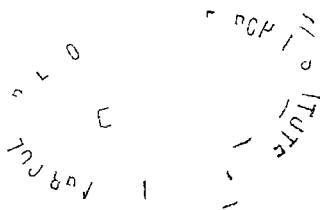


STUDIES ON THE EFFECT OF F W-450 ON  
FLOWERING, POLLINATION AND FRUIT SET  
IN BRINJAL (*Solanum melongena* L)

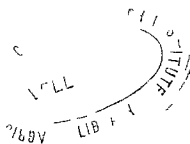


By  
LEELAMMA MATHEW, B. Sc

THESIS  
SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE IN AGRICULTURE  
(CYTOGENETICS AND PLANT BREEDING)  
OF THE UNIVERSITY OF KERALA

DIVISION OF AGRICULTURAL BOTANY  
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE,  
VELLAYANI, TRIVANDRUM

1965



C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Smt. Leelamma Mathew under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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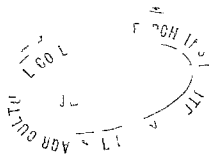
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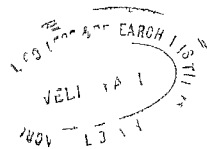


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**I N T R O D U C T I O N**





## INTRODUCTION

Since the dawn of agriculture, one of man's principal aims has been the promotion of plant growth. With the advancement of various vital phenomenon of plant life the importance of plant growth regulators came to be emphasised more and more. The practical utilization of chemical gametocides though it is in its infancy, has attracted the master minds of plant breeding all over the world. Gametocide is a newly coined term to describe the action of a chemical growth regulator which inhibits gamete development. Certain deformities and malformations following the chemical treatment have not handicapped the practical utilization of induced male sterility in certain crop plants.

In recent years exploitation of hybrid vigour or heterosis by intervarietal hybridisation has become a very promising line of economic production in a number of crop plants. Heterosis in brinjal was observed by various investigators both in India and elsewhere, in respect of a number of characters. But the presence of hermaphrodite flowers with a large amount of pollen per

anther rendered the process of emasculation costly and laborious. Careful emasculation and pollination of flowers on an extensive scale therefore became obligatory. Any kind of male sterility can eliminate this kind of cumbersome work and can facilitate the technique of crossing to make the maximum utilization of hybrid vigour or heterosis.

The numerous instances of male sterility occurring among crop plants in nature, without any direct relation to ovule sterility indicate that it may be possible to reproduce this situation artificially. Many male sterile mutants have been identified. The selective abortion of pollen by F.W.450 was reported by many authors. This phenomenon of selective male sterility may be useful in the production of hybrid seed in a great variety of crop plants. Thus the tedious process of hand emasculation is gradually getting replaced by the simpler but, more efficacious application of chemical gametocides.

Brinjal is an important fruit vegetable cultivated and consumed extensively in South India. As the utilization of improved hybrids in brinjal is likely to contribute to the substantial increase in production, a

preliminary understanding of the effect of certain well known chemical gametocides in this crop has been considered worth exploring. Hence the assessment of the effect of F.W.450 on inducing male sterility in brinjal has been the object of the work reported here.

**REVIEW OF LITERATURE**

## REVIEW OF LITERATURE

### I. Floral Biology

#### Types of clusters and flowers.

Most Brinjal varieties bear solitary fruits, while a few produce clusters of fruits up to four. Cluster bearing varieties naturally produce many flowered cymes. But single fruited varieties also commonly produce multi-flowered cymes. Often both solitary blossoms and cymes occur on the same plant.

Concerning the inflorescence Smith (1931) observed that some varieties rarely bore more than one flower per inflorescence. According to Shoemaker (1934) flowers were solitary or in clusters of two or more. Thompson and Kelley (1957) also reported that the flowers were solitary or in clusters of two or more.

Smith (1931) found that the individual flowers in the inflorescence were either long styled or short styled and that fruit set was related to style length viz., long styled flowers set fruits, while the short styled flowers aborted. The length of the style had been found by many

workers to be an important character in brinjal with regard to proper pollination and fruit set. Smith (1931) and Magtang (1936) grouped the flowers of brinjal into two classes, with regard to the position of the stigma in relation to the anther tips viz., (1) long styled flowers with the stigma protruding well above the anther tips, and (2) short styled flowers with the stigma lying between the anther tips. Smith (1931) also reported that solitary flowers were long styled while in the inflorescence the lowermost flower only had a long style and others had short styles. Magtang (1936) noted that flowers produced early and late in the season were short styled irrespective of their position on the inflorescence.

Pal and Singh (1934) in their observation with the variety Muktakezi further classified the short styled flowers into true short styled and pseudoshort styled based on the measurements of styles. According to Pal and Singh (1946) pseudoshort styled flowers set fruit with hand pollination.

Krishnamurthi and Subrahmanian (1954) reported a new category viz., medium styled flower in which the stigma was at the level of anther tips and which was capable of fruit set under natural conditions.

Cariappa (1961) found that the long styled and medium styled flowers alone produced fruits while pseudo-short styled and short styled flowers failed completely. The long styled flowers gave a higher set than medium styled flowers.

## II. Pollen studies

As a general rule the pollen grains serve best in distinguishing between and showing relationship among the higher groups of plants such as families, tribes, genera and sometimes species.

A great deal of work had been done on the morphology of pollen grains by investigators all over the world. Majority of pollen grains have got an inner wall called intine and an outer wall called exine. The outer surface of the exine may be sculptured, reticulate or smooth and with or without spines. On the surface of the pollen grains furrows may or may not be present. Wode House (1935), Lang (1937) and Erdtman (1954) studied the pollen morphology of Solanaceae and reported that they are round and may be 2-3-5-6 colporate.

### Pollen sterility.

The sterility of pollen grains had been studied by two methods.

- (1) By staining methods.
- (2) Germination in artificial media.

#### (1) Staining methods.

Zirkle (1937) had described a method for testing the viability of pollen grains in acetocarmine preparations. Pollen grains were dusted on a slide containing one or two drops of acetocarmine and mounted. They were studied after 15 minutes when the grains were properly stained. The grains which had been stained well and looked plump and normal were taken as viable. Unstained and shrivelled ones were considered as non-viable or sterile. The same technique had been used by many workers to find out the percentage of viability of pollen of various other crops.

Vietez (1952) used 2, 3, 5 triphenyl tetrazolium chloride for testing the viability of maize pollen. The best results were obtained, when the test was carried out at 50°C using a 2% solution. Oberle and Watson (1953) found that this method on peach, apple, pear and grape



pollen was ineffective. Jacopini (1954) recommended the treatment of pollen grains with 2% sodium biselenite for periods ranging from  $\frac{1}{4}$ -2 hours, depending on the species proved a rapid reliable means of determining pollen viability in stone and pome fruit trees. Grains with full germinative power turned pale yellow while non-viable grains did not change.

King (1959) recommended a peroxidase agar medium. Here the viable pollen grains could be recognised by their colourless swollen appearance while non-functional (sterile) grains became blue and did not swell.

Ostapenko (1956) by his experimental studies on the validity of pollen grains of various crop plants questioned the validity of the various staining methods. According to him these methods might be regarded as only of relative value in determining pollen viability.

#### Germination in artificial media.

There are experimental evidences to show that pollen grains can be grown in artificial media containing sucrose solutions. The concentrations of sucrose required by the pollen grains to germinate, varies between plants of different species and even within the same species.

Adams (1916) during his investigations on germination of pollen grains of apple and other fruit trees found that best pollen germination was obtained in apple in 2.5 - 10% cane sugar solution, in pear in 4-8% solution and in straw berry in 8% solution. In rasp berry and black berry best germination was obtained in 16% sugar solution. Kabel (1926) germinated pollen grains of certain fruit trees in sucrose solution and reported an optimum concentration of sucrose solution of 5% for quinces, 10% for peaches and apricot, 10-15% for plums and pears, 5-15% for apple and 15% for cherries. Dikshit (1956) found that loquat pollen germinated successfully in 1% sucrose solution. Singh (1956) studied the pollen grains of 10 varieties of peach and reported that there is a varietal variation for requirements of sucrose solution for successful germination of pollen grains.

Various workers reported that Boron can induce pollen germination and pollen tube growth in various crops. This chemical was found to occur in the cells of the pistils. Schumucker (1935) studied the pollen grains of various crops and discovered that Boron as borate was a stimulant to pollen germination and tube growth in many species. Thompson and Batjer (1950) studied the pollen of different fruit crops and found that Boron in low

concentrations 2.5-40 ppm. stimulated the pollen germination and pollen tube growth, but had a reverse effect with higher concentrations. Raghavan and Baruah (1956) reported that arecanut pollen grains cultured in sucrose medium showed a considerable degree of pollen germination and pollen tube growth. Munzner (1960) found that 0.001 to 0.01% of Boric acid had a stimulating effect on pollen germination and pollen tube growth in more than sixty angiosperm species. Singh (1960) obtained an increase in pollen germination and tube elongation in mango with addition of 20 ppm. Boric acid.

Addition of solidifying materials like agar and gelatin were used from the earlier days for the preparation of culture media for pollen grains. Iyengar (1939) used an agar-sugar medium for artificial germination of pollen grains of cotton. Johri and Vasil (1955) got an increase in the percentage of pollen germination of cotton on the above medium, when Boric acid was added to it. Agarwal, Khanna and Singh (1957) found that 1% agar medium containing 6% sucrose gave best results with pollen of Momordica charantia.

Balakrishnan (1962) reported that tomato pollen was found to germinate well in 15 and 20% sucrose

solution with addition of 100 ppm. Boric acid. Vilasini (1963) studied ten varieties of Hibiscus rosasinensis and recommended a suitable medium of 45% sucrose, 100 ppm. Boric acid and 1 g. of agar. Nair (1964) with a view to standardise a suitable medium for Bhindi pollen obtained a medium of 20% sucrose solution with 2% agar which gave the best results and was selected the solid medium for the study of germination of treated pollen grains.

#### Chemicals used for induction of male sterility.

Several attempts have been made to induce selective abortion of pollen grains through treatments with various chemicals. Some of the chemicals used for functional male sterility are reviewed and presented.

Various crop plants like wheat, tomato and onion were subjected to the studies on the chemical induction of male sterility by Chopra et al (1960) at I.A.R.I.

In wheat M.H. was used at 50, 100, 250 and 500 ppm. on 37 day old plant by spray treatments. Of which 250 ppm. and 100 ppm. were fairly effective in causing pollen sterility. With an increase in the frequency of spraying, the effect of the dosage is increased. The anthers were shrunken with shrivelled pollen.

Tomato plants were sprayed with aqueous solutions of M.H., Uracil, Thymine, Yeast, Nucleic acid and Triiodobenzoic acid (TiBA).

In onion aqueous solutions of Maleic hydrazide, Uracil, Thymine, Nucleic acid, Sodium nucleate and Triiodobenzoic acid were injected into the inflorescence prior to the onset of meiosis.

In all these cases none of the treatments tried gave complete pollen sterility, coupled with ovular fertility.

Robert et al (1961) reported 2, 4-D as a pollenicide on grape variety, Tokey. On dipping the flowering clusters in solutions of 2, 4-D, the number of seedless berries increased. This was supposed to be due to the injurious effect of 2, 4-D to pollen germination or in other words due to the action of a pollenicide.

Choudhury and George (1964) obtained pollen sterility of 90-100% lasting from 5-12 days in 2 varieties of brinjal by spraying the whole plant with M.H., N.A.A. and 2, 4-D. Spraying with 2, 4-D at concentration higher than 10 ppm. caused elongation, curling and cracking of tender parts of stem. N.A.A. induced the conversion of stamens

into miniature pistils, indicating some hormonal action in sex determination. M.H. at concentrations higher than 400 ppm. interrupted apical dominance.

F.W. 450.

The chemical gametocide F.W. 450 inhibits gamete development. This chemical induces functional male sterility in some crops without adversely affecting the female sterility. It has the composition of sodium 2,3 dichloroisobutyrate.

F.W. 450 is water soluble salt and should be considered essentially cent percent active. It is readily absorbed from foliar application of aqueous sprays. Translocation is both upward and downward, but tends to be greater towards new and rapidly growing tissues such as new leaves and especially to flowers and floral buds. Observations on spray timing with most plants indicate that F.W. 450 is probably active during the reduction division process in the formation of pollen from the pollen mother cell. The chemical accumulates to a greater extent in the anthers than in the ovules. This chemical gametocide is still under experiment and has not yet attained commercial importance.

Chemically induced male sterility in certain crop plants by F.W. 450 are the following.

Cotton:

F.W. 450 has been tested more extensively on cotton than on any other crop. Eaton (1957) studied the effect of F.W. 450 on cotton. The Empire cotton plants were found to produce no pollen grains after they had been sprayed with 1.2% solution of F.W.450. But when the flowers were hand pollinated with pollen from untreated plants normal bolls with viable seeds developed. It was indicated that the sodium salt of this chlorinated organic acid was freely absorbed by cotton plants and remains active and mobile for a long time. Here, male sterility has been effective by a late dehiscence of anthers.

Hilton (1958) observed that the chemical prevents the development of pollen grains without adversely affecting the female fertility when applied to plants at low concentrations. Pate and Duncan (1960) conducted an experiment to study the effect of F.W.450 at several treatment levels caused male and female sterility with no marked selectivity for either gamete. The results indicated that F.W.450 at the rates used in their

experiment were not suitable for use as a selective male gametocide in commercial production of hybrid seed cotton, except 0.203% which gave a selective sterility for male gametes.

Investigations of Richmond (1961) on the effect of F.W.450 having concentrations 0.25% and 0.4% showed that the four varieties of American upland cotton tested, responded similarly to the treatments and significant effects were induced in all characters except number of flowers per plot. From the data on out crossing and on boll setting in open and self pollinated plots, it was concluded that the gametocide was capable of inducing a certain amount of sterility.

Singh and Sehgal (1961) conducted a preliminary study on the effect of F.W.450 as a selective male gametocide in American cotton. The treatment resulted in a high degree of male sterility which was at its peak in third and fourth weeks from the date of application of the gametocide. But in any of the treatments or concentrations used the gametocide did not show selective action against male gametes.

Bhardwaj and Santhanam (1961) laid out a trial with *Gossypium arboreum* to determine the efficiency of the



chemical gametocide F.W.450 in producing male sterility in Asiatic cottons. The gametocide was sprayed at weekly and fortnightly intervals. The results indicated that 0.2% concentration of the gametocide sprayed six times at weekly intervals from the eleventh week after sowing induced cent percent pollen sterility during the fifth and the seventh weeks after the initial spray. However the gametocide did not appear to be highly selective and damage to female gametes also was encountered, resulting in reduced seed set per boll.

Singh and Sehgal (1964) studied the usefulness of this gametocide in producing hybrid seed in the Punjab-American 320 F variety. A higher percentage of male sterility was induced, effecting partial female sterility, resulting in a reduction in number of seeds per boll.

Ter-Avanesjan and Semenova (1964) observed the effectiveness of dichloroisobutyric acid for inducing male sterility in Gossypium hirsutum, G. barbadense, G. herbaceum, G. arboreum and numerous commercial varieties. Early maturing strains of all species were more sensitive to the treatments than late maturing forms.

The tests of Semenova (1964) with a range of gametocide on different Gossypium species showed

dichloroisobutyric acid to be effective in producing male sterility in breeding interspecific cotton hybrids. The best concentration being different for different species.

Tomato.

Moore (1959) conducted an investigation with F.W. 450 in tomato under green house and field conditions. Concentrations of F.W.450 ranging from 0.02% to 1.2% were used. Complete male sterility was induced for 10-13 days without causing female sterility at concentrations of 0.075% and 0.15% under green house conditions. In field trial four concentrations 0.075%, 0.15%, 0.3% and 0.6% were used with three subsequent applications. The lower concentration failed to produce male sterility. 0.15% produced a high percentage of sterility for 13 days with slight reduction in female sterility. The concentration 0.3% produced complete absence of pollen for 12 days, from 12 days after treatment and female fertility was also satisfactory. The highest concentration 0.6% gave complete male sterility for 19 days beginning 12 days after treatment. Pollen production was normal 37 days after application. No fruits were set when normal pollen was applied between 15 and 22 days after treatment to the

flowers of plants treated with 0.6% concentration. This was taken to indicate female sterility. Female sterility was again normal 37 days after treatment.

Balakrishnan (1963) also recorded the effect of F.W.450 on tomato. Three varieties were tried with concentrations of 0.15%, 0.3% and 0.6%. Complete male sterility was reported to have been induced for concentrations 0.3% and 0.6%. 0.15% produced fairly high male sterility for 13 days. But pollen was never completely absent. The concentrations of 0.6% and 0.3% were efficient in completely arresting the pollen production for 12 and 19 days respectively, but produced deleterious effects on plants, by causing yellowing of leaves and retardation of growth. Among concentrations used 0.6% and 0.3% proved most effective. The percentage of fruit set was considerably reduced in those concentrations. The treated plants seemed to recover in 20 days after the treatment.

#### Alfalfa.

Pederson (1959) conducted an experiment on the effect of F.W.450 on alfalfa using the concentrations of 0.5 and 1.0%. He obtained only 10% reduction in male

sterility and a reduction of 60% female fertility. There was also a marked reduction in flowering.

Miller and Hittle (1960) failed to get a significant result from the study of the effect of F.W.450 on alfalfa. Aqueous solutions sprayed at early bud and early anthesis stages had a stimulating effect on male and female fertility at low concentrations and inhibited the fertility of both at higher concentrations.

#### Sugar beets.

Preliminary green house and field experiments were conducted at Colorado by Dudley (1960) to determine the effectiveness of the chemical gametocide F.W.450 as a selective gametocide in sugar beets. The results indicated that the optimum concentrations of the chemical in the field was 1/3% at a carrier rate of 100 gallons per acre at the pollen mother stage. Such an application delayed pollen shedding by 7-10 days without seriously affecting seed yield or subsequent germination. A study in green house conditions indicated that the chemical control of pollen formation offers an effective method of forcing certain self fertile inbred lines of sugar beet to hybridise.

Rubenbauer and Schultis (1960) applied F.W.450 on sugar beets with varying concentrations. In control tests the progeny of the sugar beets contained 79.3% fruit set while in treated plants, the reduction in fertility was to 30.5% with 3% solution and it reaches up to 6% when the same concentration was applied twice to the plant. The treatment recommended is two sprayings with solutions of 0.5-1.5%.

#### Water melon.

Henz and Mohr (1959) tested the effect of F.W.450 on water melon. The initial spraying was done as the first pistillate blossoms appeared and repeated after six days. Here the chemical prevented the opening of the mature staminate buds. The effect of F.W.450 in preventing the opening of staminate buds appeared to be localised in the tip of the bud. Pinching of the tip permitted the immediate unfurling of petals. Anthers dehisced with production of apparently normal pollen inside mature unopened staminate buds. No deleterious effects upon female fertility were associated with the application of F.W.450.

Boswell (1960) reported the same experiment on water melon using concentrations of 0.6%, 0.12%, 2.0% and 2.5% or 1, 2, 3 and 4 lb. per acre respectively. The 1 lb. per acre rate was slightly phytotoxic and prevented the male flowers from opening for a period of 7 to 10 days, the 2 lb. per acre rate caused more foliar injury, the staminate failed to open for approximately 7 to 10 days after treatment. 3 and 4 lb. per acre rates caused severe injury and killing of leaves.

#### Bhindi.

Nair (1964) carried out investigations to induce male sterility in Bhindi, with F.W.450 at 0.1%, 0.15%, 0.2%, 0.25% and 0.3% concentrations. The chemical was applied at three stages of plant growth viz., 30th, 37th and 44th day after planting. A high percentage of sterility was induced in almost all concentrations at all stages of application. Increase in sterility was obtained by an increase in concentrations and a decreasing tendency from the early to the advanced stages of application. The maximum sterility was obtained in plants treated with 0.25% and 0.3% at the first stage of application. The higher concentrations showed a reduction in fruit setting.

Wheat.

Chopra, Jain and Swaminathan (1960) tried the chemical F.W.450 on wheat at I.A.R.I. But failed to get any positive result. Wheat plants were sprayed once or twice with 0.25%, 0.5%, 0.7% and 1% aqueous solutions of the chemical. Plants sprayed with lower concentrations were stunted and grass like in appearance. Plants survived showed a delay in flowering, small earheads with shorter awns and tougher glumes. The pollen grains were normal as regards their size, shape and stainability.

Maize.

Diaz Robles (1959), with a view to standardising suitable techniques for inducing male sterility, tried the chemical gametocide F.W.450. At 0.1-1.5% concentrations white spots appeared on the leaves. At 2% larger spots were produced. At 2.5-3% growth ceased, tassels and ears showed abnormal development, and pollen sterility was about 20-30% and at 4% and above, all plants died.

Rye.

Wit (1960) failed to get any response of F.W.450 for vegetative growth, flowering or even inducing male sterility in the plant.

## **MATERIALS AND METHODS**



## MATERIALS AND METHODS

The present investigation was carried out in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani, Trivendrum during the year 1964-65.

### A. MATERIALS

#### (1) Seed material.

Brinjal (Solanum melongena L.) varieties Pusa Purple Long and Pusa Purple Round were selected for studies. The seeds were obtained from I.A.R.I., New Delhi. Viability as well as the percentage of germination of the seeds were tested before being used for investigation.

#### (2) Chemical.

The chemical used was F.W.450 (Sodium 2, 3 dichloroisobutyrate). Aqueous solutions of 5 concentrations viz., 0.1%, 0.15%, 0.2%, 0.25% and 0.3% at 3 stages were tried.

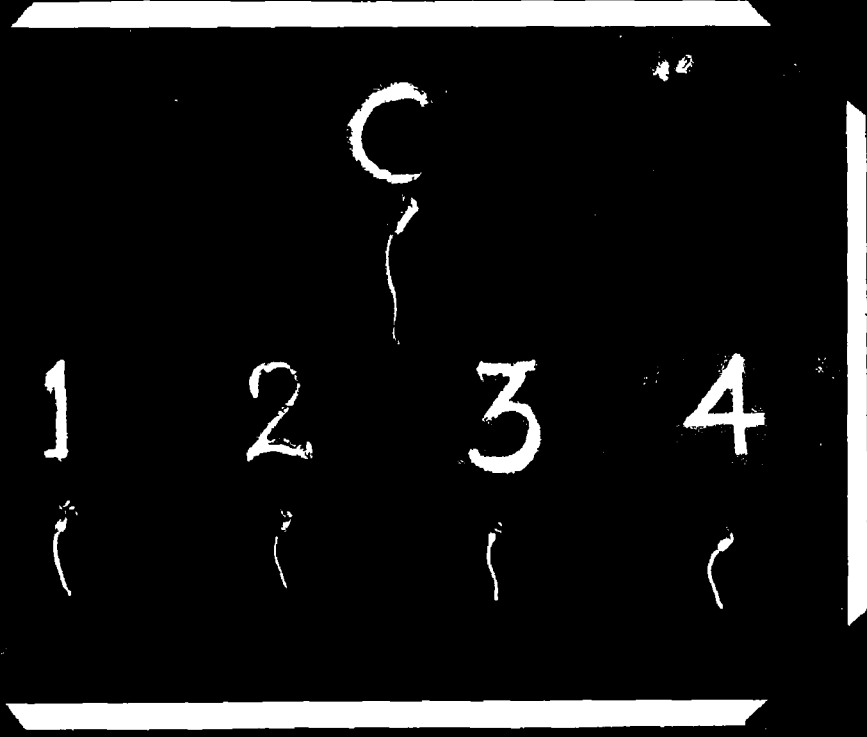


PLATE XI

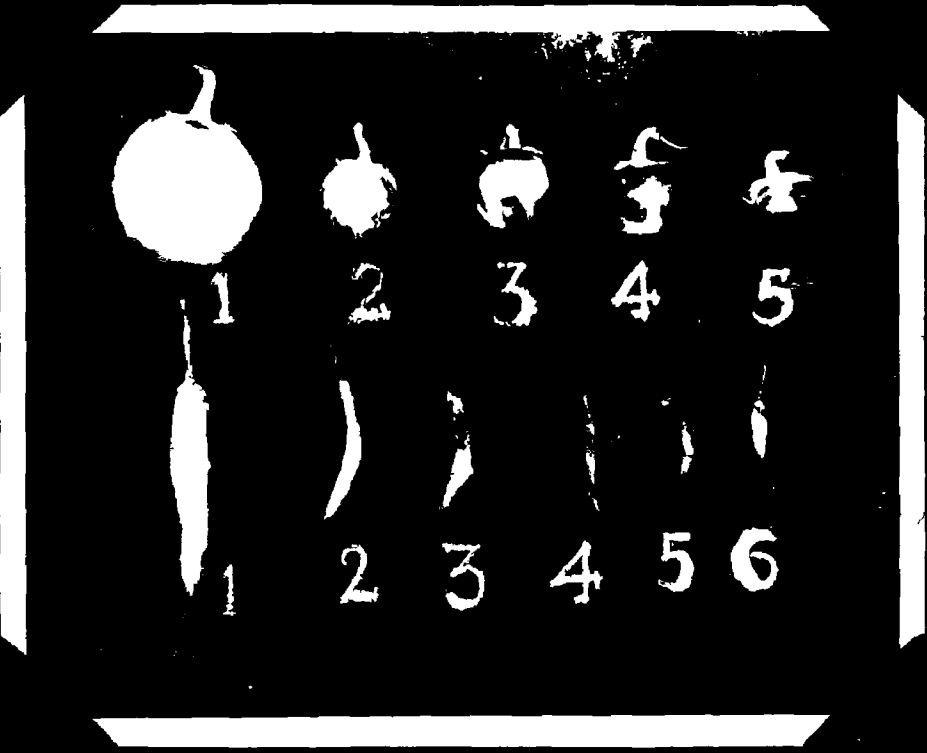


PLATE XII

(3) Treatments.

Varieties, different concentrations of the chemicals, and the stages of its application were denoted by the following symbols.

(i) Varieties.

Variety I. Pusa Purple Long - I<sub>1</sub> (Control)

Variety II. Pusa Purple Round - I<sub>2</sub> (Control)

(ii) Concentrations.

K <sub>1</sub>	-	0.1%
K <sub>2</sub>	-	0.15%
K <sub>3</sub>	-	0.2%
K <sub>4</sub>	-	0.25%
K <sub>5</sub>	-	0.3%

(iii) Stages of application.

M <sub>1</sub>	-	First stage of application.
M <sub>2</sub>	-	Second stage of application.
M <sub>3</sub>	-	Third stage of application.

Treatments 1 and 2 were the controls. Other treatment combinations were

(3) $L_1 M_1 K_1$	(13) $L_1 M_2 K_1$	(23) $L_1 M_3 K_1$
(4) $L_1 M_1 K_2$	(14) $L_1 M_2 K_2$	(24) $L_1 M_3 K_2$
(5) $L_1 M_1 K_3$	(15) $L_1 M_2 K_3$	(25) $L_1 M_3 K_3$
(6) $L_1 M_1 K_4$	(16) $L_1 M_2 K_4$	(26) $L_1 M_3 K_4$
(7) $L_1 M_1 K_5$	(17) $L_1 M_2 K_5$	(27) $L_1 M_3 K_5$
(8) $L_2 M_1 K_1$	(18) $L_2 M_2 K_1$	(28) $L_2 M_3 K_1$
(9) $L_2 M_1 K_2$	(19) $L_2 M_2 K_2$	(29) $L_2 M_3 K_2$
(10) $L_2 M_1 K_3$	(20) $L_2 M_2 K_3$	(30) $L_2 M_3 K_3$
(11) $L_2 M_1 K_4$	(21) $L_2 M_2 K_4$	(31) $L_2 M_3 K_4$
(12) $L_2 M_1 K_5$	(22) $L_2 M_2 K_5$	(32) $L_2 M_3 K_5$

## B. METHODS

### 1. Lay out.

A randomised block design, consisting of 32 treatments with 4 replications, was selected. 3 replications for breeding trials and one for pollen analysis. There were altogether 128 pots which were arranged in 4 blocks with 32 pots in each. A spacing of 3'x3' was provided.

**Fig. 1**

**Layout of the Field experiment**

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REP. III

7	16	32	19
18	4	6	25
27	1	22	23
14	3	15	17
24	30	26	21
13	29	9	31
28	11	12	8
2	5	10	20

REP. I

3	23	8	6
26	17	15	18
11	21	32	29
7	28	27	5
1	13	20	22
25	12	9	19
4	31	24	10
16	14	30	2

REP. II

12	24	3	28
4	14	11	21
7	29	6	17
31	23	32	22
26	13	16	18
27	20	2	30
15	8	1	5
10	25	9	19

REP. IV

23	27	15	4
16	29	1	26
10	24	22	28
17	11	30	8
7	25	13	12
19	21	14	5
20	18	3	2
6	32	31	9

## 2. Sowing.

Seeds of the two varieties of brinjal were sown in separate seed pans on 10-10-1964. From here healthy seedlings were transplanted in 12"x12" pots.

## 3. Preparation of pots and transplanting.

Identical pots of 12"x12" were filled with equal quantity of potting mixture. Healthy seedlings were selected and transplanted on 10-11-1964 i.e., one month after sowing.

## 4. Treatments.

Applications of the chemical were carried out with the help of an atomiser operated by connecting to a pressure pump and using water as the medium.

The first spraying was given at the onset of flower production and anthesis and subsequent sprayings were given at an interval of two weeks. In all cases at all times it was done with extreme care to obtain a thorough uniform wetting of the plants especially the terminal buds.

## 5. Characters studied.

The following characters were studied and recorded from individual plants.

### I. Floral Biology.

Number of clusters and flowers.

### II. Pollen studies.

(a) Pollen morphology.

(1) Pollen shape.

(2) Pollen size.

(b) Pollen sterility.

(1) Acetocarmine staining method.

(2) Germinating pollen grains in artificial media.

### III. Fruit set.

(1) Number of fruits per plant.

(2) Selfing.

(3) Crossing.

(4) Size of fruits.

(5) Weight of fruits.

### IV. Seed sterility.



## I. Floral biology

### Number of clusters and flowers

In order to study the effect of the chemical on clusters and flowers produced, the total number of clusters and flowers was recorded. The total number of clusters produced was noted at weekly intervals after the first spraying and the total number of flowers was taken daily.

## II. Pollen studies

### a) Pollen morphology

Pollen morphology was studied two weeks after the treatment. It was done by acetocarmine staining technique. Freshly opened flowers were collected and kept in a desiccator for two hours to obtain pollen grains for study. The shape and size of the pollen grains were studied.

(1) Pollen shape: Anthers were dusted on a slide containing a drop of acetocarmine-glycerine medium. The undehisced anthers were crushed with the help of the blunt end of the needle. It was covered with a cover glass and the shape of the pollen grains was studied.

(2) Pollen size: In order to study the size of the pollen grains an ocular micrometer was used. The micrometer was standardised under the high power of a compound microscope. The size of hundred pollen grains taken at random were measured and the mean diameter of pollen grains was calculated.

b) Pollen sterility.

Sterility of pollen grains was studied by two methods.

(1) Acetocarmine staining method. Mature flower buds which would open next day were covered with a paper bag. Anthers were collected from such flowers and dusted on a slide containing a drop of acetocarmine and covered with a cover glass. After half an hour sterile and fertile pollen grains were counted. Observations were made in ten different microscopic fields and the mean percentage of sterile grains was calculated.

(2) Pollen germination in artificial media. Pollen germination studies were conducted in three stages in order to standardise the culture media.

First stage: For the first set of observations, sucrose solutions of 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%,

22.5% and 25% were prepared. Small drops of sucrose solutions were placed on clean slides. Pollen grains were dusted on it. The drop was stirred with a needle so as to make sure that the surface of the pollen grains was wet and that they were distributed uniformly throughout the solutions. The prepared slides were then inverted and allowed to rest on two small glass rods placed on a damp blotting paper, kept in petri dishes. Germinated and nongerminated pollen grains were counted after 24 hours from ten different microscopic fields.

Three concentrations, depending on the percentage of germination and tube length, were selected and proceeded on to the second stage of experiment.

Second stage: The three concentrations, viz., 15%, 17.5% and 20% which gave the best result in the experiment, were used in combination with 50 ppm., 100 ppm. and 150 ppm. boric acid.

Third stage: Solidification media: The sucrose concentrations which gave the best result i.e., sucrose solution 15% + 50 ppm. boric acid was tried in combination with 0.5%, 1%, 1.5% and 2% of agar.

The standard medium was taken in clean sterile petri dishes. Pollen grains of the two varieties were sown uniformly in the petri dishes.

After 24 hours each petri dish was examined under the low power of the microscope. The number of germinated and nongerminated pollen grains in a microscopic field was counted. In a petri dish ten such countings were made at random and the percentage of germination was determined.

The length of tubes of pollen grains selected at random from each petri dish was measured using a standardised ocular micrometer and the mean tube length was found out.

The combination i.e., 15% sucrose solution with 50 ppm. boric acid and 1 gm. of agar which gave the best result was taken for the studies on pollen germination and tube elongation of the experimental material.

Each treatment was taken in separate petri dishes with a replication of 3 plates for each variety. The germination test in the culture media was done when the maximum sterility was attained in the three stages i.e., 15 days after the treatment in 3 stages. The percentage

of germination and the mean pollen tube growth were calculated as mentioned above.

### III. Fruit set

#### 1) Number of fruits.

Fully developed fruits from individual plants were counted and recorded. The percentage of fruit set was calculated by comparing the number of fruits produced by the control.

In order to study the source of sterility the treated plants were selfed as well as crossed.

#### 2) Selfing.

Selfing was effected by bagging method. The flower buds which open next day were bagged in the previous evening because the normal anthesis in brinjal is very early in the morning. The bags were removed, after one week.

#### 3) Crossing.

The treatments which failed to set fruits on selfing were subjected to crossing. Hand pollination was done by collecting the pollen in a watch glass and pollinating with a camel's hair brush.

4) Size of fruits.

The length as well as the girth of two selfed and two crossed fruits was recorded and the average length and girth of fruits per treatment was calculated.

5) Weight of the fruits.

Two fruits from each treatment were selected and the average weight of fruits per treatment was estimated.

IV. Seed sterility

The seeds were collected from two fruits per plant. The fertile and sterile seeds were sorted out and numbered by taking samples.

Germination of seeds.

Hundred seeds were taken at random and sown in moist filter paper kept in a petri dish. Germination counts were taken from the 4th day after sowing and continued until the 8th day. The final data were taken for analysis.

**EXPERIMENTAL RESULTS**

## EXPERIMENTAL RESULTS

The results of the investigations conducted on the effect of the chemical gametocide F.W.450 on brinjal are presented below.

### I. Floral biology

#### Total number of clusters and flowers

The total number of flower clusters and flowers in individual plants was counted and the data were statistically analysed. The productive and non-productive flowers were sorted out. The summary of the results is given in the Tables I and II. From the tables it can be seen that the treatments gave significantly different results. All the concentrations in the first stage of application gave a considerable degree of reduction in number of clusters and flowers. In the second and third stage of application, the higher concentrations significantly reduced the same.

### II. Pollen studies

#### a) Pollen morphology

The pollen grains were found to be yellow in colour. The shape was more or less similar in both the varieties.



TABLE IMean number of Clusters per plant

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	140.33	140.33	140.33
0.10%	138.67	138.33	138.33
0.15%	135.67	135.67	136.67
0.20%	133.33	134.33	136.33
0.25%	130.33	134.00	135.57
0.30%	127.33	134.00	135.67
<u>Variety II</u>			
Control	40.67	40.67	40.67
0.10%	39.51	36.67	39.67
0.15%	38.67	34.33	38.33
0.20%	38.67	30.33	35.33
0.25%	38.33	26.67	34.67
0.30%	38.33	25.33	34.27

TABLE II

Mean number of Flowers per plant

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	129.33 (46.33)	129.33 (46.33)	129.33 (46.33)
0.10%	125.33 (41.33)	125.67 (43.67)	125.33 (45.33)
0.15%	120.33 (37.67)	123.33 (41.33)	124.33 (45.00)
0.20%	117.00 (35.33)	122.33 (39.67)	123.67 (43.67)
0.25%	115.67 (33.33)	119.33 (37.33)	120.33 (41.33)
0.30%	112.33 (30.67)	117.00 (35.33)	120.67 (40.67)
<u>Variety II</u>			
Control	19.00 (15.67)	19.00 (16.00)	19.00 (15.35)
0.10%	18.33 (16.33)	16.67 (13.67)	18.33 (14.12)
0.15%	17.67 (14.67)	14.33 (11.67)	17.67 (13.82)
0.20%	17.00 (14.33)	13.67 (8.33)	17.33 (12.51)
0.25%	17.33 (12.33)	9.33 (6.33)	17.67 (11.67)
0.30%	17.00 (10.67)	8.33 (5.00)	16.67 (11.43)

Figures in the Parenthesis show the number of productive flowers.

There was no significant intervarietal variation in pollen size. The shape was found to be spherical and 2-6 colporate.

1) Pollen shape. The shape of the pollen grains was a little affected by the chemical. Pollen grains, either shrunken or irregular were observed in treatments which produced sterility.

2) Pollen size. Table III furnishes the summary of the results. Statistical analysis of the data showed a significant difference in pollen size ranging from 30.61  $\mu$  to 45.93  $\mu$ . The size of the pollen is determined by the levels of the chemical used and the stages of application.

The variety I gave a significant reduction in size of pollen at the first stage of application and with the highest concentration (0.3%). In the variety II it was found that the second stage of application was most effective in reducing the size of the pollen. In the case of third stage of application only the higher concentrations (0.25% and 0.3%) gave significant effects.

TABLE III

Size of pollen grains in  $\mu$

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	45.93	45.93	45.93
0.10%	40.85	43.76	45.00
0.15%	40.45	40.17	44.00
0.20%	38.52	40.00	42.00
0.25%	30.78	40.00	42.00
0.30%	30.33	40.00	42.00
<u>Variety II</u>			
Control	46.00	45.00	45.93
0.10%	45.61	44.00	44.33
0.15%	45.50	40.67	44.00
0.20%	45.50	38.33	44.00
0.25%	45.00	39.33	42.00
0.30%	45.00	39.33	42.00

b) Pollen sterility

1) Acetocarmine staining method. The percentage of pollen sterility was calculated from an entirely random sample at weekly intervals starting from first flower opening till the recovery of complete fertility. The calculated pollen sterility is given in the Table IV.

From the data it was understood that the percentage of pollen sterility was highly correlated with the concentrations and the time of application. The percentage of sterility was found to vary every week after treatment. The effect of a particular concentration of the chemical was quicker in the first stage of application. The degree of sterility was found to be decreasing as the stages of application advanced. In all the concentrations a higher degree of sterility was obtained. The highest concentration (0.3%) gave a more lasting effect when compared to the low level of the chemical (0.1%). As the concentrations of the chemical increased there was a proportionate increase in the percentage of sterility.

The variety I showed cent percent sterility in the third and fourth week at the first stage of application. Cent percent sterility was obtained in the case of

Percentage of pollen sterility - Aceto-carmine staining technique

Treatments	Week interval						
	1	2	3	4	5	6	7
<b>I stage of application</b>							
<b>Variety I</b>							
Control	5 08	5 30	5 30	5 30	5 58	6 62	7 00
0 10%	6 00	14 33	96 21	55 84	29 21	10 00	7 12
0 15%	6 42	16 21	100 00	96 41	75 27	10 33	7 00
0 20%	8 92	22 78	100 00	100 00	84 34	10 33	7 41
0 25%	9 32	32 58	100 00	100 00	86 63	19 00	7 42
0 30%	9 57	35 41	100 00	100 00	88 33	19 00	7 89
<b>Variety II</b>							
Control					5 00	5 00	6 67
0 10%					86 20	10 33	6 00
0 15%		No flower			86 67	11 00	6 12
0 20%					86 67	13 21	6 39
0 25%					87 00	19 00	7 00
0 30%					88 33	20 20	7 00
<b>II stage of application</b>							
<b>Variety I</b>							
Control	5 08	5 30	5 00	5 00	6 00	9 00	10 00
0 10%	5 00	9 33	65 20	85 00	5 20	10 33	10 00
0 15%	5 34	15 72	70 21	87 11	65 23	10 00	10 00
0 20%	5 67	21 45	76 76	98 35	87 00	18 67	10 00
0 25%	6 00	27 21	89 67	100 00	89 33	18 00	10 33
0 30%	6 11	28 21	96 27	100 00	90 35	18 00	10 67
<b>Variety II</b>							
Control	5 88	5 88	5 00	6 00	6 21	6 33	6 91
0 10%	5 91	12 58	69 78	93 24	58 20	9 38	6 94
0 15%	6 23	16 27	77 41	95 34	61 27	9 18	6 97
0 20%	6 51	16 34	85 24	96 78	66 71	18 11	7 11
0 25%	8 29	17 54	95 35	100 00	70 18	19 00	8 00
0 30%	9 17	25 34	100 00	100 00	75 00	19 24	8 00
<b>III stage of application</b>							
<b>Variety I</b>							
Control	5 30	5 00	5 55	5 58	5 68	8 31	
0 10%	5 45	15 87	36 24	30 21	13 24	8 44	
0 15%	10 27	25 72	70 21	45 82	20 10	8 91	
0 20%	12 38	28 21	85 21	54 71	58 11	8 92	
0 25%	15 44	55 21	100 00	95 21	68 48	9 32	
0 30%	16 15	58 43	100 00	100 00	88 21	9 21	
<b>Variety II</b>							
Control	5 88	5 88	5 00	6 21	6 34	9 88	
0 10%	6 89	10 33	35 21	25 14	18 21	9 19	
0 15%	6 89	10 41	73 41	63 21	43 24	9 33	
0 20%	7 00	11 52	88 21	76 21	69 23	10 00	
0 25%	7 14	15 58	99 25	96 41	83 14	10 00	
0 30%	7 34	20 31	100 00	100 00	89 88	11 24	

variety II during the third and fourth weeks at the second stage of application.

2) Pollen germination. Observations on the preliminary trials were made at three stages and are presented separately.

(i) Sucrose solution alone. The results obtained during the study of pollen germination in sucrose solution are presented in the Table V.

Various concentrations were tried with a view to fixing the optimum concentration. It was found that in the concentrations from 2.5% - 10% the pollen grains failed to germinate. From 10% onwards pollen germination was observed but the percentage of germination was very low and the pollen tube was very short. The percentage of germination and the rate of tube elongation were found to be high in the sucrose solution ranging from 12.5% to 20%. Higher concentrations gave very poor germination.

(ii) Sucrose solution with Boric acid. The Table VI presents the summary of the observations of pollen germination and tube growth in sucrose solutions with Boric acid.

TABLE V

Standardisation of Media

Sucrose solution alone

<u>Concentration of the sucrose solution</u>	<u>Mean percentage of germination</u>	<u>Mean pollen tube length in <math>\mu</math></u>
2.5%	-	-
5.0%	-	-
7.5%	-	-
10.0%	39.50	43.71
12.5%	61.28	105.28
15.0%	91.89	132.34
17.5%	85.00	112.54
20.0%	72.52	95.43
22.5%	52.00	47.34
25.0%	45.00	30.84



TABLE VI

Standardisation of Media

Sucrose solution with Boric acid

Concentration of sucrose solution with Boric acid	Mean percentage of germination	Mean pollen tube length in $\mu$
15.0% + 50 ppm.	96.60	145.27
17.5% + 50 ppm.	88.95	139.56
20.5% + 50 ppm.	77.09	135.43
15.0% + 100 ppm.	68.33	144.27
17.5% + 100 ppm.	63.50	128.57
20.0% + 100 ppm.	58.81	122.67
15.0% + 150 ppm.	55.57	80.21
17.5% + 150 ppm.	49.38	56.24
20.0% + 150 ppm.	38.21	44.21

Boric acid was found to increase the percentage of germination and the tube growth. 15% of sucrose solution with the lowest concentration of Boric acid (50 ppm.) gave the best results. The percentage of germination and the pollen tube elongation were very poor with 100 ppm. Pollen germination was not marked at higher concentrations (200 ppm.).

(iii) Sucrose solution in combination with Boric acid and agar. The summary of the results is presented in the Table VII. Among the four doses of agar used, 1 gm. of agar with 15% sucrose solution and 50 ppm. Boric acid was found to be the best combination.

#### Percentage of sterility in culture media

From the Table VIII it can be seen that the percentage of pollen germination and the pollen tube growth were affected by the chemical.

The percentage of sterility varied with the concentrations of the chemical and the stages of application. Cent percent sterility was obtained at the higher concentrations (0.25% and 0.3%). The highest concentration gave a higher percentage of sterility and the lowest concentration gave a lower percentage of

TABLE VII

Standardisation of Media

Sucrose and Boric acid in combination with Agar

<u>Agar in combination with 15% sucrose and 50 ppm. Boric acid</u>	<u>Mean %age of pollen germination</u>	<u>Mean pollen tube length in <math>\mu</math></u>
0.5 gm.	80.21	138.00
1.0 gm.	96.38	143.61
1.5 gm.	90.41	140.00
2.0 gm.	86.37	138.00

TABLE VIII

Percentage of pollen sterility in Culture media

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	8.18	8.88	9.67
0.10%	45.62	38.23	26.27
0.15%	51.75	45.45	28.32
0.20%	65.32	62.32	28.14
0.25%	100.00	89.62	30.21
0.30%	100.00	100.00	48.34
<u>Variety II</u>			
Control	9.17	10.23	10.58
0.10%	10.31	40.18	30.48
0.15%	13.37	45.25	35.26
0.20%	15.28	58.27	48.17
0.25%	18.32	100.00	49.73
0.30%	18.41	100.00	58.27

sterility. There was a decrease in the effect of the chemical from the first stage of application to the advanced stages. Even the lowest concentration gave cent percent sterility during the first stage of application. But in the second and third stages of application the sterility was decreased.

#### Length of pollen tube

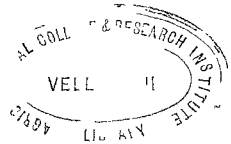
Summary of the results is furnished in the Table IX. There was a significant difference in pollen tube growth. The reduction in pollen tube elongation was found to be determined by the concentration of the chemical and the stages of application. The higher concentrations gave a considerable degree of reduction in pollen tube growth during the first stage of application in the case of variety I and second stage of application in the case of variety II.

The anthers were undehisced and hence a failure in pollen germination was noticed in certain cases. In cases where the anthers dehisce and the pollen grains germinate they showed a reduction in vigour and a staggering nature of growth.

TABLE IX

Length of pollen tube in  $\mu$

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	148.53	146.27	143.25
0.10%	73.37	106.47	120.33
0.15%	69.48	81.56	101.43
0.20%	38.27	66.43	93.21
0.25%	2.33	43.21	89.34
0.30%	2.21	34.73	89.00
<u>Variety II</u>			
Control	146.21	146.15	144.23
0.10%	140.53	65.30	99.41
0.15%	139.12	56.17	94.32
0.20%	139.13	34.31	88.55
0.25%	137.54	00.00	65.00
0.30%	134.61	00.00	60.27



### III. Fruit set

The summary of the results of the total number of fruits set is presented in the Table X and XI.

From the data it can be seen that there was a reduction in number of fruit set when compared to the control. The fruit setting was very much reduced in the plants treated with higher concentrations of the chemical. The first stage of application is more effective in the case of variety I and the second stage of application in the case of variety II.

The induced sterility may be either on the male phase or on the female phase. In order to confirm the source of sterility the treated plants were selfed as well as crossed with the control plant.

The data of the percentage of fruit setting and weight of the fruit set were recorded.

#### Selfing

The summary of the results furnished in the Table XII, illustrates the percentage of fruit setting. It was found to be determined by the concentrations of the chemical and the stages of application. The mean number

TABLE X

Number of fruit set per plant

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	26.30	26.3	26.3
0.10%	19.67	19.33	23.33
0.15%	17.33	18.33	23.31
0.20%	15.67	17.33	22.67
0.25%	15.33	16.67	22.33
0.30%	13.33	13.67	21.67
<u>Variety II</u>			
Control	12.00	12.00	12.00
0.10%	10.90	10.67	11.00
0.15%	10.00	8.33	9.66
0.20%	9.67	6.33	8.67
0.25%	9.33	5.67	8.34
0.30%	9.01	4.67	7.22



TABLE XI

Analysis of variance table for total  
number of fruit set per plant

Source	S.S.	df	Variance	F ratio
Total	5348.96	95		
Treatment	4244.29	31	136.91	7.68 **
Treatment Vs. control	143.13	1	143.13	8.08 **
Between levels of Chemical	224.48	4	56.12	3.14 **
Between stages	152.35	2	76.17	4.27 **
Error	1104.73	62	17.82	

\*\*Significant at 1% level

TABLE XII

Percentage of fruit setting on selfing

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	15.00	15.00	15.00
0.1%	5.00	6.98	11.00
0.15%	4.67	6.11	9.00
0.20%	3.00	3.89	8.91
0.25%	2.33	2.67	8.67
0.30%	2.00	2.67	8.00
<u>Variety II</u>			
Control	10.33	10.33	10.33
0.10%	10.00	6.00	9.33
0.15%	10.00	5.33	9.00
0.20%	9.67	5.00	8.67
0.25%	9.33	4.00	7.67
0.30%	9.33	2.67	7.33

of the fruit set was highly reduced by the chemical treatment when compared with control plants.

The chemical in all concentrations showed a higher degree of sterility and it was confirmed by selfing. There was a gradual increase in sterility from the lowest to the highest concentrations. There was found to be a decreasing tendency from early to the advanced stages of application. The mean number of fruit set per plant varied from 2 to 15 in the case of variety I and 2.67 to 10 in the case of variety II.

### Crossing

For determining the ovule fertility and to confirm the reduction in fruit setting due to male sterility a number of crosses were made.

The summary of the results of the percentage of fruit setting on crossing furnished in the Table XIII, shows that the treatments were not significantly different. Only the highest concentration showed a reduction in fruit setting on crossing.

TABLE XIII

Percentage of fruit setting on crossing

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	99.67	99.67	99.67
0.10%	99.00	99.33	99.33
0.15%	99.33	99.00	99.00
0.20%	98.00	98.33	99.33
0.25%	98.00	98.00	99.00
0.30%	93.33	96.00	96.67
<u>Variety II</u>			
Control	100.00	100.00	100.00
0.10%	100.00	100.00	99.33
0.15%	100.00	100.00	98.67
0.20%	99.67	99.00	98.67
0.25%	99.33	99.00	98.00
0.30%	99.33	93.43	98.00

### Size of fruits

Size of selfed fruits. The Tables XIV and XV give the summary of the results of the size of fruits. The treatments were found to be significantly different.

The length and girth of the fruits were directly related to the concentration of the chemical and stages of application. It was found that the increase in the concentration was accompanied by a decrease in the length and girth of the fruits. The highest concentrations 0.25% and 0.3% gave a maximum reduction in size. The first stage of application had a pronounced effect in reducing the size of fruits when compared with the second and third stages of application in the case of variety I. For the variety II the maximum reduction in size of fruits were obtained in the second stage of application.

Size of crossed fruits. The data relating to the size of crossed fruits are presented in the Tables XVI and XVII. The data show that there was a slight reduction in size of the fruits when compared with the control. But between treatments there was no significant difference. The size of the fruits was found to be the same in all the treatments.

TABLE XIV

Length of selfed fruits in cm.

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	26.91	26.91	26.91
0.10%	18.67	19.92	21.64
0.15%	18.33	19.32	20.13
0.20%	15.41	16.67	18.67
0.25%	14.67	14.82	18.33
0.30%	13.33	13.87	17.24
<u>Variety II</u>			
Control	11.67	11.67	11.67
0.10%	10.67	9.67	9.67
0.15%	9.67	9.33	8.70
0.20%	9.60	8.00	8.33
0.25%	9.60	8.33	8.00
0.30%	9.00	7.33	8.00

TABLE XV

Girth of selfed fruits in cm.

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	15.33	15.33	15.33
0.10%	12.67	13.67	13.67
0.15%	12.50	13.33	13.33
0.20%	10.33	10.33	12.21
0.25%	9.33	10.00	10.33
0.30%	8.00	9.33	10.00
<u>Variety II</u>			
Control	30.21	30.21	30.21
0.10%	26.24	24.00	26.67
0.15%	25.67	22.60	24.33
0.20%	25.33	22.00	23.33
0.25%	24.33	21.67	23.00
0.30%	24.00	20.00	22.67

TABLE XVI

Length of crossed fruit in cm.

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	26.33	26.33	26.33
0.10%	24.33	24.67	25.67
0.15%	24.33	25.67	25.67
0.20%	24.10	25.33	25.33
0.25%	24.00	25.00	25.33
0.30%	23.00	25.00	25.00
<u>Variety II</u>			
Control	12.67	12.67	12.33
0.10%	11.67	11.90	11.67
0.15%	11.67	11.81	11.67
0.20%	11.33	11.67	11.67
0.25%	11.33	11.33	11.67
0.30%	11.00	10.33	11.67



TABLE XVII

Girth of crossed fruit in cm.

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	17.67	17.67	17.67
0.10%	15.67	15.90	15.87
0.15%	15.67	15.71	15.74
0.20%	15.33	15.33	15.67
0.25%	15.33	15.33	15.60
0.30%	14.00	15.00	15.00
<u>Variety II</u>			
Control	32.41	32.41	32.41
0.10%	29.67	29.67	29.67
0.15%	29.67	29.33	29.67
0.20%	29.33	29.33	29.67
0.25%	29.33	28.33	29.00
0.30%	29.33	27.00	29.00

### Weight of fruits

Weight of selfed fruits. The mean weight of the fruits corresponding to different concentration is given in the Table XVIII, in order of merit.

The levels of the chemical and the stages of application were found to have a major role in reducing the weight of the fruits. As the concentrations were increased gradually, the weight of selfed fruits was found to decrease. The highest reduction in weight of the fruits was obtained in the first stage of application, in variety I. Variety II showed maximum reduction in the second stage of application.

Weight of crossed fruits. The mean weight of the crossed fruits are given in the Table XIX.

The weight of the crossed fruits was little affected by the treatments. The data showed that the treatments were not significantly different. There was a significant difference between the treatments and control, but not between concentrations.

TABLE XVIII

Weight of selfed fruits in gm.

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	225.00	225.00	225.00
0.10%	65.33	86.33	125.33
0.15%	50.00	61.67	110.00
0.20%	49.67	58.00	91.67
0.25%	45.33	55.00	79.67
0.30%	40.33	53.33	78.34
<u>Variety II</u>			
Control	425.33	427.67	425.00
0.10%	409.41	206.33	285.43
0.15%	395.47	205.00	256.67
0.20%	394.23	191.00	175.67
0.25%	388.33	109.46	169.21
0.30%	382.43	95.43	156.43

TABLE XIX

Weight of crossed fruits in gm.

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	267.00	267.00	267.00
0.10%	255.34	256.67	255.98
0.15%	255.14	256.51	255.81
0.20%	254.31	256.46	255.69
0.25%	254.00	255.33	255.67
0.30%	254.00	255.00	255.67
<u>Variety II</u>			
Control	420.33	420.41	420.31
0.10%	404.51	404.00	400.90
0.15%	404.33	403.67	400.00
0.20%	404.00	403.38	400.00
0.25%	404.00	403.00	400.00
0.30%	404.00	403.00	400.00

#### IV. Seed sterility

Selfed fruits. The percentage of seed sterility was calculated from an entirely random sample. The calculated percentage of seed sterility is given in the Table XX.

From the data it is seen that the percentage of seed sterility was highly correlated with concentrations of the chemical and the stages of application. There was a linear increase in the percentage of seed sterility as the concentration increased.

In all the treatments germination was completed within ten days after sowing. In the controls complete germination was observed by the sixth day after sowing. Delayed germination was noticed in almost all cases. The Table XX reveals that the germination of seeds was much affected by the chemical treatment. The germination percentage was highly reduced in the case of plants treated with higher concentrations (0.25% and 0.3%).

Crossed fruits. The germination percentage of seeds from crossed fruits is given in the Table XXI. The table evokes that the germination is little affected by the treatments. The seeds obtained from plants treated with highest concentrations failed to show a reduction in germination percentage.

TABLE XX

Germination percentage of seeds in selfed fruits

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	89.49	89.49	89.49
0.10%	79.67	86.41	87.67
0.15%	71.34	69.34	80.24
0.20%	58.33	60.21	63.33
0.25%	54.24	59.00	61.24
0.30%	48.41	58.63	61.33
<u>Variety II</u>			
Control	75.64	75.64	75.64
0.10%	70.67	60.67	62.41
0.15%	59.33	56.21	59.33
0.20%	56.33	54.33	57.67
0.25%	56.73	53.24	55.67
0.30%	48.87	36.17	52.34

TABLE XXI

Percentage of seed germination in crossed fruits

Treatments	Stages of application		
	I	II	III
<u>Variety I</u>			
Control	99.35	98.00	98.00
0.10%	99.33	92.27	96.82
0.15%	99.21	87.67	96.33
0.20%	89.67	86.38	95.00
0.25%	84.33	86.00	93.67
0.30%	84.17	86.00	93.00
<u>Variety II</u>			
Control	80.67	80.67	80.67
0.10%	80.00	80.33	80.00
0.15%	79.33	79.67	80.00
0.20%	79.00	79.33	79.33
0.25%	79.00	78.33	79.33
0.30%	77.67	76.33	78.33

## DISCUSSION



## DISCUSSION

Ever since the phenomenon of hybrid vigour has been observed, necessary considerations have been bestowed upon, for its exploitation in crop improvement programmes. It has thus become a fascinating as well as an efficient tool in plant breeding in recent years. However, its successful utilization in enhanced crop yield depends on the economics of hybrid seed production. Extensive research works have been resorted to since the last few decades to isolate spontaneously occurring male sterile lines in nature which, if used, could minimise the labour in hybrid seed production.

Investigations have also been made, through treatments with various chemicals to induce selective abortion of pollen grains. Positive results were obtained by various workers in the successful induction of pollen sterility in different crop plants through gametocides like Maleic hydrazide, 2, 4-D and the like. This appears to offer some promise in several crop plants which facilitate the elimination of manual labour of emasculation in hybrid seed production. It is especially important and useful in crops in which daily manual emasculation on

a large scale is economically prohibitive and maintenance of naturally occurring sterile lines is extremely difficult.

### Floral biology

A flower cluster of brinjal consists of one to many flowers. The number of such clusters varies from variety to variety. The mere number of flowers in brinjal is not necessarily an index of its productive capacity. According to Smith (1931), Pal and Singh (1934, 1946) and Krishnamurthi and Subrahmanian (1956) in brinjal long styled and medium styled were productive while pseudoshort styled and short styled were unproductive. Similar results were obtained in the present investigation also.

### Number of clusters and flowers

The results presented in the Tables I and II show a high reduction in the total number of clusters and flowers produced by individual plant. Different concentrations of the chemical at all stages had decreased the number of clusters and flowers (Fig. 2 A&B). The maximum reduction in the number of clusters and flowers were noted in the first stage of application for the variety I and second stage of application for the variety II. This

**Fig. 2**

**Graph showing the total number  
of flowers per plant**

**A. Variety I - B. Variety II**

- 1. First stage of application**
- 2. Second stage of application**
- 3. Third stage of application**

(TOTAL FLOWERS) VARIETY - I.

Fig 2 A

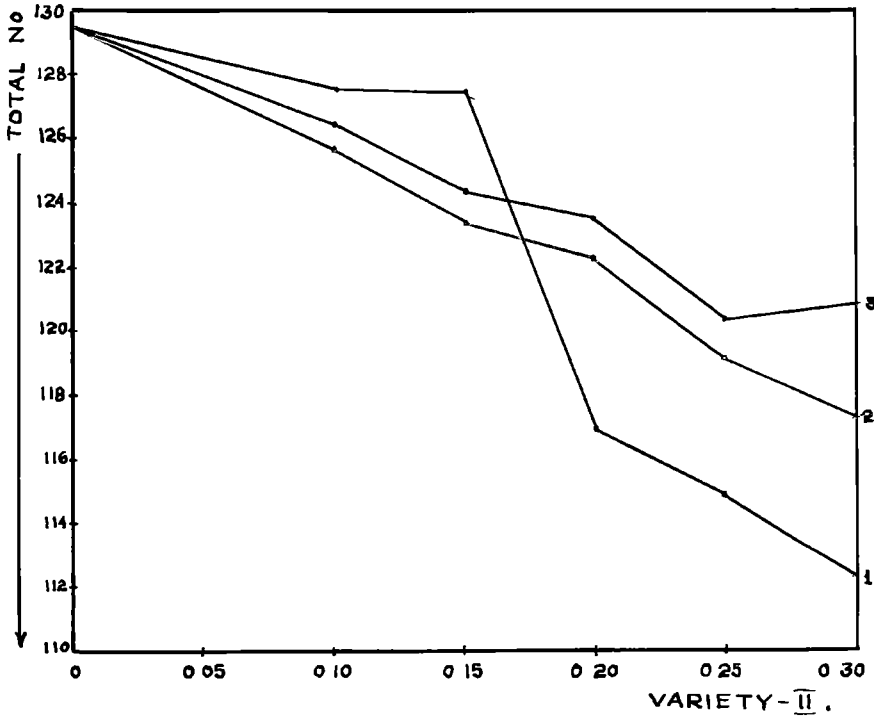
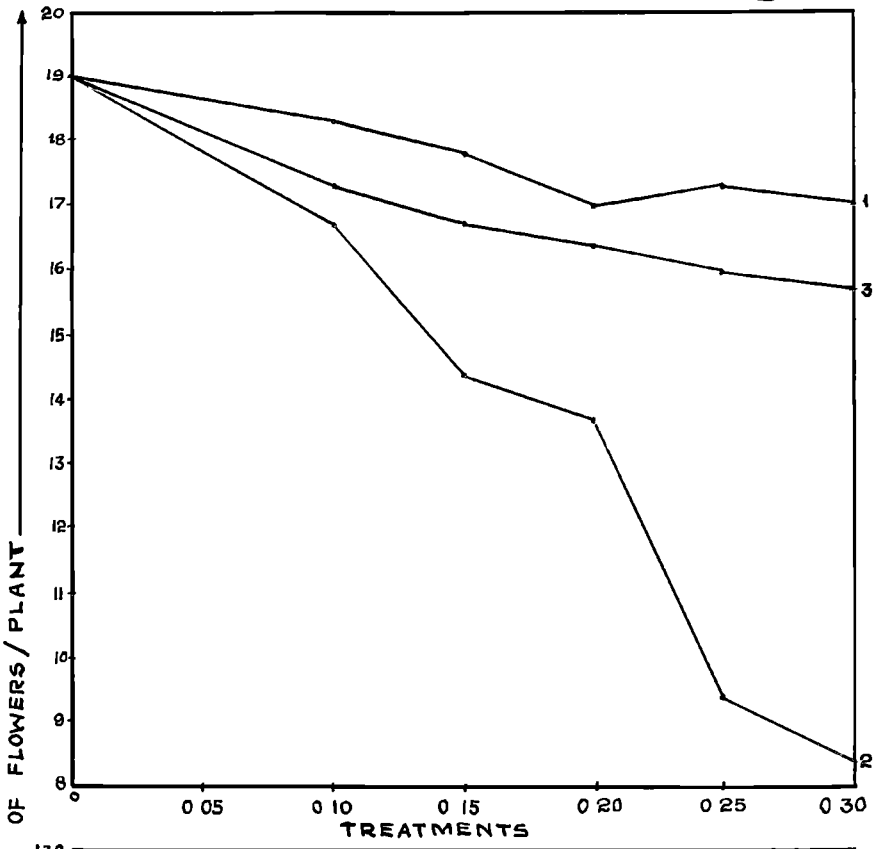


Fig 2 B

VARIETY - II.

was due to difference in flowering time of both varieties. Variety II was late maturing than variety I. Almost all the plants in the variety I produced flower buds at the time of the first spraying. Variety II did not develop any flower buds at that time. At the time of second spraying flower buds were produced by the variety II.

The size and shape of the flowers were much affected by the chemical treatments (Plates IV, V and VI). The results are in agreement with the previous authors like Pate and Duncan (1959) and Bocanegra et al (1960) who obtained in cotton a reduction in size of flowers and bolls with the chemical treatment. The effect of the concentration however varied with the age of the plant. Old plants required higher dosages for showing the same effect which the lower concentrations produce in plants treated at an early stage of growth. There was found to exist an inhibitory action on the part of the chemical. The inhibitory action was found to be determined by the concentration of the chemical and its availability. The inhibitory action was more in the young plants.

#### Pollen studies

Pollen morphology. The shape of the pollen grains of brinjal is spherical and 2-6 colporate. It is in

agreement with the ideas of Wodehouse (1935), Lang (1937) and Erdtman (1952) who studied the pollen morphology of Solanaceae. The shape was affected by the chemical (Plates VII and VIII). The mature pollen grains at dehiscence were deformed in shape, small, shrivelled and empty and did not take stain in acetocarmine. Confirmatory results were obtained by Chopra et al (1960). There was a gradual reduction in size of pollen from low concentration to higher concentrations and a decrease in the effect was found from early stages to the advanced stages. The highest concentration gave the maximum reduction. This was due to the action of the chemical in the actively dividing cells. According to various investigators absorption is faster in the young actively growing leaves than in mature leaves. As the plant grew old the capacity of the plant to accumulate the chemical was found to decrease.

Pollen sterility. Anthers of the male sterile plants were completely or partially barren in some and in others the pollen was present but the anthers did not dehisce. Cent percent sterility was obtained by Eaton (1957), Pate and Duncan (1960), Santhanam (1960), Mendez (1960) and Bocanegra (1960).

Acetocarmine staining method revealed a marked variation in pollen viability in both varieties (Plates VII and VIII). The results obtained are almost similar to those reported by Rolm and Haas (1958).

Anther dehiscence was affected in the first week after the treatment. Even in the case of lowest concentration the pollen produced was found to be defective as determined by their stainability. The lower concentrations 0.1%, 0.15% and 0.2% produced fairly higher degree of sterility for two weeks from two weeks after the treatment. The higher concentrations 0.25% and 0.3% were efficient in completely arresting pollen production for two weeks. This is in line with the work of Moore (1959).

The percentage of sterility was increased for a period and after reaching the maximum of each concentration for few days, depending on the concentration and the stages of application, there was a gradual decrease. The pollen viability was normal after few weeks. This is similar to the results obtained by investigators like Moore (1959) and Santhanam (1961).

Cent percent sterility was obtained during the first stage of application for variety I and second stage



of application for variety II. In the later stages of application only the higher concentrations (0.25% and 0.3%) gave cent percent sterility, for one week and two weeks respectively. Confirmatory evidence to the above conclusion had been reported by Eaton (1957), Moore (1957), Pederson-(1959), Pate and Duncan (1960) and Santhanam (1961).

The exact mechanism of the action of the chemical is not known. Hilton (1958) showed that E.W.450 competed with pantoate for the site on the enzyme which synthesise pantothenate. According to Chopra et al (1960) the chemical is active during the reduction division of the pollen mother cell to produce pollen grains.

According to Ostapenko (1959) in acetocarmine staining technique all pollen grains which were completely or less viable may take stains. So it is not an accurate test.

Germination trials of pollen grains conducted in artificial media revealed that a medium containing 15% sucrose with 50 ppm. boric acid and one gram of agar is most suitable for brinjal. Similar medium was used by Iyengar (1938), Agarwal et al (1953), Johri and Vasil (1955) etc.



In the present studies it was found that 50 ppm. boric acid can increase pollen germination. Higher concentrations reduced pollen germination and tube elongation. The result is in favour of the previous investigators that boric acid when added to the basic medium is capable of increasing pollen germination. Thompson and Batjer (1950) in different species of fruit trees and Raghavan and Baruah (1956) in arecanut obtained results to show that boric acid enhances pollen germination. Madhava Rao and Abdul Khader (1961) have also reported that germination of pollen in sapota could be enhanced by the addition of 100 ppm. boric acid to the medium.

Presence of 1% agar along with sucrose and boric acid in the medium is found to increase the percentage of germination. Similar reports were obtained from various authors like Iyengar (1939), Johri and Vasil (1955).

But the treatment gave a higher percentage of sterility in the nutrient medium (Plates IX and X) and (Fig. 3 A&B). The maximum sterility was obtained in the case of plants treated with higher concentrations (0.25% and 0.3%) and the sterility was decreased towards the low level of concentration.

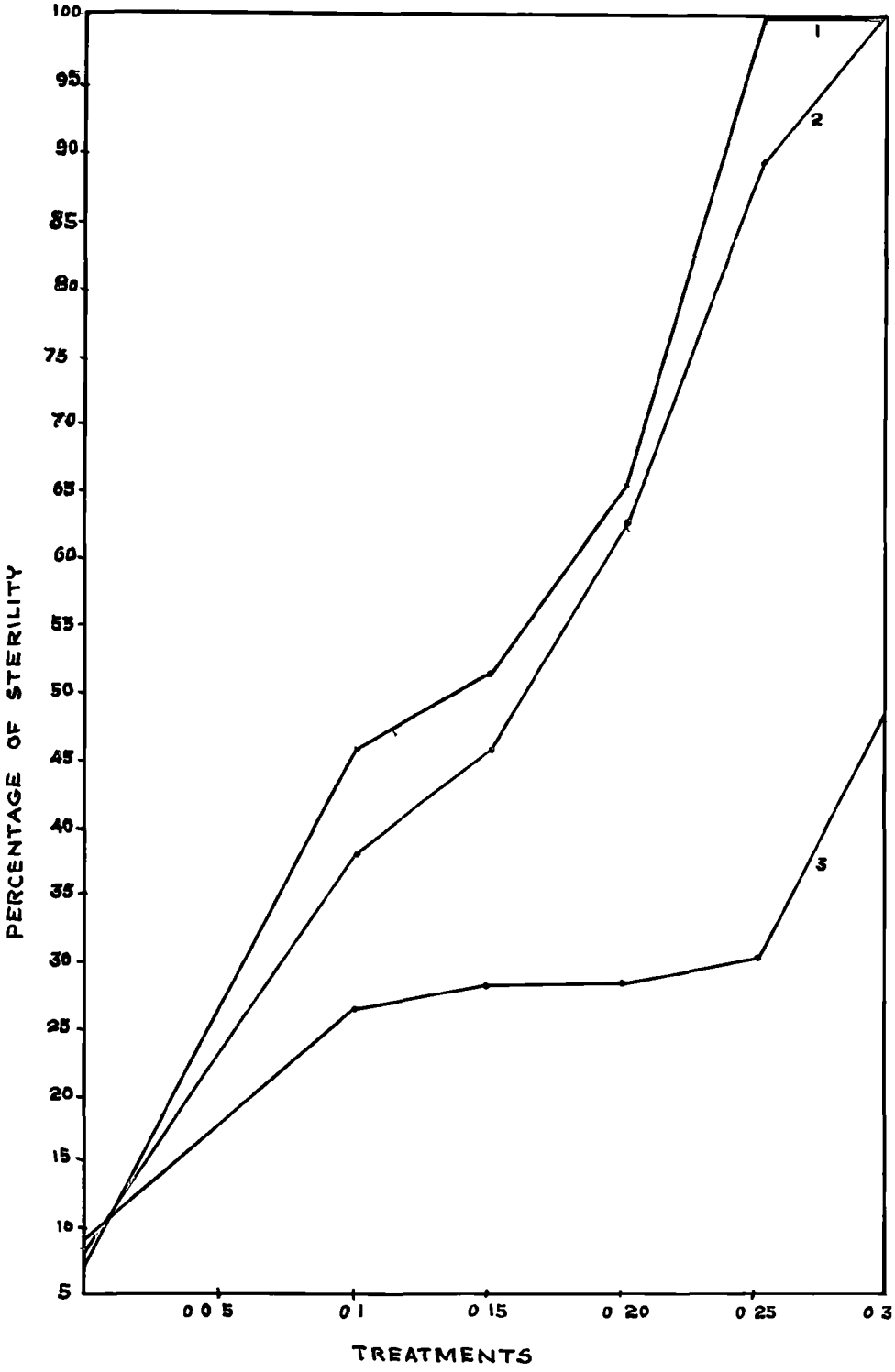
Fig. 3

Graph showing the percentage of  
sterility in culture media

A. Variety I - B. Variety II

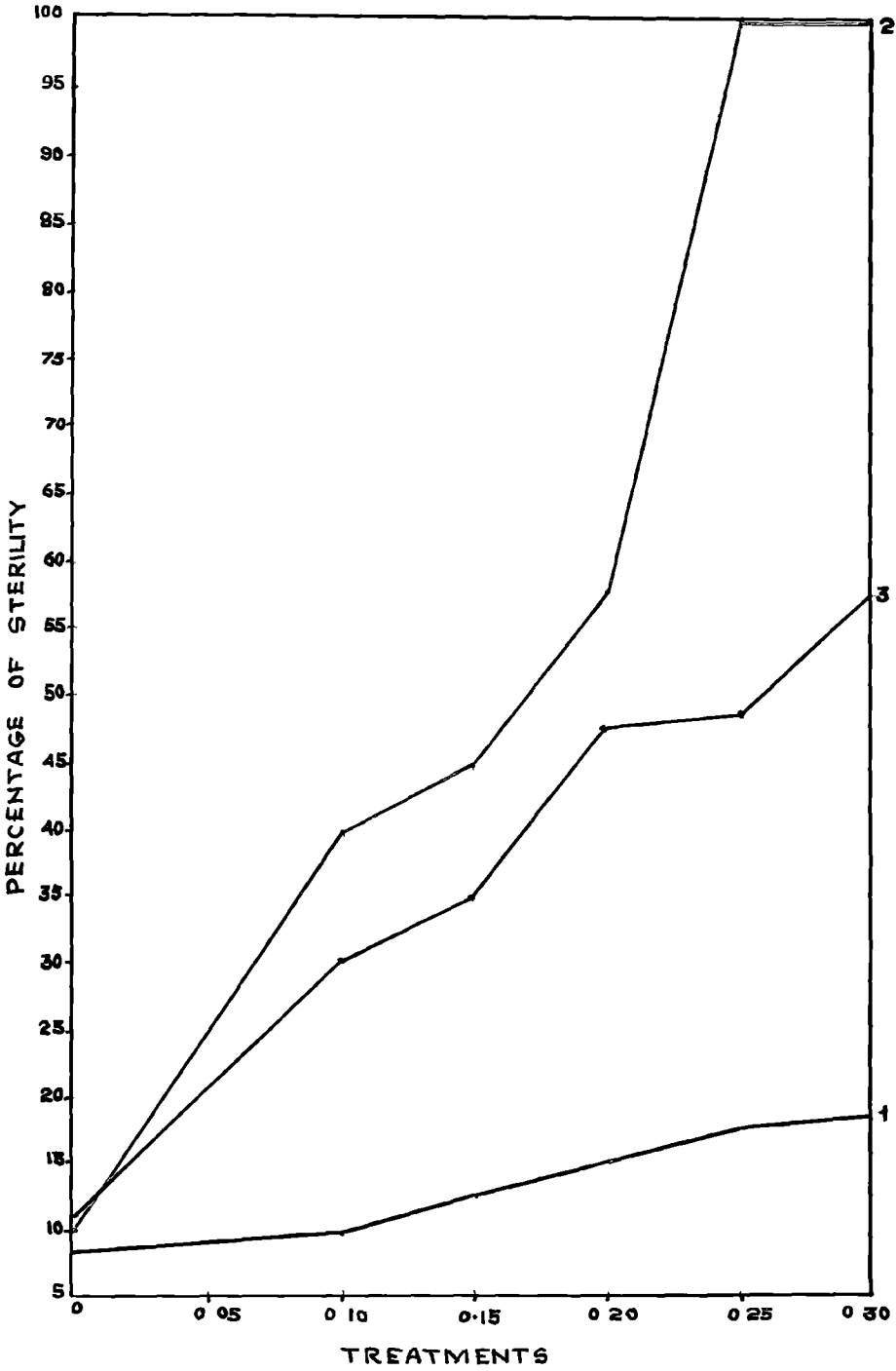
1. First stage of application
2. Second stage of application
3. Third stage of application

VARIETY- I -



VARIETY II

Fig  
3 B



Reduction in pollen tube growth also was noted. (Fig. 4 A&B). The length of pollen tube was found to decrease as the concentration was increased. The maximum reduction in growth was observed in plants treated with the higher concentrations. The first stage of application was more effective for the variety I and the second stage of application for the variety II due to difference in flowering time.

The enzymes present in the pollen grains may control the germination and elongation of pollen tube. The reduction in germination and tube elongation of treated pollen grains may be due to the inhibition of those auxins by the chemical. The inhibitory action of the chemical was reported by Starnes and Hardley (1960). The young plants were more susceptible to the chemical action. As the plants grew old only higher concentrations (0.25% and 0.3%) were found to be effective. The extent of penetration of the chemical was more during the early stage of plant growth than the advanced stage of growth.

#### Fruit set

The total number of fruits produced by the different treatments was found to be less than the control (Fig. 5 A&B).

**Fig. 4**

**Graph showing the length of pollen tube in  $\mu$**

**A. Variety I      -      B. Variety II**

- 1. First stage of application**
- 2. Second stage of application**
- 3. Third stage of application**

Fig. 4 A

VARIETY I

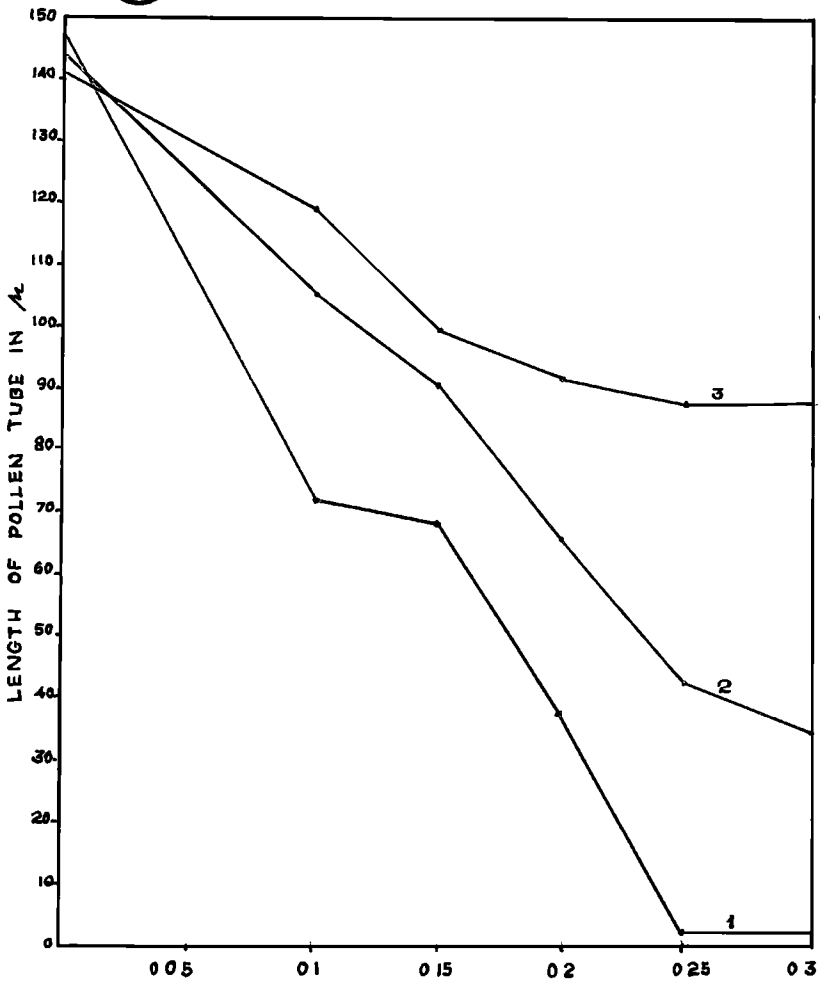
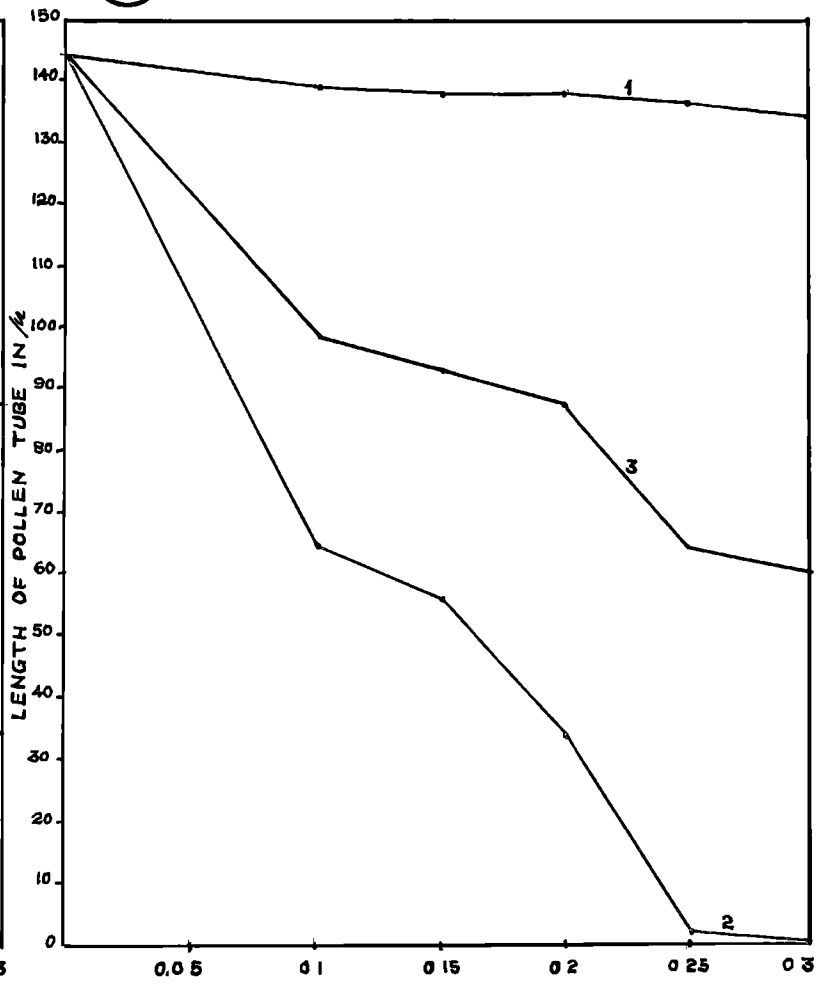


Fig. 4 B

VARIETY II



TREATMENTS

**Fig. 5**

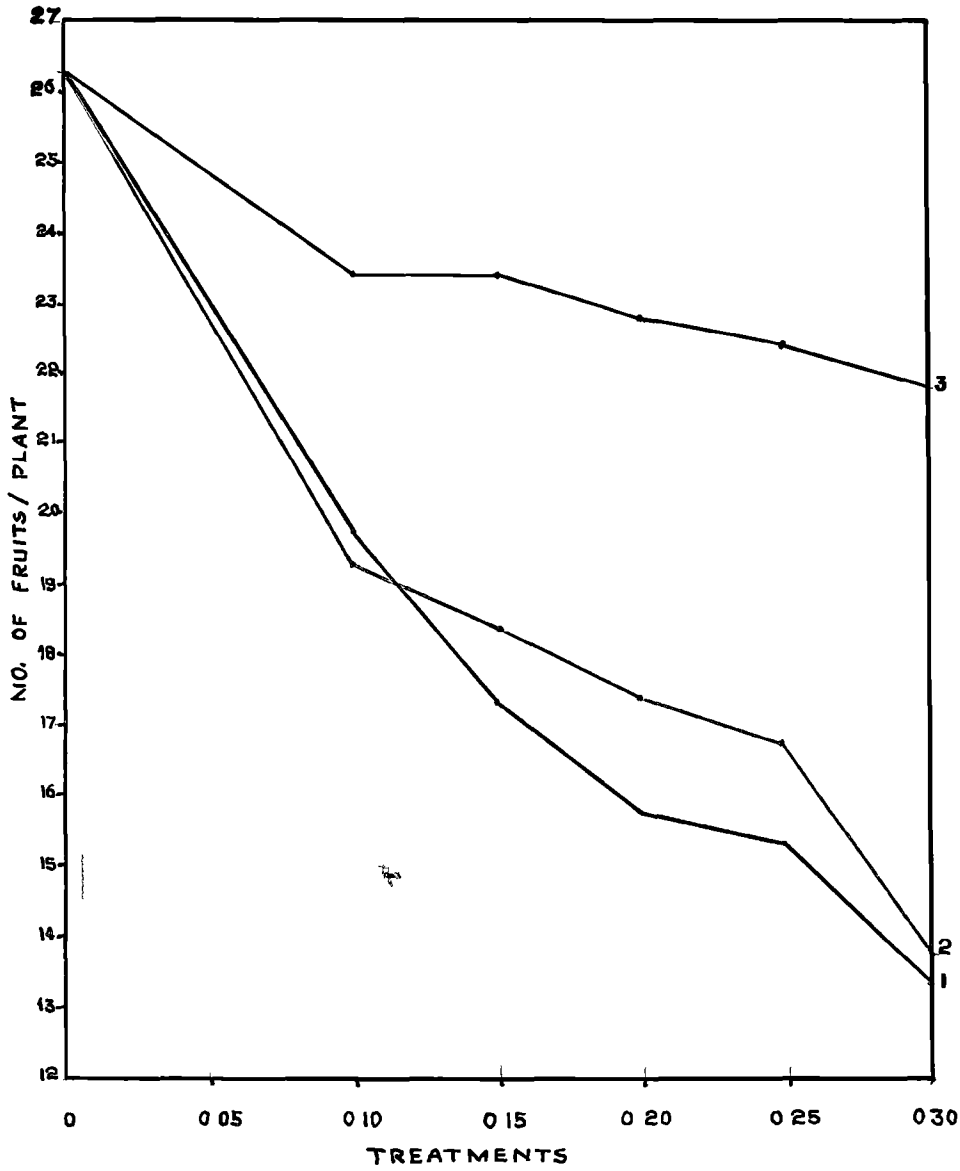
**Graph showing the total number of fruits per plant**

**A. Variety I      -      B. Variety II**

- 1. First stage of application**
- 2. Second stage of application**
- 3. Third stage of application**

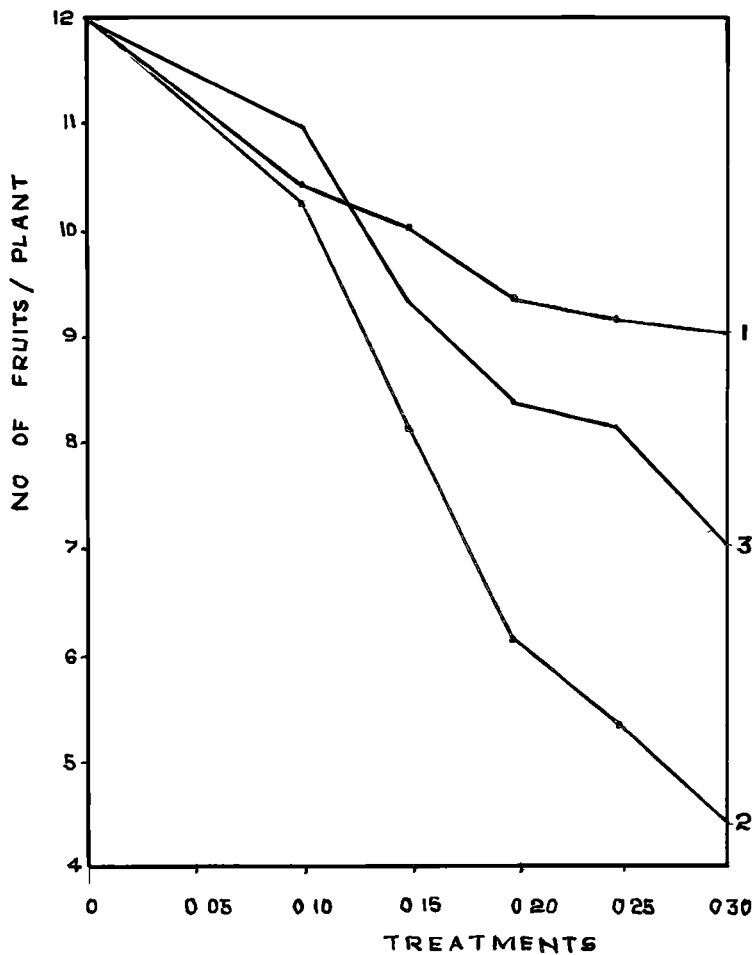


VARIETY I



VARIETY 11

Fig  
5 B



In the treated plants the number of fruits were inversely proportional to the increasing concentration.

Moore (1959), Pate and Duncan (1960) and Singh and Sehgal (1961) obtained a reduction in yield which was dependent upon the concentration of the chemical and the stages of application. In the present investigation also comparable results were obtained.

The reduction in fruit setting was found to be due to the undevelopment of anthers and induced sterility of pollen grains. Abscission of flower buds found at the time of treatments was not uncommon during the present investigation.

According to Chopra et al (1960) the chemical acts at the reduction division process during microsporogenesis. The chemical accumulates to a greater extent in the anthers than in the ovules. The present investigation confirmed the findings of above authors.

The summary of the results presented in the Table XIII explains that the treatments were not significantly different. The percentage of fruit setting on crossing was little affected by the chemical treatment. The plants treated with highest concentration 0.3% showed a

slight reduction in fruit setting. This reduction in fruit setting on crossing probably due to ovule sterility. Similar conclusions were made by investigators like Eaton (1957), Moore (1959), Pate and Duncan (1960) and Singh and Sehgal (1961). The lower concentrations gave complete pollen sterility coupled with complete ovule fertility. Eaton (1957), Rubenbauer and Schultis (1960) and Wit (1960) arrived at similar conclusions.

From the present result it appears likely that young plants were more susceptible to the effect of the chemical. So in both the varieties the reduction in fruit setting was noticed at an early stage of application.

The Tables XIV and XV reveal that the chemical affected markedly the length and girth of fruits. (Plates XI and XII). Such conclusions were obtained by Moore (1959).

The effect of the chemical was found to be dependent upon the stages of application and the concentrations of the chemical. The extent of reduction in length and girth of fruits were more in the plants treated at an early stage of application. According to

Moore (1959) it may be due to the inhibition of the chemical and may be more at an early stage of growth of the plant and when treated with higher concentrations.

The results presented in the Tables XVI and XVII reveal that the chemical had no significant effect on length and girth of crossed fruits. Among various concentrations tried there was no significant difference. Confirmatory evidence to the above conclusion had been reported by Singh and Sehgal (1961). A slight reduction in size of fruits on crossing was noted in the case of plants treated with the highest concentration. This was due to partial female sterility.

From the data presented in the table it appears that the weight of selfed fruits was affected by the chemical treatment. Similar results were obtained from the work conducted by Moore (1959) and Pate and Duncan (1960) in the boll weight of cotton.

The effectiveness of the chemical in reducing the weight of selfed fruits was found to vary with the concentration of the chemical and the stage of application. Older plants required higher doses as compared to the younger plants.

The results presented in the Table XIX are in agreement with the findings of Singh and Sehgal (1961) who obtained a reduction in boll weight in cotton with the higher concentrations indicating female sterility. The lower concentrations showed a selective action towards male phase.

#### Seed sterility

The percentage of germination of seeds in selfed fruits revealed that the chemical had brought about marked differences in germination. Comparable results were obtained by Bocanegra et al (1960) in cotton. It was found that young plants were more affected by the chemical. Old plants required higher dosages for showing the same effect which the lower concentration produced in the plants treated at an early stage of growth. The maximum reduction in germination percentage was showed by the highest concentration in both the varieties.

The data presented in the Table XXI shows that there was no marked differences in the germination percentage of seeds in the crossed fruits. A slight reduction was obtained in the plants treated with highest concentration (0.3%).

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**SUMMARY AND CONCLUSIONS**

## SUMMARY AND CONCLUSIONS

Experiments were conducted at the Agricultural College and Research Institute, Vellayani during the year 1964-65 to study the effect of a chemical gametocide F.W.450 on certain aspects of flowering, pollination and fruit set in brinjal.

The chemical was applied in five concentrations viz., 0.1%, 0.15%, 0.2%, 0.25% and 0.3% at three stages. The first spraying was given when the first flower was undergoing anthesis. Subsequent sprayings were given at an interval of 15 days in between two sprayings.

In brinjal two types of flowers were found

- (1) productive flowers with long or medium styles and
- (2) unproductive flowers with pseudoshort or short styles.

The chemical was found to decrease the size and number ✓ of clusters and flowers.

The sterility of the treated plants was determined by pollen studies and by artificial pollination.

Pollen studies were conducted by staining methods and by germination of pollen grains in artificial media.



Frequent observations in both the varieties revealed complete absence of dehiscent anthers in the treated plants. Such plants showed cent percent sterility. The pollen of male sterile plants were small, shrivelled and empty and did not take stain in acetocarmine. Pollen fertility counts at weekly intervals revealed that there was a gradual reversion to the fertile condition. Complete sterility lasted for a period of upto two weeks, beginning from two weeks after the treatment.

Germination studies of treated pollen in 1% agar medium also revealed that there was a marked difference in germination of pollen grains and pollen tube growth. The percentage of sterility was higher in the culture media than that obtained from the staining method. The effect of the chemical was more in the early stage of growth than in later stages.

There was a marked reduction in the percentage of fruit setting on selfing. A reduction in fruit setting was obtained with the increase in the concentration. The effect of the chemical was found to decrease as the stages advanced.

The size and weight of the selfed fruits were also found to decrease as the concentration increased. The effect was much pronounced in the early stage of application. The maximum reduction in size and weight of fruits was shown by the plants treated with higher concentrations (0.25% and 0.3%).

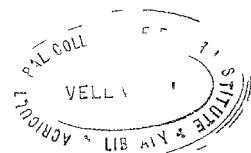
Male sterility of treated plants was further evaluated by applying normal pollen from control to the chemically emasculated flowers. The fruit setting on crossing was normal in almost all cases. A slight reduction in fruit setting was obtained in the case of plants treated with the highest concentration (0.3%).

The size and weight of the crossed fruits also were found to be unaffected by the chemical. In cases where a slight reduction in size and weight was found, it was interpreted as due to female sterility. The lower concentrations proved as a selective male gametocide.

The germination percentage of seeds in selfed fruits showed a marked reduction in germination, while the percentage of germination in crossed seeds was found to be unaffected by the treatment.

It has been proved that all the concentrations of the chemical gametocide F.W.450 applied at the blooming period produced cent percent sterility. But the highest concentration (0.3%) caused female sterility which thus put up a limitation to the use of higher concentrations. The present investigation thus envisages a meticulous standardization of the concentrations of the gametocide with the time of application, which would definitely produce complete male sterility in brinjal.

As has been mentioned earlier, male sterility gets rid of mechanical emasculation for large scale hybrid seed production. Thus the results obtained in the present studies are of great importance in the field of Agriculture.



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R E F E R E N C E S

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ILLUSTRATIONS

Plate I

General layout of the field experiment



PLATE I

**Plate II**

**Variety I Pusa purple long**

**Plate III**

**Variety II Pusa purple round**



PLATE II



PLATE III



**Plate IV**

**A comparative study of control and F.W.450  
treated flowers during the First stage of  
application**

**Plate V**

**A comparative study of control and F.W.450  
treated flowers during the Second stage of  
application**



PLATE IV



PLATE V

Plate VI

A comparative study of control and F.W.450  
treated flowers during the Third stage of  
application



PLATE VI

**Plate VII**

**Pollen from F.W. 450 sprayed plants**

**Effect of Gametocide spray on pollen maturity**

**Plate VIII**

**Pollen from 'Control' plants**

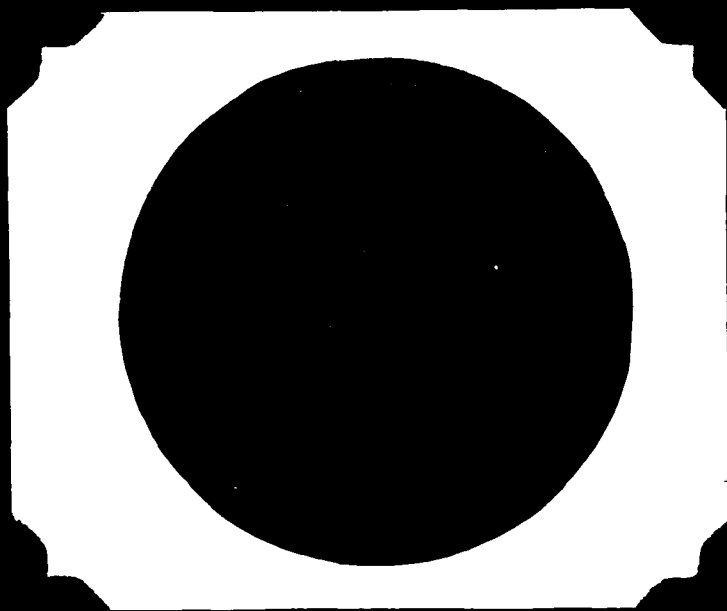


PLATE VII

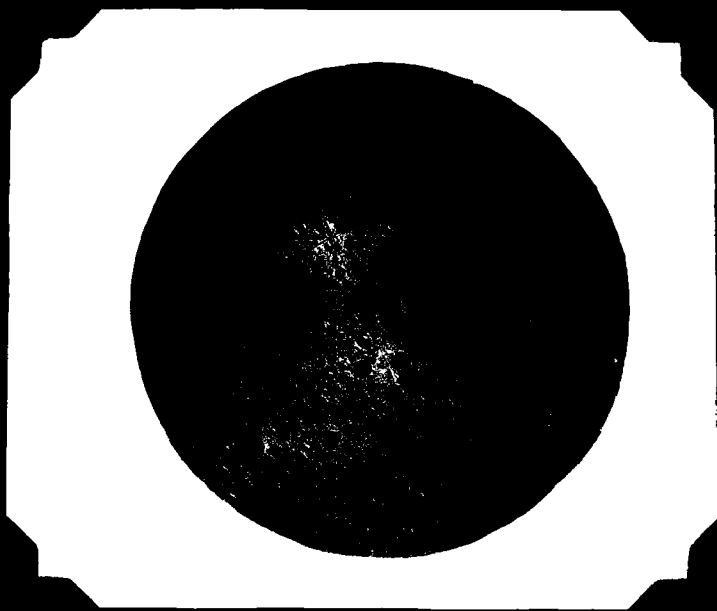


PLATE VIII

**Plate IX**

**Pollen from treated plants**

**Effect of gametocide on pollen germination**

**Plate I**

**Pollen from control**

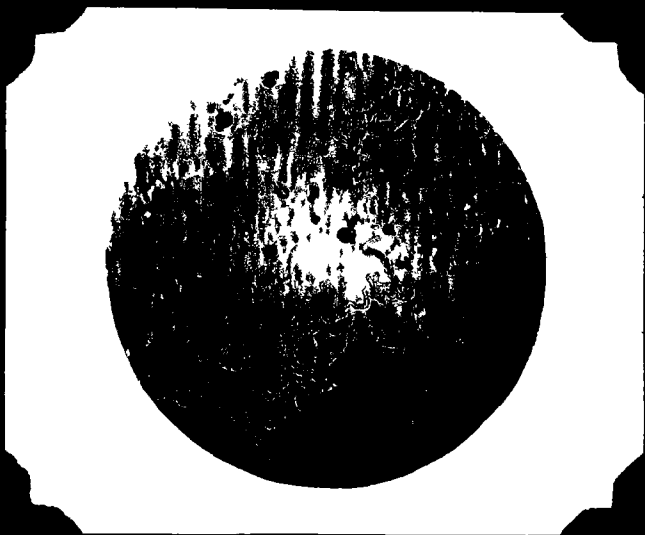


PLATE IX

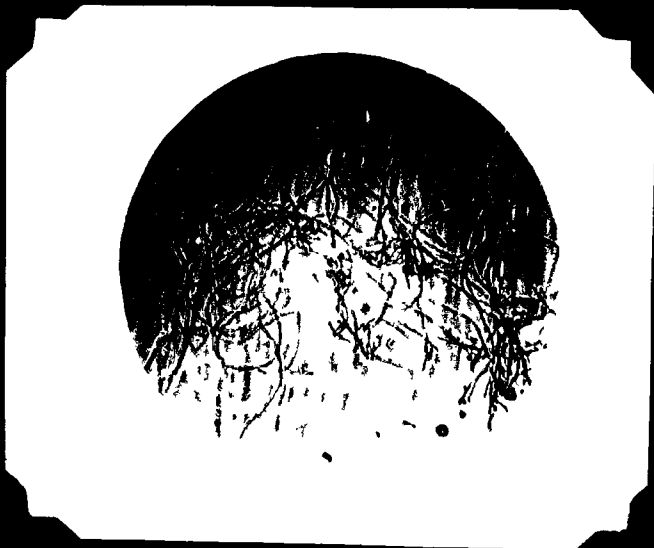


PLATE X



**Plate XI**

**Selfed fruits after one week**

**Effect of gametocide on fruit development**

**Plate XII**

**Selfed fruits (mature)**