium, as calcium dihydrate can maximize the medium efficiency. Agar was used to give uniformity to the medium and avoid damage to the pollen tube during the assessment (Ferreira *et al*., 2007). The sustainability is a simple technique, reasonable and provides results quickly, making it very attractive for works concerning the pollen grains.

Various dyes may be used for this purpose such as, Acetocarmine, Triphenyl Tetrazolium Chloride (TTC), Aniline blue and Malachite green with acid fuchsin. The viability of the pollen grain was not only inclined by intrinsic factors such as its state of biological maturation, origin, genetic characteristics, the plant nutrition and by extrinsic factors such as the components of the culture medium, pollen density in the medium, temperature and incubation time, collection period, but also by environmental circumstances such as temperature and humidity (Stanley and Linskens, 1974).

Almeida *et al.* (2002), reported that the *in vitro* germination of pollen is highly interrelated with fertilization in the field, in other words, *in vivo*. However, fertilization inclines to be smaller than *in vitro* germination due to the impact of factors such as stigma receptivity, genetic barriers, temperature and relative humidity. In view of these facts, it is necessary to raise and provide data about the probability of pollen grains, because it was a primary and essential condition for the success of the hybridization in the genetic improvement programs (Kaefer *et al*., 2016).

Usman (2004), reported that if the pests and diseases of fruit vegetables are not accurately managed, it could have an economic influence on the level of production by reducing crop yield, quality and subsequently low return. Pests and diseases such as flea beetle, cotton stainer, aphids, thrips, grasshopper, and diseases include leaf spot, bacterial wilt, root-knot nematode, powdery mildew and early blight were common diseases accompanied with okra (Kucharek, 2004).

Pesticides are compounds that were used to kill the pests and insects which attack and harm crops. Different kinds of pesticides have been implemented for the protection of crops. Though pesticides have some benefits they themselves execute threat to the crops and serious negative impacts on environment. Excessive use of pesticides leads to the imbalance of biodiversity.

Pesticides have effective mitigation of harmful bugs, but unfortunately, the hazards associated with their use have beaten their beneficial effects. Non selective insecticides slaughters non-target plants and animals along with the targeted ones. Besides, with the passage of time, few pests also develop genetic resistance to pesticides. So, pesticides have extreme effects on non target species and affect animal and plant biodiversity, aquatic as well as terrestrial food webs and ecosystems (Mahmood *et al.*, 2016).

The chemical pesticides used in crop fortification, to reduce the damage caused by pathogens and pests in agricultural fields, pose many long term threats and risks to living beings due to their harmful side effects. They are known to cause cancers and fetal impairments and they persist in the environment for many years. Based on the dormant application and solid oppressive activity against pests, these synthetic pesticides control the market and have a substantial impact on the making of products.

On the other hand, biological pesticides, also known as biopesticides, biological controls or biocontrol, are used to manage pests or diseases and most remarkably used in agriculture. The biological pesticides have the following benefits on the greens. Biological control products naturally target a narrow range of pests or diseases while non target organisms, such as birds, bees, fish, humans and beneficial soil organisms, remain unaffected. Subsequently biological controls are naturally occurring, and at the end of their life, they entirely biodegrade and leave no destructive remainders on the crop or in the environment. This feature helps encourage the safety and wellbeing of individuals who works on fields.

Biological resistor, as a part of IPM, works to accomplish sustainable management of pests and diseases, maintaining the pressure well below economically damaging levels. Pests and diseases do not progress resistance to biological controls. Since the rate of application will only change with pest or disease pressure, farmers can precisely predict input costs. Records have revealed that pests tend to become resistance to conventional pesticides thus proving that it is not a long term solution, something that never occurs with the use of organic pesticides.

As the regular consumers, became conscious of the dangers posed by synthetic chemicals, demand for farm products that have experienced organic treatments rises. This makes the use of these chemicals a potential risk as there was a glaring prospect of experiencing huge losses due to the consumer shunning your product. In the present work the effects of chemicals, on the morphological features of pollen grains were studied. A comparative study was done along with the biological pesticides. The effects on the pollen grains were noted on the application of chemical and biological pesticides. Two different pesticides, each from both chemical and biological pesticides were carefully chosen on the basis of commonly used insect repellent.

OBJECTIVES OF THE STUDY

- To study the morphological features of pollen grains of *Abelmoschus esculentus* L.
- To observe and study the *in vitro* germination and viability of the pollen grains of *Abelmoschus esculentus* L.
- To analyze the morphology and *in vitro* germination on application of a chemical pesticide and biological pesticide.

CHAPTER 2

REVIEW OF LITERATURE

2.1 POLLEN GRAINS

Pollen physiology, particularly germination and viability, has established substantial attention for its application in plant breeding, conservation, adaptation, and understanding the physiological behavior of different taxa. Pollen has considerable potential to achieve genetic transformation. Pollen grains from different plants require a varied range of growth factors like water, sugar solution, inorganic salts and vitamins for effective germination. Pollen stored at low temperature has a better germination capacity than at high temperature. It has been widely recognized that the temperature and relative humidity of the storage environment are two important factors that intensely influence the viability of stored pollen. Pollen storage is the most effective method of irresistible hybridization barriers between plants, flowering at different times and growing in different regions (Khan and Perveen, 2006).

The characteristic features of pollen grains mostly consist in the size and shape of the grain, the number and arrangement of the germinal apertures and the sculpturing of the exine (Wodehouse, 1928). Even the number of pollen grains placed upon a stigma stimulates both the development of pollen tubes and subsequently the progeny which result from fertilizations by gametes from these pollen tubes (Ter-Avanesian, 1978).

2.2 *ABELMOSCHUS ESCULENTUS* L.

Okra is considered to be an easily available, low cost vegetable crop with various nutritional values and potential health benefits (Elkhalifa *et al.*, 2021). *Abelmoschus* species are self pollinators but they express allogamy levels up to 63% (Hamon and Koechlin, 1991). Okra is a cost effective and economically affordable natural source with abundant reservoirs of carbohydrates, proteins, fatty acids, vitamins, fiber, and minerals, with various other bioactive phytochemicals that are important for human well being (Elkhalifa *et al.*, 2021). Although they are potentially available, they exhibit some disorders due to the excess use of pesticides.

2.3 PEST CONTROL

The application of chemical insecticides dates back to India since 1948 while production initiated in 1952 with the establishment of a DDT & BHC manufacturing plant near Calcutta. At present, in India, almost 150 pesticides were registered with legal application. Despite their benefits, the unregulated and indiscriminate application of pesticides had raised serious apprehensions about the environment and human health. Long term exposure, even at low concentrations, causes serious health problems such as immunosuppression, hormonal disruption, decreased intelligence, reproductive abnormalities, and cancer. The apparent reason lies in research data which indicates that only 0.1% of pesticide applications target the rest of the pests, 99.9% stay and infiltrate the environment.

The consumption pattern was also more biased towards insecticides. Thus, the agrochemical application pattern in India was not similar to that of the world at large. In India, 76% of the pesticides used were insecticides, compared to 44% worldwide. The application of herbicides and fungicides was therefore less cumbersome (Bhardwaj and Sharma, 2013).

A large number of studies have been carried out on the biological control for pest, in spite of these studies, the pesticides are still common and consistent source for the farmers to resist pests. The unrestrained use can lead to many adverse effects, such as, poisoning of the human beings and animals, induction of the pesticide resistance, bioaccumulation as well as the pesticide residue problems, and the devastation of environment.

Millions of pesticides were being used in the world and this tendency has been increasing with the passage of time. On the basis of data conducted, Imidacloprid owe its popularity among the farmer. Imidacloprid was marketed as an insecticide that acts as an early plant growth enhancer and belongs to a moderately new class of insecticide, known as neonicotinoids, which has a high activity against sucking insects. It is colorless, crystal in nature and quite water soluble. In the earlier studies it was revised that it has the potential to leach up to the groundwater levels (Baig *et al.*, 2012).

Acute pesticide intoxication is an imperative public health problem worldwide. Most of the mortalities are due to poisoning with organophosphorus insecticides which are an integral part of agriculture. Due to their very high intrinsic toxicity, continuous efforts were being made to develop novel pesticides of low toxicity and high potency. Perpetually such compounds were released into the market without proper data on direct human toxicity.

Imidacloprid is a neonicotinoid insect repellent belonging to the Chloromycetin nitroguanidine chemical family. It works on the nervous system through an acetylcholine receptor blockade. It was regularly used to kill fleas present on pet animals, termites and bees. The studies reports that the accidental exposure of these chemicals can cause severe gastrointestinal symptoms along with respiratory distress and neuropsychiatric features (Kumar and Kumar, 2013).

Imidacloprid was the most extensively used active ingredient in neonicotinoid insecticides and its activity was focused on whole plant protection. It acts in contradiction of homopteran insects, such as rice larvae or aphids, as well as certain other insects such as thrips, whiteflies, termites, lawn insects and certain beetles. This compound was usually used on rice, corn, sunflower, rapeseed, potato, sugar beet, vegetable and fruit crops.

The level of Imidacloprid decreases during growth, and very low levels were anticipated at the time of flowering. The problem of declining pollinator numbers has been compounded with the increasing use of Imidacloprid formulation for seed coating on sunflower, maize and rapeseed in Western European countries. Parallel studies on Imidacloprid have shown that they were toxic to bees which were the major pollinators of the field (Bonmatin *et al.*, 2005).

Mancozeb is a widely used fungicide approved for use in agriculture in many countries with long perseverance in the environment and subsequent bioaccumulation in tissues and biological fluids. Persistent organic pollutants (POPs) play an important role among environmental pollutants, whose application can cause adverse health effects in animals and humans. Different types of POPs are widely used in agriculture and present a very strong persistence on plants, with bioaccumulation in the food web. Mancozeb is considered as one of the major endocrine disruptors which possibly leading to low fecundability, miscarriage, preeclampsia, polycystic ovary syndrome (PCOS), endometriosis, and alterations in the menstrual cycle. This negatively affect female reproductive competence both in adulthood and during embryonic development (Bianchi *et al.,* 2020).

Mancozeb is an extensively practiced fungicide in the fields by farmers. Owing to injudicious and random use of these pesticides in water bodies like ponds, lakes, river and low lying water areas were continuously getting polluted through surface run off, sediment transference from treated soil and direct application as spray for controlling pests. These toxicants indirectly produce considerable damage to aquatic life including economically important species of fishes. Revelation to pesticides produces molecular and biochemical changes in fish which lead to cellular disinfections (Allayie and Vardhan, 2016).

According to the studies, Mancozeb was found to reduce the nitrification at all concentrations studied, which was in good agreement with previous reports. Mancozeb concentrations above 10 ppm can have an inhibitory effect on the specialized group of nitrifying bacteria and the inhibitory effect was consistent above 250 ppm to 500 ppm (Walia *et al.,* 2014). The long term use of these chemicals has a negative impact on the environment. The Environmental Protection Agency (EPA) has withdrawn several fungicides from the market due to groundwater contamination and adverse effects on wildlife and human health (Siddiqui *et al.,* 2008).

The most common method of bruchid control was chemical methods, namely fumigation with Ethylene Dibromide (EDB), Aluminum phosphide, and Carbon disulfide or dusting with Malathion, Carbaryl, and Permethrin. Due to chemical toxicity, high cost, residual effect and environmentally harmful phytosanitary actions, treatments were more accessible. The use of neem leaves and seeds was an age old practice in India. Rather than using neem in its native form, the current trend was to use neem extracts, especially Azadirachtin, Nimbin, Nimbidine, Salanin, etc. in various concentrations increases its efficiency. It was an effective treatment to improve seed quality (Manjula *et al.,* 2021).

Experiments were conducted to examine the allelopathic potential of *Ocimum basilicum* extract as a biodegradable herbicide against broadleaf and grass weeds. The leaves and flowers of *O. basilicum* were used to formulate tri solvent extracts (Methanol, Acetone and Distilled water) to analyze their effects on maize and soybean seedlings and dominant weeds in pots at Giza and in the fields of Sids stations. Their extracts inhibit the germination and growth of *Amaranthus* weed seeds more than soybean and corn plants. The effect of *Ocimum* extracts was more operative on weeds with 2 to 4 leaves followed by the root, seedling growth and germination percentage was less affected by *Ocimum* extracts (Mekky *et al.,* 2019).

Biopesticides were mostly microbial biological pest control agents that were applied in the same way as chemical pesticides. The most beneficial advantages of biopesticides were that, they were harmful residues that went unnoticed. They can be economical than chemical pesticides when produced locally. They can be more effective than chemical pesticides in the long run. They were decomposable.

The application of chemical pesticides and the practice of highly toxic pesticides that endanger agricultural workers were unacceptable. Government agencies should recognize these alternatives and provide proactive support to farmers so that they can switch to ecological, sustainable and healthy ways of farming. Thus, the use of biopesticides as a component of Integrated Pest Management (IPM) programs can significantly reduce the use of conventional (chemical) pesticides, while achieving the same level of crop yield. The effective use of biopesticides requires a good understanding of pest management (Bhardwaj and Sharma, 2013).

CHAPTER 3

MATERIAL AND METHODS

3.1 SELECTION OF PLANTS

For the morphological studies of pollen grains, healthy saplings of *Abelmoschus esculentus* L. were collected from Vegetable and Fruit Promotion Council Keralam *(*VFPCK*)*, Kakkanadu, Kerala. And was grown under controlled conditions (Figure 1).

Figure 1: ***Abelmoschus esculentus* L. plant**

3.2 COLLECTION OF POLLEN GRAINS

Pollen grains of *Abelmoschus esculentus* L. were collected early in the morning from the plants. Acetolysis was carried out according to the procedure followed by Erdtman (1960). Acetolysed pollen grains were mounted in glycerol and examined under the light microscope at different magnifications. Measurements of pollen diameter were taken with the assistance of the ocular micrometer inserted in the eyepiece of the microscope.

3.3 PREPARATION OF POLLEN GRAINS

3.3.1 ACETOLYSIS OF POLLEN GRAINS

Anthers from the flower of *Abelmoschus esculentus* L. were cautiously removed and placed in test tube, crushed with glass rod in 70% alcohol and then filtered. The residue left in the test tube after decantation of alcohol was covered with glacial acetic acid, centrifuged and the residue covered with fresh Acetolysis mixture prepared by mixing 9 parts of acetic anhydride and 1 part of concentrated sulphuric acid, the latter being put drop by drop. The tube with the mixture was then kept in a hot water bath until the pollen grains turned brownish black. The test tube was then chilled and centrifuged and this centrifuged Acetolysis mixture was decanted and again centrifuged with glacial acetic acid and then decanted. The procedure was repeated three times using distilled water. After decanting water, 50% glycerin was added and centrifuged, small quantity of glycerin jelly was placed on warming the slide, carefully pollen sample were added from test tube, it was then covered with cover slip, and kept for microscopic observation (Basarkar, 2017).

3.3.2 MICROSCOPIC OBSERVATION

The pollen grains were observed under light microscope at 4X, 10X, 60X and 100X magnification using a light microscope, to study the morphological features of the specimen.

3.3.3 STORAGE OF POLLEN GRAINS

Pollen was removed from its flower and directly stored in 1.5ml microfuge tubes and rehydrated before each experiment. After removing from freezer, pollen was kept at room temperature for about 30 minutes.

3.4 GERMINATION AND VIABILITY OF POLLEN GRAINS

To evaluate pollen grain germination and viability, the male inflorescence was protected with a polyethylene bag on the day before sampling, to avoid contamination. Flowers in anthesis were picked from the field on the next day. The pollen grains were excised from the anthers and were stained on a glass slide, covered with a coverslip, and observed under an optical microscope (10x lens). The experiments were randomized with three replications.

3.4.1 SUCROSE METHOD

Sucrose was considered to be an excellent source of carbon for *in vitro* culture of cells. Various concentrations of sucrose solutions were prepared to study the growth of pollen grains. The pollen grains were kept in 5%, 10%, 15%, 20%, and 25% sucrose solution and was observed at regular interval to check the germination of pollen tubes.

3.4.2 BREWBAKER'S AND KWACK'S MEDIA ANALYSIS

For the preparation of 100 ml media 15% sucrose, was dissolved in distilled water. The pH of the media was adjusted to 7.0 ± 0.2 using a pH meter. The composition of the media given in Table 1.

SL. NO.	COMPONENT	CONTENT
1	Sucrose	15g/l
2	Boric acid	0.1g/l
3	Calcium nitrate	0.3g/l
4	Magnesium sulphate	0.2g/l
5	Potassium nitrate	0.1g/l

Table 1: **Composition of Brewbaker's and Kwack's media for pollen germination**

3.4.3 GERMINATION MEDIA

For all experiments, two diverse versions of growth media were used for pollen germination, a liquid version and a solid version containing 0.5 % agar. For the preparation of solid media, the media was prepared in the subsequent composition given in Table 2 with a PH adjusted to 7.0 ± 0.2 and was left to cool to 42° C. The media was then transferred onto a glass slide to form a layer with a thickness of about 0.5 mm. Hydrated pollen grains were dusted on to the media. The observations were made at normal room temperature. Pollen was observed at regular intervals.

SL. NO.	COMPONENTS	CONTENT
1	Sucrose	18g/l
2	Boric acid	1.5mg/l
3	Calcium chloride	1mg/l
4	Calcium nitrate	1mg/l
5	Magnesium sulphate	1mg/l
6	Potassium chloride	1mg/l

Table 2: **Composition of Germination media**

Per cent pollen germination was computed using formula:

$$\textbf{Pollen Germination (\%)} = \frac{\textit{Number of pollen grains germinated}}{\textit{Total number of pollen grains}} \times 100$$

3.4.4 ACETOCARMINE TEST

For the test, carmine powder was dissolved in glacial acetic acid and made to 100ml using distilled water (Table 3). The mixture was boiled, cooled and filtered and kept in a refrigerator. Two drop of stain was placed on slide and pollen grains were dusted onto it and covered with coverslip. The pollen viability was recorded after 10 min. The dark red-colored grains were counted as viable pollens.

SL. NO.	COMPONENT	CONTENT
1	Carmine powder	2g/l
2	Glacial acetic acid	95ml/l

Table 3: **Composition of Acetocarmine solution**

3.4.5 IODINE POTASSIUM TEST

The test required potassium iodide and iodine. Distilled water was used to make up to 100ml. Two drops of the dye were mixed thoroughly with the pollen grains (Table 4). A cover slip was placed and waited for 10 minutes. Darkly stained pollen grains were counted to be viable under the microscope.

The tests were repeated to know the germination and viability of pollen grains after treating with chemical and biological pesticides.

SL. NO.	COMPONENT	CONTENT
1	Potassium iodide	1g/l
2	Iodine	0.5g/l

Table 4: Composition of Iodine Potassium solution

The pollen viability percentage was calculated using formula:

$$\textbf{Pollen Viability (\%)} = \frac{\textit{Number of stained pollen grains}}{\textit{Total number of pollen grains}} \times 100$$

3.5 TREATMENT WITH CHEMICAL AND BIOLOGICAL PESTICIDES

To study the difference in the morphological features of pollen grains, they were treated with chemical and biological pesticides. Sucrose solution of 15% was taken as the control solution. The treatments were carried out *in vitro,* along with sucrose solution in the ratio 1:10 for both chemical and biological pesticides. These pollen grains were observed under scanning electron microscope to assess the variations. List of chemicals and biological pesticides are shown below (Table 5).

SL. NO.	COMPONENT	NATURE
1	15% Sucrose solution	Control
2	TATAMEDIA (Imidacloprid)	Chemical
3	SAAF (Mancozeb)	Chemical
4	NEEM OIL (Azadirachtin)	Biological
5	AGROPLUS (*Ocimum tenuiflorum*)	Biological

Table 5: Chemical and Biological pesticides used for analysis

CHAPTER 4

OBSERVATIONS AND RESULTS

4.1 SELECTION OF PLANTS

The saplings bought from VFPCK flowered after 86 days of growth. The flowers were yellow with reddish brown at the centre, solitary axillary inflorescence, Pedicellate, bracteolate in the form of epicalyx, hermaphrodite, complete, actinomorphic, pentamerous and hypogynous. Stamens were numerous, monadelphous, filaments united to form a tube and anthers protruding above the middle of the tube and anthers were monothecous. Epipetalous staminal tube united with the corolla, reniform, basifixed, filament short and introrse (Figure 2).

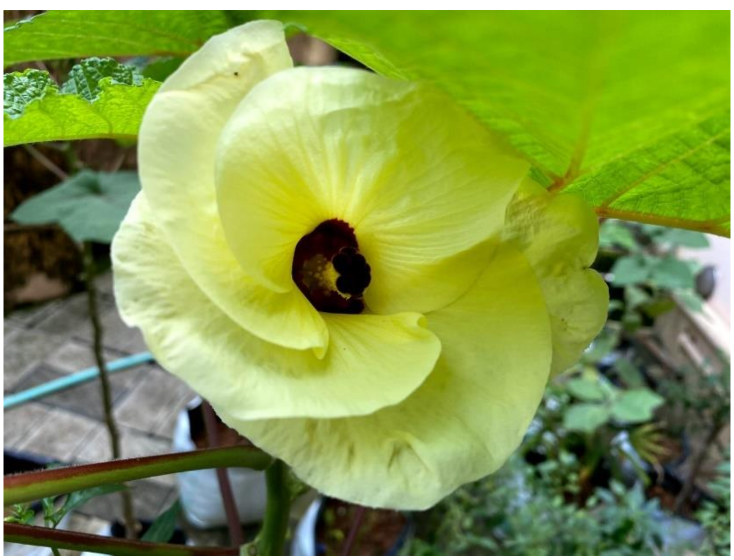

Figure 2: ***Abelmoschus esculentus*** **L. flower**

4.2 MICROSCOPIC OBSERVATIONS

Pollen grains of *Abelmoschus esculentus* L. were spheroidal in form, porolate with an echinate sculpture. Pollen exine always comprises of sexine and nexine, the latter was copious than the former. Spines were evenly distributed over the surface of the grain and vary in length, shape and density.

The microscopic observations showed that, the pollen grains were porolate, spheroidal, pantoporate and isopolar in nature with spines, ranges a size of 120 -140 µm. Pore number varies from 22 - 45, pores were large, 6 - 9 µm in diameter, annulate. The exine showed sculptures. The shape of the spine was long, straight, sharp and pointed to the apex and a few blunt, length 23.74 – 33.3 µm, width 10.53 – 32.2 µm. Spines and apertures were arranged horizontal and random (Figure 3).

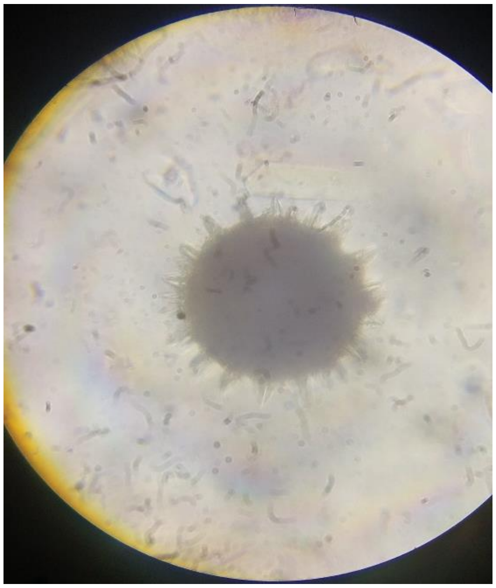

Figure 3: Pollen grain of *Abelmoschus esculentus* L. at 60X

4.3 GERMINATION AND VIABILITY OF POLLEN GRAINS

4.3.1 SUCROSE METHOD

The pollen grains placed in sucrose solution of five different concentrations shown notable changes in each concentrated solution. Germination was first observed in 15% sucrose solution after half an hour. Germination occurred faster in 10% and 15% of sucrose solutions when compared to 5%, 20% and 25% of solutions. The percentage of germination was higher in 15% of solution than any other concentrations. The pollen tubes germinated at 88.9% and 83.3% in 15 % and 10 % of sucrose solution respectively (Figure 4).

The test was carried out to study the viability of the pollen grains and shown pollen tube germination in all concentrations which specify the viability of the pollen grains. The viability of the pollen grains remains up to 48 hours after removing them from the flower.

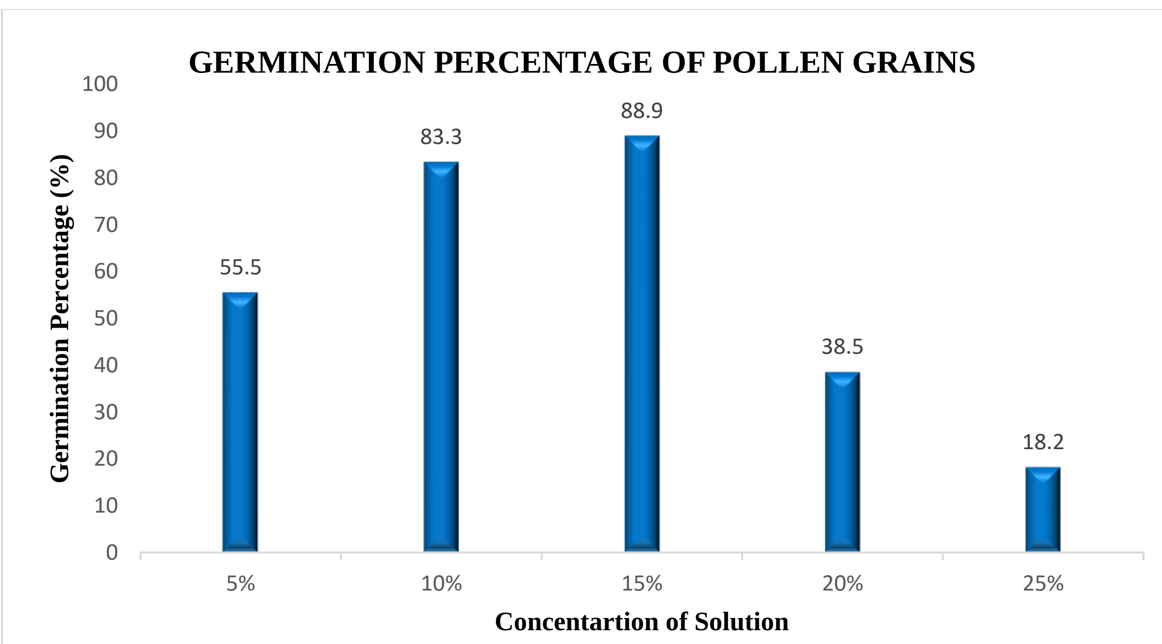

Figure 4: Graph on germination percentage of pollen grains

4.3.2 BREWBAKER’S AND KWACKS MEDIA ANALYSIS

Brewbaker’s and Kwacks media is the most relevant and frequently used one for the pollen germination. The test results shown in Figure 5 and 6. The diameter of the pollen grain and pollen tube was (24 ± 5) µm and (39 ± 5) respectively at 10 X. The viability of the pollen grains remined up to 48 hours and up to 24 weeks when maintained at *in vitro* condition by hanging drop method.

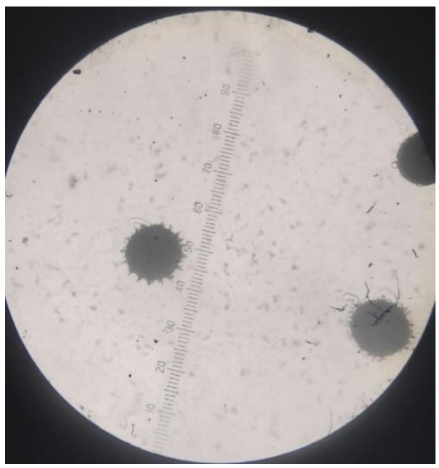

Figure 5: Pollen grains in Brewbaker’s and Kwack’s media

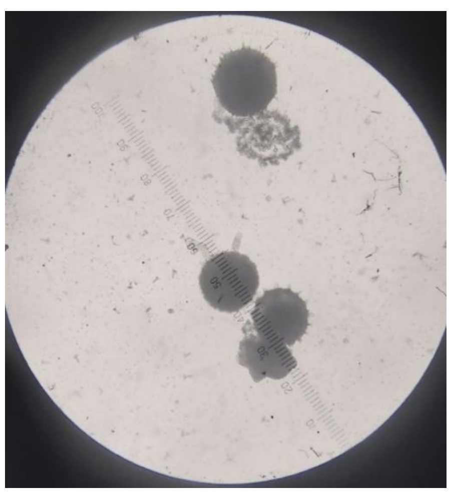

Figure 6: Pollen tube germinated in Brewbaker’s and Kwack’s media

4.3.3 GERMINATION MEDIA

The pollen tube germinated in the media was shown in Figure 7. The length of the pollen tube were note from day 1 to day 5 of germination. The maximum length of pollen tubes shown on the 3rd day of incubation 58µm (Figure 8). A graph was plotted on the germination percentage of pollen grains in the media (Figure 9).

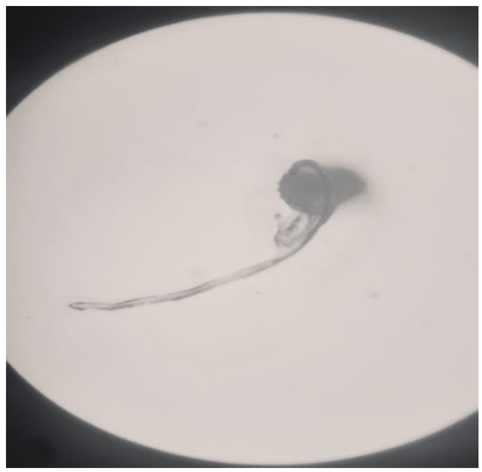

Figure 7: Pollen germinated on Agar medium

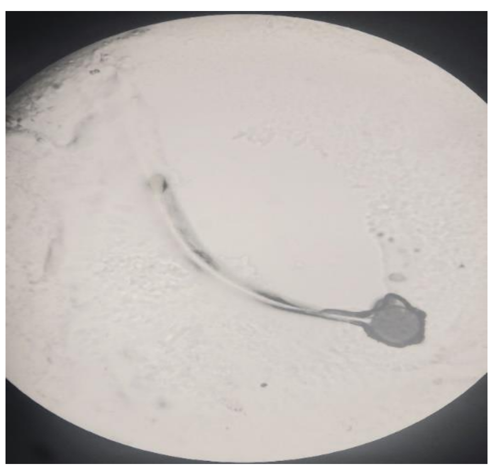

Figure 8: Pollen germinated on Agar medium

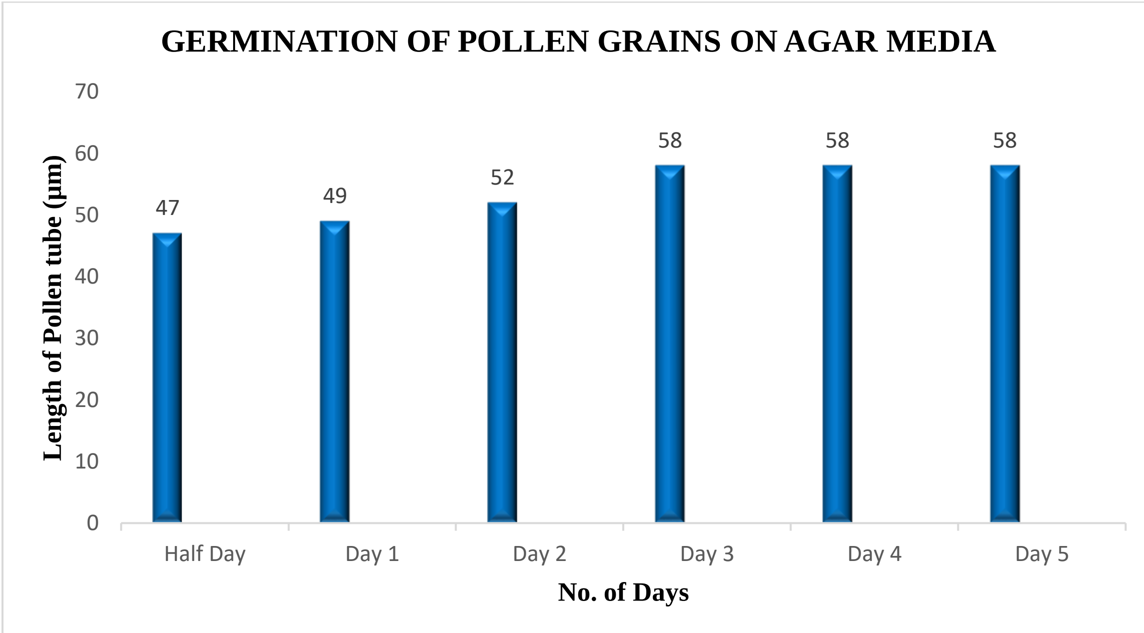

Figure 9: Graph on germination of pollen grains in Agar media

4.3.4 ACETOCARMINE TEST

The pollen grains which were viable stained red and which were non viable did not take up the stain (Figures 10). The viability of the pollen grains was retained for 48 hours in *in vitro* condition. A graph on the viability of pollen grains plotted in Figure 12.

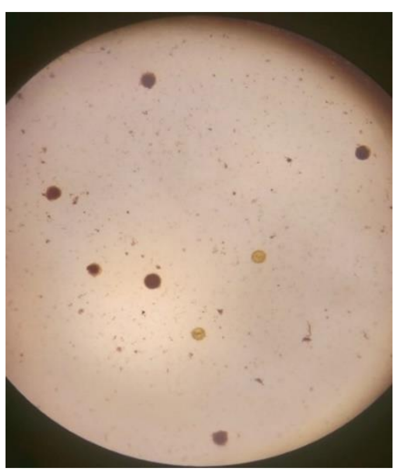

Figure 10: Pollen grains tested with Acetocarmine Solution

4.3.5 IODINE POTASSIUM (IK2) TEST

The viability was exhibited in pollen grains which were darkly stained and non viable pollen grains appeared yellow in color (Figure 11). Figure 12 shows the viability percentage of pollen grains. Acetocarmine and Iodine Potassium tests were non germination methods for the examination of viability in pollen grains. The viability remained for 48 hours was recorded.

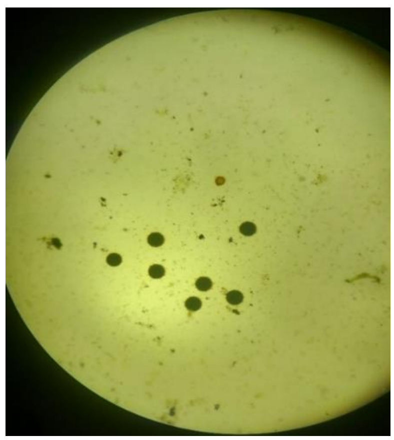

Figure 11: Pollen grains stained dark exhibited viability in Iodine Potassium solution

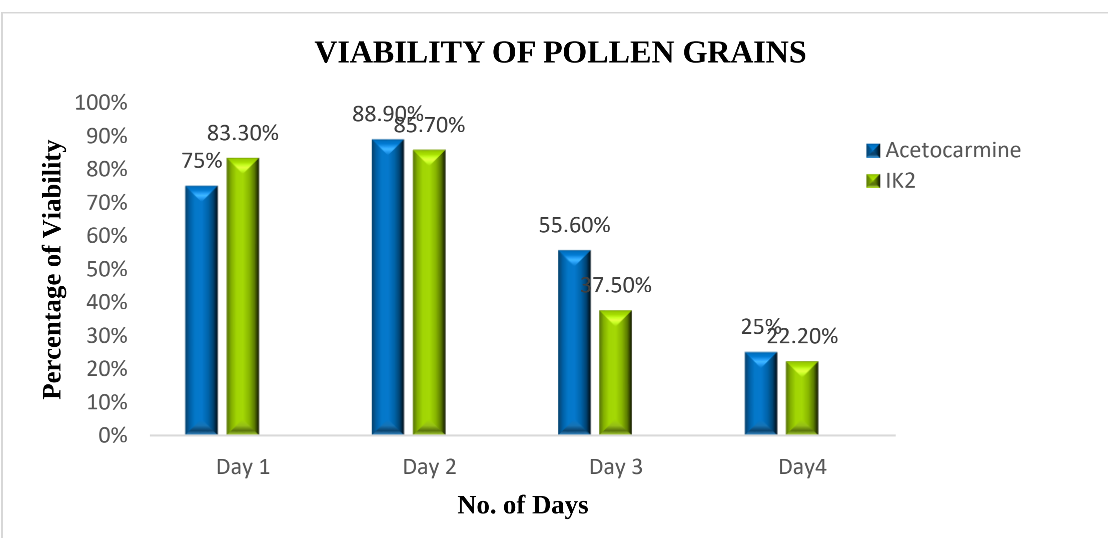

Figure 12: Graph on viability of pollen grains

4.4 GERMINATION AND VIABILITY OF POLLEN GRAINS TREATED WITH CHEMICAL AND BIOLOGICAL PESTICDES

4.4.1 BREWBAKER'S AND KWACK'S MEDIA

The germination percentage of pollen grains in Brewbaker's and Kwack's media was maximum in the Neem oil (Azadirachtin) 87.99%, when compared with the control (15% sucrose solution) Neem oil (Azadirachtin) 87.79%. Agroplus (O. *tenuiflorum*) also exhibit a higher germination percentage than control. Germination percentage of Agroplus (O. *tenuiflorum*), Tatamedia (Imidacloprid) and Saaf (Mancozeb) were 87.85%, 87.10% and 86.82% respectively. The minimum germination percentage was shown in the pollen grains treated with Mancozeb (Figure 13).

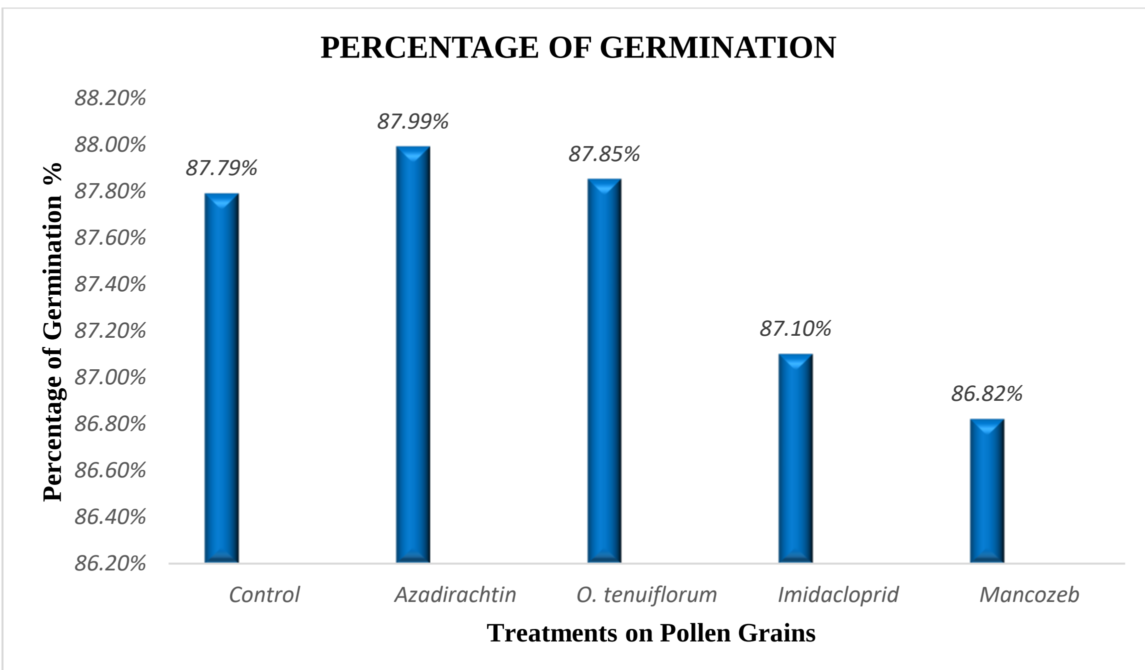

Figure 13: Germination of pollen grains in Brewbaker's and Kwack's medium

4.4.2 GERMINATION OF POLLEN GRAINS ON AGAR MEDIA

The pollen grains treated with biological pesticides Neem oil (Azadirachtin) and Agroplus (O. *tenuiflorum*) showed maximum pollen tube germination 58µm in the agar medium when compared with the control 56µm. The pollen grains treated with Tatamedia (Imidacloprid) and Saaf (Mancozeb) exhibited a length of 53µm and 54µm respectively. Tatamedia (Imidacloprid) showed minimum growth of pollen tube when compared with the control solution (Figure 14).

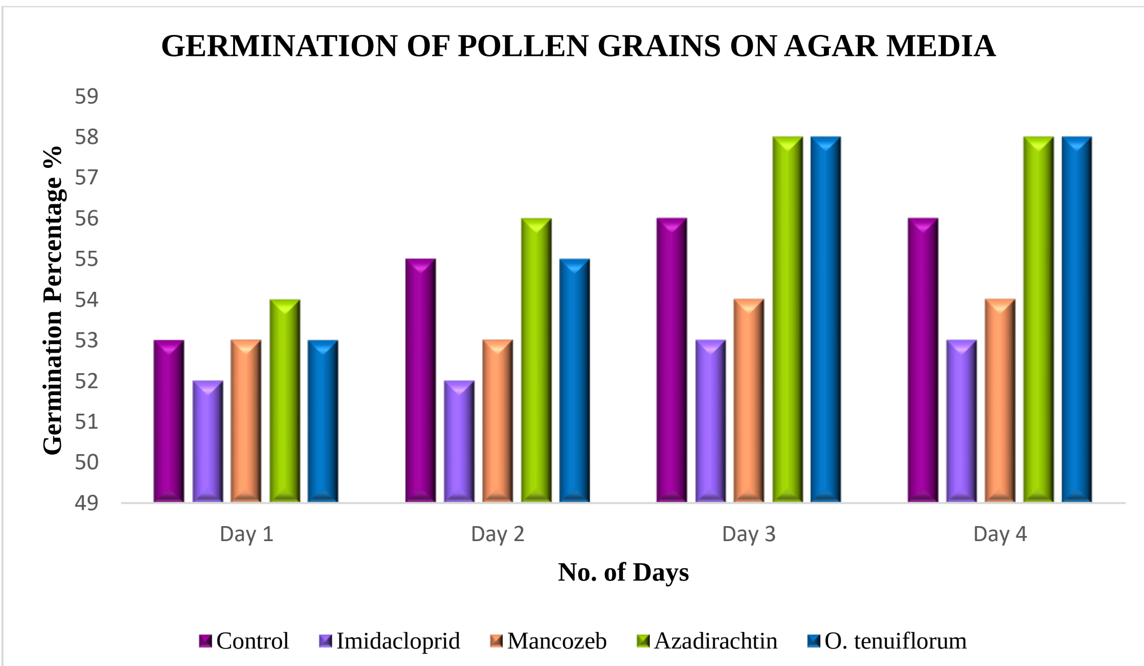

Figure 14: Graph on germination of pollen grains treated with Chemical and Biological pesticides on Agar media

4.4.3 ACETOCARMINE AND IK2 TESTS

The viability of the pollen grains treated with chemical and biological pesticides exhibit the following results (Figure 15). The maximum viability of pollen grains was exhibited in pollen grains treated with biological pesticide Neem oil (Azadirachtin) 80% and 87.5%, both in Acetocarmine and Iodine Potassium tests respectively in comparison with the control. The viability percentage of pollen grains treated with Agroplus (O. *tenuiflorum*), Tatamedia (Imidacloprid) and Saaf (Mancozeb) was 73%, 60% and 50% respectively in Acetocarmine test. The Iodine Potassium test exhibits a viability percentage of 80%, 71.42% and 46.67% for Agroplus (O. *tenuiflorum*), Tatamedia (Imidacloprid) and Saaf (Mancozeb) respectively. Saaf (Mancozeb) exhibits lowest percentage of viability for both the tests.

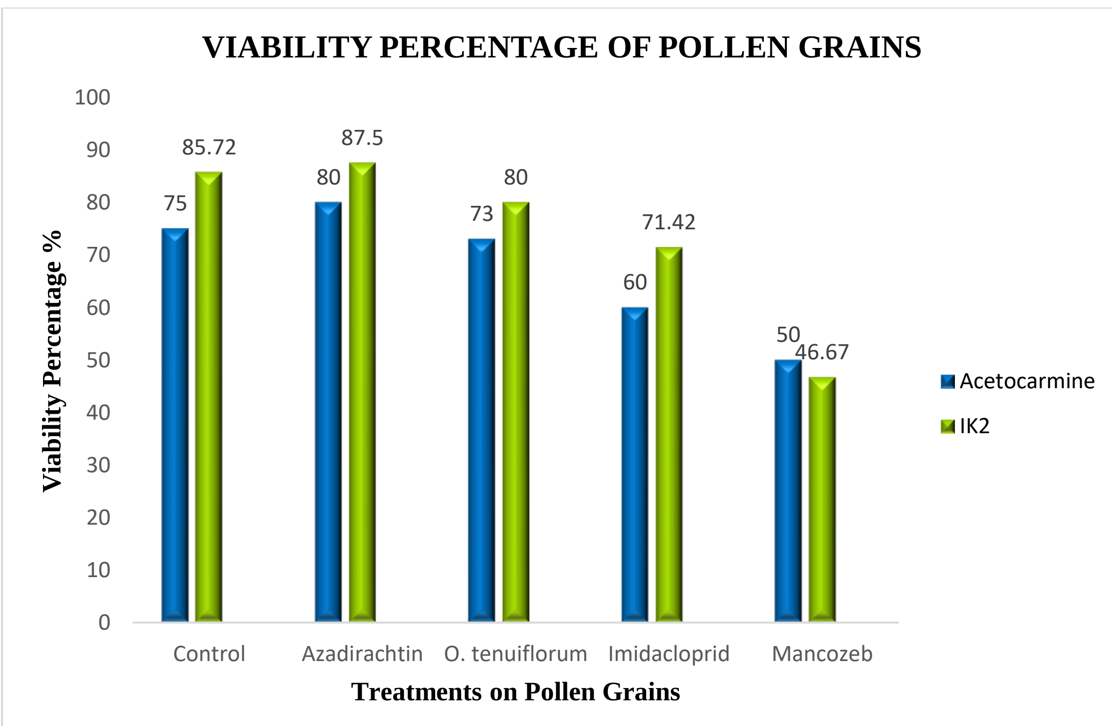

Figure 15: Graph on viability of pollen grains treated with Chemical and Biological pesticides

4.5 TREATMENT WITH CHEMICAL AND BIOLOGICAL PESTICIDES

The pollen grains which were treated with chemical and biological pesticides were observed using scanning electron microscope (Table 6).

The results demonstrations the size of pollen grains in their equatorial view to be (124.18 ± 1) µm, (128.84 ± 1) µm, (140.36 ± 1) µm, (33.05 ± 1) µm and (12.42 ± 1) µm for control, Neem oil (Azadirachtin), Agroplus (*Ocimum tenuiflorum*), Tatamedia (Imidacloprid) and Saaf (Mancozeb) respectively. The polar views of control, Neem oil (Azadirachtin), Agroplus (*Ocimum tenuiflorum*), Tatamedia (Imidacloprid) and Saaf (Mancozeb) displayed to be (123.74 ± 1) µm, (121.41 ± 1) µm, (139.41 ± 1) µm, (29.45 ± 9) µm and (11.30 ± 1) µm respectively. And their E/P ratio was 1.004, 1.064, 1.007, 1,222 and 1.099 respectively.

The basal length, width and distance between two spines were recorded in the analysis. (18.35 ± 3) µm, (20.40 ± 2) µm and (18.41 ± 2) µm were the basal length of spines of control, Neem oil (Azadirachtin) and Agroplus (*Ocimum tenuiflorum*) respectively. (8.39 ± 1) µm, (9.82 ± 2) µm and (9.76 ± 1) µm were the width of pollen grains of control, Neem oil (Azadirachtin), and Agroplus (*Ocimum tenuiflorum*) respectively. The distance between two spines were (22.28 ± 1) µm, (20.03 ± 3) µm and (22.73 ± 1) µm for control Neem oil (Azadirachtin) and Agroplus (*Ocimum tenuiflorum*) respectively (Figure 16, 17 and 18). No spines were observed for the pollen grains treated with chemical pesticides Tatamedia (Imidacloprid) and Saaf (Mancozeb) (Figure 19 and 20).

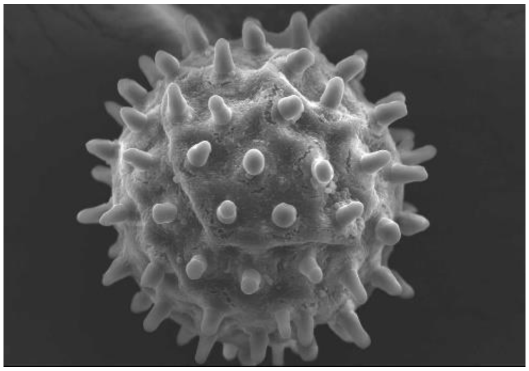

Figure 16: Pollen grain treated with Control solution

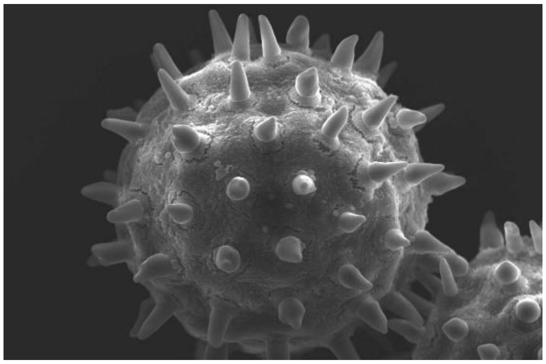

Figure 17: Pollen grain treated with Azadirachtin

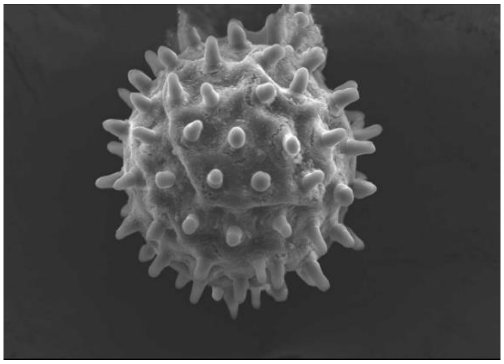

Figure 18: Pollen grain treated with *Ocimum tenuiflorum*

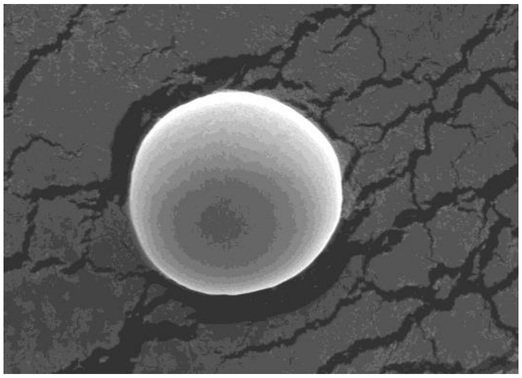

Figure 19: Pollen grain treated with Imidacloprid

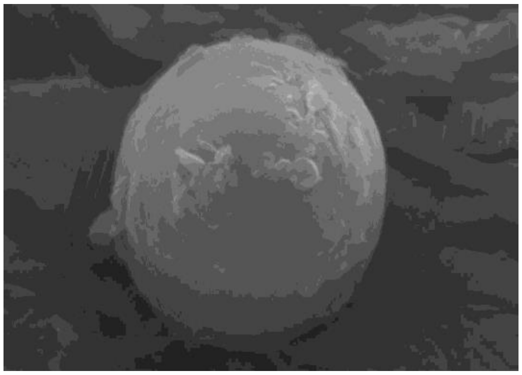

Figure 20: Pollen grain treated with Mancozeb

Sl. No.	Treatment	Equatorial (µm)	Polar (µm)	E/P ratio (µm)	Spine length (µm)	Spine width (µm)	Distance b/w two spines (µm)
1	Control	124.18 ± 1	123.74 ± 1	1.004	18.35 ± 3	8.39 ± 1	22.28 ± 1
2	NEEM OIL (Azadirachtin)	128.84 ± 1	121.41 ± 1	1.064	20.40 ± 2	9.82 ± 2	20.03 ± 3
3	AGROPLUS (*Ocimum tenuiflorum*)	140.36 ± 1	139.41 ± 1	1.007	18.41 ± 2	9.76 ± 1	22.73 ± 1
4	TATAMEDIA (Imidacloprid)	33.05 ± 1	29.45 ± 9	1.222	-	-	-
5	SAAF (Mancozeb)	12.42 ± 1	11.30 ± 1	1.099	-	-	-

Table 6: Results of SEM analysis on the pollen grains treated with Chemical and Biological pesticides

DISCUSSION

In the present work the morphological features of pollen grains, germination and viability, advantages and disadvantages on the application of chemical and biological pesticides were focused.

MORPHOLOGICAL FEATURES

The characteristic features of pollen grains mostly comprise on their size and shape, the number and positions of the germinal apertures and the sculpturing of the exine (Wodehouse, 1928). The pollen grains of *Abelmoschus esculentus* L. were spheroidal, porolate, pantoporate and isopolar in nature with an echinate sculpture. The exine of pollen grains was composed of sexine and nexine. Spines were evenly distributed over the surface of the grain and with spines, ranges a size of 120 - 140 µm. Pore number varies from 22 - 45, with large pores, 6 - 9 µm in diameter and annulate.

GERMINATION AND VIABILITY OF POLLEN GRAINS

The germination and viability of pollen grains are vital for studies of reproductive biology (Soares *et al.*, 2008). If the diameter of the pollen tube was higher than, the diameter of pollen grains, were considered viable in the germination media. Both germination methods and non germination methods were studied to understand the germination and viability of pollen grains. In the germination methods sucrose solutions of different concentrations were studies and 15% was found to be accurate for the germination of pollen growth and the length of the pollen tubes, were double the diameter of pollen grains.

Based on the sucrose solution method, 15% concentration of sucrose was selected for the analysis of Brewbaker's and Kwack's media which is commonly used for pollen grain germination studies. The pollen tubes were germinated and viability was tested. In both germination and non germination methods, the viability of pollen grains retained up to 48 hours in *in vitro* condition. The viability can be retained up to 24 weeks, using hanging drop method. Khan and Perveen (2006), emphasized that, the germination and viability of pollen grains and its application in plant breeding, conservation, adaptation and understanding the physiological behaviors and potency in genetic transformation. They also stressed on the germination capability of *Abelmoschus esculentus* L., for 48 weeks in different storage conditions.

The non germination methods also indicated the viability check with the difference in color change of the pollen grains. Those which were viable took the red color and non viable were colorless in Acetocarmine test. In Iodide Potassium test the viable and non viable become blue and pale color respectively. The change of color in the pollen grains was due to the difference in their cytological structure.

GERMINATION AND VIABILITY OF POLLEN GRAINS TREATED WITH CHEMICAL AND BIOLOGICAL PESTICIDES

The pollen grains treated with biological pesticides Neem oil (Azadirachtin) and Agroplus (O. *tenuiflorum*) showed maximum germination percentage in Brewbaker's and Kwack's medium. The pollen tube growth was also exhibited maximum in biological pesticides. The pollen grains treated with chemical pesticides exhibited a low germination percentage and pollen tube germination in the media when compared to the control solution.

The viability of the pollen grains treated with biological pesticides were similar to that of control. It was evident that the of use these biopesticides were not harmful for their growth. But the viability of pollen grains treated with chemical pesticides were reduced.

TREATMENT ON CHEMICAL AND BIOLOGICAL PESTICIDES

In scanning electron microscopy (SEM) the differences arose with the use of chemical and biological pesticides were evident. The pollen grains treated with chemical pesticides like Tatamedia (Imidacloprid) and Saaf (Mancozeb) showed drastic change in their structure when compared to biological pesticides and control. In the SEM analysis it was evident that the size, shape and ornamentations of the pollen grains were changed with the use of chemical pesticides. The spines were totally extinct from the surface of both pollen grains which were chemically treated. On the other hand, the pollen grains which were biologically treated were much more alike to control. In specific the pollen grains treated with Neem oil (Azadirachtin) was more operative.

Studies specified that, the extreme use of pesticides was based on different motives, which includes the unawareness of the farmers, accessibility of low priced pesticides, and unlicensed supplies, hence, the management process for controlling pesticide practice requires intrusion for quality improvement. Pesticides resistance is a result of elevated dosage that can also lead to aggravating environmental contaminations. Pesticide has massive unfavorable consequences for the environment, including higher poison resistance in arthropods that, in some cases, has caused the whole pest (including carriers) control operation to fail, resulting in huge economic loss and hygienic damages (Khayatnezhad and Nasehi, 2021).

Baig *et al.,* (2012), stated that chemical pesticides like Imidacloprid has the potential to percolate up to the groundwater levels. And these chemicals can cause severe gastrointestinal symptoms along with respiratory diseases and neuropsychiatric features (Kumar and Kumar, 2013). It was supposed that the level these chemicals decreases with the plant growth and time of flowering. Yet their presence seemed significant in the upper part of plants like flower, nectar and pollen when studied with decrease in the non target insects like honey bees (Bonmatin *et al.,* 2005).

The bioaccumulation of Mancozeb had caused aggressive health effects in animals and humans. It had also led to low fecundability, miscarriage, preeclampsia, polycystic ovary syndrome (PCOS), endometriosis, and alterations in the menstrual cycle. This negatively affect female reproductive capability both in adulthood and during embryonic development (Bianchi *et al.,* 2020). In the analysis, the pollen grains treated with Mancozeb displayed to be more globular without any sculptures along with few depositions of the chemicals on their surface. Chemicals used in the pesticides have both direct as well as indirect consequences on human health and environment. Global demand was for the safe, non toxic, nutritious food products. These products can be obtained only by good agricultural practices (Meena and Mishra, 2020).

The treatment with neem extracts, especially Azadirachtin, in various concentrations was effective to improve seed quality (Manjula *et al.,* 2021). The treatment with Neem oil (Azadirachtin) on pollen grains were effective when compared to control and chemical pesticides. The application of biopesticides can improve and restore the fertility of the soil and guarantee the sustainable agricultural production using green technology. Using biopesticides can diminish the demand for energy and consumption of chemical fertilizers and restore the productivity of agroecosystems and wastelands (Kumar *et al.,* 2021).

SUMMARY AND CONCLUSION

The present study was a comprehensive attempt to evaluate the effects of chemical and biological pesticides on the pollen grains of *Abelmoschus esculentus* L. The plant saplings of *Abelmoschus esculentus* L. were collected from VFPCK and was grown for flowers and matured flowered were collected before anthesis. To prevent contamination, they were bagged. Pollen grains were taken carefully by removing the petals of the collected flowers. The collected pollen grains were stored in refrigerator for prolonged used. The pollen grains were subjected to modified acetolysis method for the morphological studies of pollen grains under light microscope. Pollen grains were observed at different magnifications to study and understand the features which make them distant from other families.

Spheroidal, porolate, pantoporate and isopolar pollen grains with spines, ranges a size of 120 - 140 µm were observed under microscope. The pores were large and number varies from 22 - 45 with 6 - 9 µm in diameter. The exine showed sculptures with long, straight, sharp and pointed spines to the apex with a few blunt ones.

The germination and viability of pollen grains were examined by means of germination and non germination methods which included sucrose method, Brewbaker's and Kwack's media analysis, agar media, Acetocarmine and Iodide Potassium tests. This study depicts that, the germination percentage of pollen grains and viability of pollen grains in *in vitro* and *in vivo* conditions were noted to be 48 hours.

The germination and viability of pollen grains treated with biological and chemical pesticides were also recorded. The pollen grains treated with biological pesticides showed elevated results on both germination and viability. Treatment of chemical and biological pesticides on pollen grains were assessed using SEM analysis. The morphological variations arose due to their applications and changes were examined and were noted. The application of Neem oil (Azadirachtin) was effective in maintaining the pollen grains. While, the other pesticides comparatively exhibited a negative impact on the pollen grains.

The study exhibits the morphological variations brought due to the application of chemical pesticides. They adversely affect the structure of the pollen grains. The sexine of the pollen grains completely detaches off and the spheroidal structure became round and smaller in size. The studies highlight that, the use of chemicals like Saaf (Mancozeb) and Tatamedia (Imidacloprid) can infiltrate into the ecosystem and effects all forms of life and from the structural studies the effects on pollen grains were evident. The use of biological pesticides was more beneficial because they do not harm the ecosystem and life forms associated with it, without negative impacts on the pollen structure. They are also studied to have least effect on the other necessary organisms associated with plant growth.

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