REPRODUCTIVE BIOLOGY AND ENZYME STUDIES IN *OCIMUM* Spp.

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

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VELLANIKKARA, THRISSUR
KERALA, INDIA.

1997

DECLARATION

I hereby declare that the thesis entitled "**Reproductive biology and enzyme studies in *Ocimum* spp.**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled "**Reproductive biology and enzyme studies in *Ocimum* spp.**" is a record of research work done independently by **Mrs.Fancy Parameswaran**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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ACKNOWLEDGEMENT

I wish to express my profound gratitude to **Dr.K.T.Presannakumari**, Assistant Professor, AICRP on Medicinal and Aromatic Plants, College of Horticulture and the Chairperson of my advisory committee, for her valuable guidance and help during the course of this investigation and preparation of the thesis.

I am grateful to the members of my Advisory Committee, **Dr.K.Pushkaran**, Professor and Head, Department of Plant Breeding and Genetics; **Dr.A.Augustin,** Assistant Professor, AICRP on Medicinal and Aromatic Plants and **Dr.K.Nandini,** Assistant Professor, K.H.D.P. for their valuable advice and suggestions during the entire course of research work.

It gives me great privilege to express my obligation towards Dr.Luckins C. Babu, Associate Professor, College of Forestry and Smt.M.R.Bindhu, Assistant Professor, College of Horticulture, for the help and guidance extended.

Sincere thanks are also due to Sri.K.K.Sunilkumar and Sri.K. Manojkumar for their timely help in the analysis of the data.

I am gratefully obliged to Miss.T.K.Indira, Research Assistant, for her sincere help during the conduct of the study.

A word of thanks to Sri.Joy for the neat typing of the thesis.

It is with immense pleasure that I thank all my friends especially Miss.Vandana Venugopal and P.Sindhumol for the sincere help provided at and beyond the times of need.

I also place on record my sincere thanks to my in-laws for their kind co-operation.

I owe a lot to my mother who kept encouraging me at all critical junctures.

I am forever indebted to my husband without whose moral support and sacrifice this work would have never found conclusion.

Last but not least I bow my head before Almighty whose blessings enabled me to make this endeavour a grand success.

FANCY PARAMESWARAN

In memory of
my beloved father

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Introduction

INTRODUCTION

The family Labiatae, consisting of about 180 genera and 3500 species (Willis, 1966) is world wide in distribution, with its main centre in the Mediterranean region. Mukerjee (1940) described 420 species from the Indian subcontinent of which 152 occur in the Western Himalayas. This family, which is important in perfumery, has a broad odour spectrum ranging from floral odour of lavender to minty odour of peppermint, spicy odour of *Ocimum* to woody odour of patchouli.

The genus *Ocimum*, a fascinating group of aromatic plants comprises of about 160 species distributed in warmer parts of hemispheres from sea level upto 1800 m. Among the different species, *O. canum, O. basilicum, O. gratissimum* and *O. tenuiflorum* are common in South India including Kerala (Sobti *et al.*, 1976).

Ocimum species form a rich source of a variety of essential oils and aroma chemicals useful in perfumery, cosmetics and indigenous system of medicine. Oil of some species has fungicidal, bactericidal and insecticidal properties also. From the industrial point of view *Ocimum* species with oil rich in camphor, citral, geraniol, linalool, linalyl acetate, methyl chevicol, eugenol, thymol etc. are important and can be harnessed for successful utilization in industry. In India the requirement of most of these are met by imports and the demand is increasing. The products of *Ocimum* worth several lakhs of rupees are annually imported into our country.

The different species of *Ocimum* have varying composition of chemical constituents making them of different quality and odour. It has been reported by

various authors that biosynthesis of various chemical constituents in *Ocimum* is gene controlled. By adopting proper breeding techniques, plants of high economic value can be produced. But for the success of any crop improvement programme a thorough knowledge of reproductive biology, pollen morphology and seed characters is essential. Besides, pollen morphology has great relevance to our understanding of systematic relationships of different species. The importance of seed physiology need not be over emphasised. Seeds being the main propagule in *Ocimum*, an understanding of the seed characters like seed dormancy, moisture content, seed density etc. is also essential in crop improvement programmes. Being a crop with tremendous potential for the present, the present investigation entitled "Reproductive biology and enzyme studies in *Ocimum* spp." was undertaken with the following objectives.

1. To investigate and compare the floral biology, anthesis and mode of pollination in four different species of *Ocimum* viz. *O. tenuiflorum, O. gratissimum, O. canum* and *O. basilicum*.

2. To make a comparative evaluation of physiological attributes of seed like density, germination and dormancy in four different species.

3. To estimate total phenol and phosphorylase enzyme activity in the seeds of different *Ocimum* species.

Review of Literature

REVIEW OF LITERATURE

About 160 species of the genus *Ocimum* are reported in the world. Out of which around 30 species are found in the tropics and subtropics of the Old and New World (Paton, 1991).

2.1 Reproductive biology

Very little work has been reported on the reproductive biology of *Ocimum*. To project the overall dimension of the present topic, information available in other related crops are also reviewed hereunder.

2.1.1 Flowering pattern

In tulsi, flowering season was observed from August to March, with its peak period from September to December, and flowers were borne on current seasons growth (Singh and Sharma, 1980)

Flowering pattern in sweet basil has been described by Putievsky (1983). According to them flowering was continuous with new flowers appearing throughout the growing season. Proportions of leaves and stems decreased and the inflorescence ratio increased as the plant increased in maturity. The sequence of expansion of inflorescences and flowers in sweet basil was from apex downwards.

Raju (1989) observed that *O. americanum* and *O. basilicum* flowered once a year and they produced flowers over a long period.

Echeverry *et al.* (1990) reported that *O. basilicum* L. propagated from seeds started flowering on 137th day and flowering lasted upto 197th day, when

vegetatively propagated the plants flowered on the 91st day and the cycle lasted until 160th day. When raised from cuttings, *O. gratissimum* and *O. tenuiflorum* flowered between 136 and 195 days and 147 and 175 days respectively.

In Lavendula, Herrara (1991) observed that blooming period extended for a period of 40-50 days.

Marjoram plants took 61.9 days for first flowering and flowering continued for a period of 1 month (Kumar *et al.*, 1994).

2.1.2 Floral morphology

The value of comparative studies of floral morphology in the determination of ontogenetic and phylogenetic concepts were demonstrated by Bessey (1898), Smith (1926), Chester (1927), Arber (1931, 1936) and Fraster (1937).

Stauffer (1937) made a comparative study of floral anatomy and concluded that the ovule-attachment region appeared to be the most reliable anatomical character in tracing phylogeny of mints.

Comparative studies of floral morphology of the Labiatae were done by Hillson (1959).

According to Kumari (1980) an active upward and lateral growth of the nutlets at the stylar base to accommodate the growing ovules was the probable cause of gynobasy in Lamiaceae and with the progress in gynobasy, the placental region became more condensed and occupied the basal position in the ovary with two anatropous ovules in each carpel.

Singh and Sharma (1980) observed that in *O. sanctum* L. the inflorescence was a raceme having usually six flowers in each verticil. They also reported the average length of fully developed bud as 7.12 mm.

A new variety *O. nudicaule* var. *unisifolia*, was described to include plants with long calyxes, short racemes and large seeds with striate surfaces. Plants with seeds having rugous surfaces, were placed in *O. nudicaule* var. *nudicaule* (Ribeiro *et al.*, 1980).

Herrara (1985) observed that flowers of *Lavendula stoechas* L. (Lamiaceae) were hermaphrodite and tubular, with dark purple corolla and secrete minute amounts of sugar-rich nectar.

Raju (1989) reported that flowers of *O. americanum* and *O. basilicum* were short-lived (3-4 hours), bisexual and zygomorphic.

Fahn (1952, 1953) considered the disc in Lamiaceae as an outgrowth of the thalamus, while Kartashova (1960) regarded it as the proliferation of basal part of the ovary.

According to Kumari (1986) the disc received vascular supply mostly from the gynoecial bundles which indicated its association with the gynoecium.

Zer and Fahn (1992) observed that the nectary of *Rosmarinus officinalis* L. has the form of a four-lobed, asymmetrical disc situated around the base of the ovary.

2.1.3 Anthesis

In tulsi, anthesis started with slight cracks observed at the upper and lower lips of corolla and the peak period of anthesis was between 9.30 am and 11 a.m (Singh and Sharma, 1980).

In Lavendula Herrara (1991) observed that anthesis of central flowers was remarkably synchronous and restricted to the first ten days of the blooming period. Within any inflorescence, the anthesis of central flowers never overlapped that of lateral flowers.

In marjoram anthesis took place from 8 am to 3 pm and the peak period (35%) was between 12 noon and 1 pm (Kumar *et al.*, 1994).

2.1.4 Anther dehiscence and stigma receptivity

In tulsi, maximum dehiscence of anthers was observed between 11 am and 12 noon and a partial dehiscence of anthers took place before anthesis (Singh and Sharma, 1980).

Raju (1989) observed that in *Ocimum americanum* and *Ocimum basilicum* flowers were short lived with anthers dehiscing in the bud.

In marjoram, Kumar *et al.* (1994) observed that anther dehiscence took place from 10 am to 4 pm with a peak period from 12 noon to 3 pm. Sunitha and Farooqi (1990) observed similar results in davanna.

Kumar *et al.* (1994) reported that in marjoram stigma was found receptive from a day prior to anthesis and remained so until a day after anthesis, however it was maximum (60%) on the day of anthesis.

2.1.5 Mode of pollination

Investigation of the cultivars of the basils carried out by Darrah (1974) revealed that pollination was chiefly carried out by honey bees, and some cross pollination between species was also observed, but the hybrid was not viable.

In *O. basilicum* natural crossing at varietal level was detected to the extent of 66.7 per cent using seedling pigmentation as gene marker (Krishnan, 1981). He also noted that honey bees act as pollinators for the crop.

From a study on reproductive ecology of *O. americanum* L. and *O. basilicum* L. Raju (1989) concluded that flowers of both offered nectar and pollen as rewards and were pollinated by day flying insects. In Andhra Pradesh, where these species were studied, bee species such as *Apis florea, Apis cerana, Amegilla species* and *Pseudapis oxybeloides* and the butterfly *Surandra quercetorum* were the most frequent and consistent visitors and could be pollinators for both plant species.

According to Tesi *et al.* (1991) basil could be considered as an autogamous species, although degree of cross pollination ranged from 5-10 per cent. In cultivar Nano self-incompatibility mechanism existed and cross pollination went upto 25 per cent.

2.1.6 Pollen morphology and pollen fertility

Pollen morphology has long been recognized as an important parameter in determining the natural relationship among genera and families. Angiosperm families in general can be divided arbitrarily into two types based on the pollen features. Some are stenopalynous, with uniform pollen morphology; the others are eurypalynous, with nonuniform pollen morphology. Labiatae is a stenopalynous family.

Though taxonomically this family is well known, reports on pollen morphology is restricted. In Europe, Erdtman (1945), studied the pollen morphology of a number of Labiatae members.

From North America, Waterman (1959) studied the pollen grains of Michigan Labiatae.

Singh and Sharma (1980) observed that in *O. sanctum*, pollen grains were oval to round in shape with 4-5 germpores and size ranged from 36.4 to 48.55 μ in methyl-green glycerine jelly.

Gill and Chinnappa (1982) made investigations on pollen morphology of 49 West-Himalayan Labiatae and encountered two basic types. The tribe Ocimoideae was represented by 6-colpate grains.

Detailed studies on pollen morphology of the genus *Origanum* and allied genera were done by Husain and Heywood (1982).

Pollen morphological studies in tribe Ocimeae were conducted by Harley *et al.* (1992).

Kumar *et al.* (1994) reported that pollen grains of marjoram were dull white in colour, oblong to oval in shape with 43.74 μm in length and 27.70 μm in breadth.

Staining technique to study the fertility of pollen grains was adopted by Zirkle as early as in 1937. He introduced acetocarmine staining technique.

In tulsi, Singh and Sharma (1980) observed a fertility percentage of 88.36 to 94.15 in acetocarmine stainability.

2.2 Seed characters

2.2.1 Seed weight

In *Ocimum sanctum*, 1000-seed weight decreased with plant age, season and development (Dey and Choudhuri, 1982). It showed a minimum value in May and increased thereafter.

A comparison of single seed weight and 100 seed weight in faba bean varieties was done by Higgins and Sparks (1989).

Ismail *et al.* (1990) reported that in *Ocimum basilicum* L. with increasing age, seed dry weight decreased.

Putievsky (1993) observed that 1000-seed weight in sweet basil was affected by branch position, stem age and plant maturity.

2.2.2 Seed germination

A brief light exposure was essential for the germination of dark imbibed seeds of *O. americanum* Linn. (Varshney, 1968).

Ivanov (1975) reported that treatment of basil seeds with sulphuric acid for 3 min, advanced germination by 3-4 days and increased it by 45 per cent.

In some Labiatae during aging, germination energy and germination percentage of seed decreased (Tsvetkov *et al.*, 1975).

Wanner *et al.* (1977) studied the effect of long chain fatty acids on seed germination.

Dey and Choudhuri (1982) stated that the germination percentage and rate of germination of tulsi seeds started decreasing from October onwards, reaching a minimum value in May and increased thereafter. Seeds were positively photoblastic and those seeds collected from April to June when treated with hormones, promotors and chilling temperature improved the germination percentage but had no effect on those collected in May.

Putievsky (1983) observed that optimum temperature for germination of oregano was a day/light regime of 24/19°C, but sweet basil was less sensitive and germinated well at temperature regimes between 18/13° and 30/25°C.

Seeds of *O. americanum* L. displayed an absolute light requirement for germination, and the minimal length of the daily photoperiod required to induce a high germination decreased with increasing seed age (Amritphale *et al.*, 1984). They

also suggested that scarification treatment did not allow the seeds to bypass the light requirement, but it enhanced the germination considerably.

Chaudhury and Bordoloi (1986) reported that in *O. gratissimum* highest seed germination of 89.7 per cent was obtained during August sowing and lowest percentage of 14.5 was recorded under December sowing. In general germination percentage was above 75 in the seeds sown from March to September.

When seeds of *O. basilicum, O. gratissimum* and *O. minimum* were tested, germination percentage obtained were 28, 8.75 and zero, respectively (Echeverry *et al.*, 1990).

In growth cabinet, glass house and field studies, fresh and exumed seeds of *O. basilicum* gave 100 per cent germination with alternating temperature of 20/10, 32/19, 38/24 and 40/32 for 13/11 h, but did not germinate at constant temperature and in dark; but occurred with a 13 h photoperiod (Ismail *et al.*, 1990).

Aerts *et al.* (1991) studied the inhibition of *Ocimum* seed germination by cinchona alkaloids.

Ushitani (1991) reported that germination in sweet basil was slower during December, January and February compared to March, April and May.

Putievsky (1993) reported that in sweet basil, seeds with highest percentage of germination were obtained from plants which were almost dry.

Effect of temperature, light and stimulated drought on germination of *O. basilicum* was studied by Hamada *et al.* (1993). They showed that maximum temperature for germination was 40°C and at alternating temperature of 30/20°C

germination was 4 per cent and corresponding figures for 12 h light/12 h dark were 100 per cent. Seed germination decreased with decreasing osmotic pressure.

Ecophysiological aspects of seed germination were investigated in aromatic plants thyme, savory and oregano (Thanos *et al.*, 1995).

2.2.3 Seed dormancy

Causes of seed dormancy appear to vary in different crops. Mayer and Anderson (1963) had listed six possible causes of seed dormancy viz., (1) impermeability of seed coat to water, (ii) mechanical resistance of seed coat, (iii) impermeability of seed coat to oxygen, (iv) presence of rudimentary embryo, (v) dormancy of embryo and (vi) presence of germination inhibitors.

Varshney (1968) reported that imbibed seeds of *O. americanum* L. entered a secondary dormancy (skotodormancy) under prolonged darkness.

Leaf canopy induced dormancy was exhibited by two plants of the family Labiatae viz., *Origanum vulgare* L. and *Prunella vulgaris* L. (Silvertown, 1980).

In *O. nudicaule*, changes in hemicellulose polysacharide composition in dormancy and sprouting were studied by Ribeiro *et al.* (1992).

Absence of secondary dormancy induction was noted in savory and oregano seeds, which germinated promptly under favourable light condition (Thanos *et al.*, 1995).

2.3 Biochemical studies

2.3.1 Phenol

Endogenic substances like the polyphenols and free amino acids were directly related with tulsi seed germination and viability during storage (Tsvetkov *et al.*, 1975).

Studies on retaining the sowing qualities of some essential oil plant seeds revealed that seed constituents such as sugars, amino acids and polyphenols were directly associated with viability (Tsvetkov *et al.*, 1975). They also revealed that polyphenol content rose substantially during storage.

Inheritance pattern of some phenolic compounds of essential oil of *Ocimum* was studied (Sobti *et al.*, 1978). They also discussed the genetic basis of inheritance of phenolic compounds.

Decline in germination percentage of the seeds of *Ocimum sanctum*, was however associated with fall in polyphenol content in the seeds and polyphenol in the seeds showed a similar trend as eugenol content in leaf with age (Dey and Choudhuri, 1980). They also suggested that there was a rapid rate of polyphenol biosynthesis during the early growth phase and high amount of polyphenols possibly migrated to seeds.

Studies on phenolic constituents from clocimum oil was done by Puri *et al.* (1982).

2.3.2 Phosphorylase enzyme action

In sweet potato phosphorylase and yam starch phosphorylase, glucose 1 phosphate was specific for enzyme activity (Kamogawa *et al.*, 1968).

Four forms of phosphorylase were isolated from maize (Tsai and Nelson, 1969), nine from potato tubers (Gerbrandy and Doorgeest, 1972), five from banana fruits (Singh and Sanwal, 1976), one from *Dioscorea rotundata* tuber (Oluoha, 1990) and two from *Dioscorea dumentorum* tuber (Oluoha and Ugochukwu, 1994).

Activation of phosphorylase from *Dioscorea cayenensis* by Mg^{2+} and Ca^{2+} was reported by Oluoha and Ugochukwu (1991), while in *Dioscorea rotundata* enzyme was inhibited (Oluoha, 1990).

Hg^{2+} and Zn^{2+} inhibited the enzymatic activity of yam phosphorylase (Hamdan and Diopoh, 1991).

Pyridoxal-5'-phosphate was identified as prosthetic group of yam phosphorylase and it was essential for enzyme activity (Oluoha and Ugochukwu, 1994).

Materials and Methods

MATERIALS AND METHODS

Investigations on the reproductive biology and phosphorylase enzyme activity in four species of *Ocimum* were carried out at the College of Horticulture, Vellanikkara during the period from October, 1994 to Semptember, 1996.

A. Materials

Four different species of *Ocimum* viz., *O. tenuiflorum, O. gratissimum, O. basilicum* and *O. canum* collected and maintained in the Department of Plant Breeding and Genetics were utilized for the study (Plate 1a-d). These are assigned to two groups viz., Sanctum group (*O. tenuiflorum* and *O. gratissimum*) and Basilicum group (*O. basilicum* and *O. canum*).

B. Methodology

3.1 Reproductive biology

3.1.1 Time taken for inflorescence development

In order to study the time taken for the full development of inflorescence from visual observation stage of formation, 10 young flowering shoots from 5 different plants in each species were tagged soon after appearance of flower bud in the leaf axil. Time taken for the opening of first flower in an inflorescence from visual observation stage of formation was recorded.

3.1.2 Inflorescence characters

Type of inflorescence, length of fully developed inflorescence, number of flowers per inflorescence and distance between verticils were recorded.

Plate 1a. Single plant of *Ocimum tenuiflorum*

Plate 1b. Plant of *Ocimum gratissimum*

Plate 1c. Single plant of *Ocimum canum*

Plate 1d. Single plant of *Ocimum basilicum*

Observations were taken from 10 different inflorescences of each species and the average was computed.

3.1.3 Floral morphology

Fresh flowers of the four species were collected. Hand sections, both L.S. and T.S., were taken and examined under microscope. Description of the morphological features of the four different species were made.

3.1.4 Anthesis

3.1.4.1 Time of flower opening and peak period of anthesis in an inflorescence

Initial investigations were conducted by tagging 10 mature inflorescences of each species. Next morning at 4 am inflorescences were observed and at 4 pm all opened flowers were removed and inflorescences were again observed at 4 am on the third day to see whether any flower opening took place between 4 pm and 4 am. After preliminary studies inflorescences of uniform age facing different directions in five different plants of each species were labelled. The number of flowers opened in each inflorescence were recorded at bihourly intervals from 4 am to 4 pm. The counting was continued from the commencement till completion of flowering in an inflorescence. Percentage of flowers opened at bihourly intervals was estimated for determining the peak time of flower opening on each day and peak period of anthesis in an inflorescence.

3.1.4.2 Time of anther dehiscence and stigma receptivity

The colour and appearance of anthers were examined with handlens at bihourly intervals in fully mature flower buds of each species to find out the time of

anther dehiscence in a flower. The stigmatic surfaces were also observed for any change in colour or appearance, in the same buds, at the same intervals of time to find out the stigma receptivity.

3.1.5 Mode of pollination

This experiment was conducted with a view to find out the role played by different pollinating agents. Twenty inflorescences of uniform age were tagged in five different plants before commencement of anthesis. Only mature buds were retained in these and others were removed. Out of these five were covered with butter paper cover and five were kept uncovered until completion of anthesis. Another set of five was covered with butter paper cover, but the cover was removed at the time of flower opening and sprayed with water. After that cover was placed again, until the flowers fell. Another set of five was subjected to the activity of wind and insects by removing the cover at the time of flower opening alone. Extent of fruit set in each case was determined and expressed as percentage. Observations on various insects visiting the flowers were also recorded.

3.1.6 Pollen morphology and pollen fertility

For determining morphology, pollen samples were taken from fully opened flowers and acetolysed according to the method described by Erdtman (1960). Then the sculpturing on the exine was examined under the microscope and classification was done following the procedure suggested by Moore and Webb (1978).

Fertility of pollen was assessed on the basis of stainability of pollen grains in acetocarmine-glycerine mixture. Pollen grains were collected from the

newly opened flowers and stained in a drop of acetocarmine-glycerine mixture on a clean slide and kept aside for one hour. All the pollen grains that were well filled and stained were counted as fertile and others as sterile. Two fields of five different slides prepared from each species, were observed under microscope. The values were expressed as percentage.

Pollen diameter was measured using an ocular micrometer, after calibration. Mean as well as range of size was computed.

3.2 Seed characters

3.2.1 Seed moisture content

Moisture content of the seed was estimated by gravimetric method. Based on moisture content the species were classified into different groups (1) High moisture content (10-12%) (2) Medium moisture content (8-10%) (3) Low moisture content (6-8%).

3.2.2 1000-seed weight

Four samples containing thousand seeds each were taken from each species and weighed. Average of four values were taken to estimate 1000-seed weight. Species with 1000-seed weight less than 1 g were grouped as low and more than 1 g as high.

3.2.3 Seed density

Known weighed quantity of seed was immersed in distilled water taken in a measuring cylinder. Water displaced by seed was noted. From that seed density was calculated according to the formula

$$\text{Seed density} = \frac{\text{Weight of seed in air} \times \text{Specific gravity of water}}{\text{Weight of water displaced}}$$

3.2.4 Dormancy and germination percentage

Period of dormancy was determined by germination test conducted at regular intervals. Germination test was started on the day of harvest. For the test 100 seeds of each species were placed on Whatman No.1 filter paper in petridish and moistened with distilled water. Germination count was taken on the 10th day. A sound seed that had not germinated was considered to be dormant. Based on this the germination percentage and dormancy percentage were worked out. Dormancy percentage is a measure of the intensity of dormancy. Test was repeated at ten days interval until the germination percentage exceeded 80. Based on the results of this study, the period of dormancy was ascertained. Species with dormancy period less than 40 days were rated as low, between 40-80 days rated as medium and those with more than 80 days were rated as high.

3.3 Biochemical studies

3.3.1 Estimation of total phenol

Reagents

1) 80% ethanol

2) Folin-ciocalteau reagent

3) Na_2CO_3 20%

4) Standard solution (100 μg catechol in 1 ml water)

Total phenol was estimated by Folin-ciocalteau method (Mahadevan and Sridhar, 1986).

One gram freshly harvested seed of each species was crushed in a mortar and pestle along with 10 ml alcohol and then centrifuged for 20 minutes. From the alcoholic extract 4 ml was taken in a graduated test tube to which 1 ml of Folin-ciocalteau reagent and 2 ml of Na_2CO_3 solutions were added and after 30 minutes absorbance read at 650 nm in a spectrophotometer.

Total phenol content was calculated from a standard curve of catechol and was expressed as mg/g of sample

Content of phenol in 4 ml of extract =

$$Y\mu g = \frac{\mu g \text{ standard}}{\text{Absorbance of standard}} \times \text{absorbance of sample}$$

content of phenol in 1 g of sample = Y x 2.5 μg

Based on phenol content species were grouped into three ie., low (< 1.25 mg/g), medium (1.25-2.25 mg/g), high (> 2.25 mg/g).

3.3.2 Phosphorylase enzyme estimation

Phosphorylase enzyme of the seed was estimated according to the method described by Linskens *et al.* (1964). Species were classified into different groups based on phosphorylase enzyme activity. Those having an OD value less than 0.15 as low, between 0.15 and 0.2 as medium and more than 0.2 as high.

3.3.2.1 Preparation of reagents

1) Citrate buffer

21.01 g citric acid dissolved in 1000 ml - A

29.41 g sodium citrate dissolved in 1000 ml - B

9.5 ml of A mixed with 41.5 ml of B and pH adjusted to 6 to make citrate buffer

2) Saturated ammonium sulphate

Dissolved the ammonium sulphate in hot water till a precipitate was seen at the bottom. Cooled and decanted the saturated ammonium sulphate solution. Adjusted the pH to 6 by adding liquid ammonia drop by drop.

3) 5% starch

Dissolved 2.5 g soluble starch in 50 ml distilled water. Starch solution was prepared fresh for each analysis.

4) 0.26 M G I-P pH 6.0

Dissolved 0.968 g of G I-P in 10 ml of distilled water and adjusted the pH to 6.

5) Stopping reagent

Dissolved 2.5 g ammonium molybdate in 100 ml distilled water. Added 10 ml of 5N H_2SO_4 and 710 ml of water for preparing stopping reagent.

6) ANSA (1 amino 2 naphthol 4 sulphonic acid) reagent

First 12 g sodium metabisulphite and 1.2 g sodium sulphate were well powdered in a mortar and pestle. 200 mg of ANSA was added to it and again ground to fine powder. Finally the above mixture was dissolved in 100 ml distilled water.

7) 5N H_2SO_4

To 300 ml distilled water 70 ml concentrated H_2SO_4 was added, cooled and the volume made up to 500 ml.

8) 0.018 M ammonium molybdate

Dissolved 11.125 g ammonium molybdate in 500 ml distilled water.

3.3.2.2 Extraction

One gram seed of each species was ground in chilled mortar and pestle along with little quantity of acid washed sand and 10 ml extraction buffer. The extract was centrifuged at 15,000 rpm at 5°C for 20 minutes. Cold saturated ammonium sulphate of 20 ml quantity was added so that the saturation level of ammonium sulphate was about 65%, and kept overnight in refrigerator for precipitation and flocculation of enzyme. Next day the extract was centrifuged at 15,000 rpm for 20 minutes at 5°C. Supernatant was discarded and the enzyme at the bottom of the tube was dissolved in 1 ml of extraction buffer (pH 6). This enzyme solution was used for further analysis.

3.3.2.3 Procedure

To 1 ml of enzyme solution, added 0.25 ml starch and 0.2 ml G I-P and kept at room temperature. After 30 minutes, 7.15 ml stopping reagent, 0.9 ml 5N H_2SO_4 and 0.5 ml ANSA were added in a sequence and kept it as such for 10 minutes and read the absorbance at 660 nm. Phosphorylase enzyme activity was expressed in terms of OD value.

Results

RESULTS

Results of the study on reproductive biology and phosphorylase enzyme activity in four species of *Ocimum* are presented below.

4.1 Reproductive biology

4.1.1 Time taken for inflorescence development

The time taken for the full development of inflorescence in four different species are presented in Table 1.

4.1.2 Inflorescence characters

Inflorescence is a verticillaster, in all the four species under study (Plate 2). A pair of bracts are found subtending each verticil (Plate 3). Flower opening proceeded from bottom to top in all the four species, but very rarely opening started from middle also. The average length of inflorescence, number of flowers per inflorescence and distance between verticils are presented in Table 2.

4.1.3 Floral morphology

Comparison of the morphological characters of the flowers of four species viz., *O. tenuiflorum, O. gratissimum, O. canum* and *O. basilicum* are presented in Table 3 and depicted in Plates 4-8. Details of a single flower, L.S, C.S of ovary, floral formula and floral diagram of the four species under study are given in Fig. 1-5.

Plate 2. Inflorescence of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*

Plate 3. Bracts of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*

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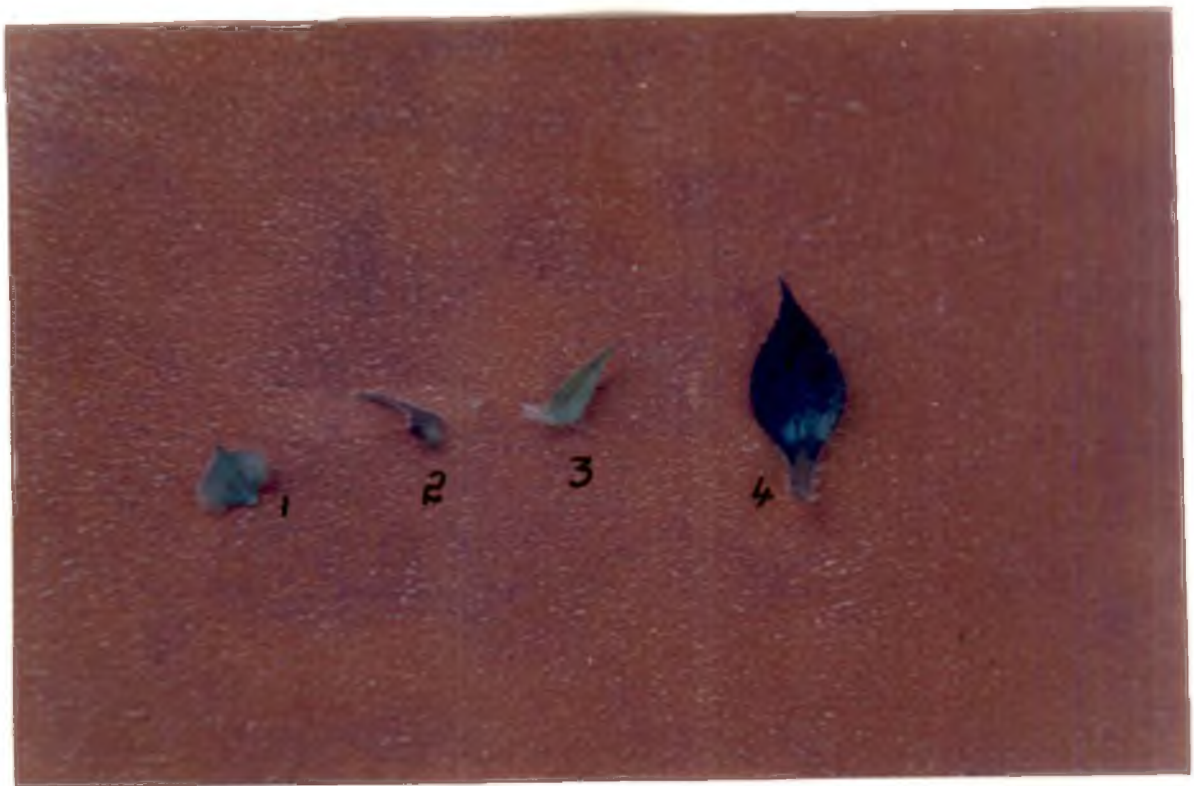
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Table 1. The average number of days for inflorescence development in different species of *Ocimum*

Species	Time for inflorescence development (days)
O. tenuiflorum	13
O. gratissimum	21
O. canum	6
O. basilicum	7
SEm±	0.91
CD (0.05)	2.62

Table 2. The average length, number of flowers per inflorescence and distance between verticils in different *Ocimum* species

Species	Length (cm)	Number of flowers	Distance between verticils (mm)	Number of flowers per unit length
O. tenuiflorum	7.5	47	6.5	6.4
O. gratissimum	11.6	67	7.7	5.8
O. canum	9.6	70	8.5	8.0
O. basilicum	10.9	42	12.5	4.4
SEm±	1.12	4.3	0.43	0.37
CD (0.05)	3.22	12.3	1.24	1.06

Table 3. Floral characters in four different species of *Ocimum*

Character	*O. tenuiflorum*	*O. gratissimum*	*O. canum*	*O. basilicum*
1	2	3	4	5
Bract				
Shape	Round	Elliptical	Elliptical	Elliptical
Size	2mm x 2mm	5mm x 2mm	4mm x 2mm	7mm x 3mm
Hairyness	Sparsely hairy	Sparsely hairy	Hairs of > 1.5 mm long	Sparsely hairy
Pedicellate/ Sessile	Sessile	Sessile	Pedicellate	Pedicellate
Colour	Purple	Green with purple colour along the margin	Green	Purple
Pedicel				
Length	3 mm	3 mm	2 mm	3 mm
Colour	Purple	Green	Green	Purple
Hairyness	Hairy	Hairy	Hairy	Hairy
Calyx				
Size	2 mm	3 mm	3 mm	3 mm
Colour	Purple	Green	Green	Purple with green tinge
Hairyness	Upper lip glabrous lower lip pubescent	Pubescent	Pubescent	Sparsely haired
Nature of calyx throat	Open	Closed	Open	Open
Corolla				
Shape	Funnel shaped	Funnel shaped	Funnel shaped	Funnel shaped

Contd.

Table 3. Continued

1	2	3	4	5
Colour	Purple	Dull white	White	Cream coloured with a purple tinge
Size	3-4 mm	3-5 mm	4-5 mm	7 mm
Hairyness	Sparsely hairy	Sparsely hairy	Hairy	Sparsely hairy
Relative size of upper and lower lip	Upper lip prominent	Not much difference between upper and lower lip	Upper lip prominent	Lower lip more prominent
Androecium				
Stamen colour	Yellow	Yellow	Cream	Cream
Attachment of filament to anther lobe	Dorsifixed	Dorsifixed	Dorsifixed	Dorsifixed
Length of stamen protruding out of corolla	2 mm	1 mm	1-2 mm	2-4 mm
Gynoecium				
Nature of style	Gynobasic	Gynobasic	Gynobasic	Gynobasic
Colour of style	Purplish	Cream	Cream	Cream with a purple tinge
Length of style	5-6 mm	6 mm	6 mm	10 mm
Stigma	Bifid	Bifid	Bifid	Bifid
Placentation	Axile	Axile	Axile	Axile

Plate 4. Flowers of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*

Plate 5. Calyx of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*

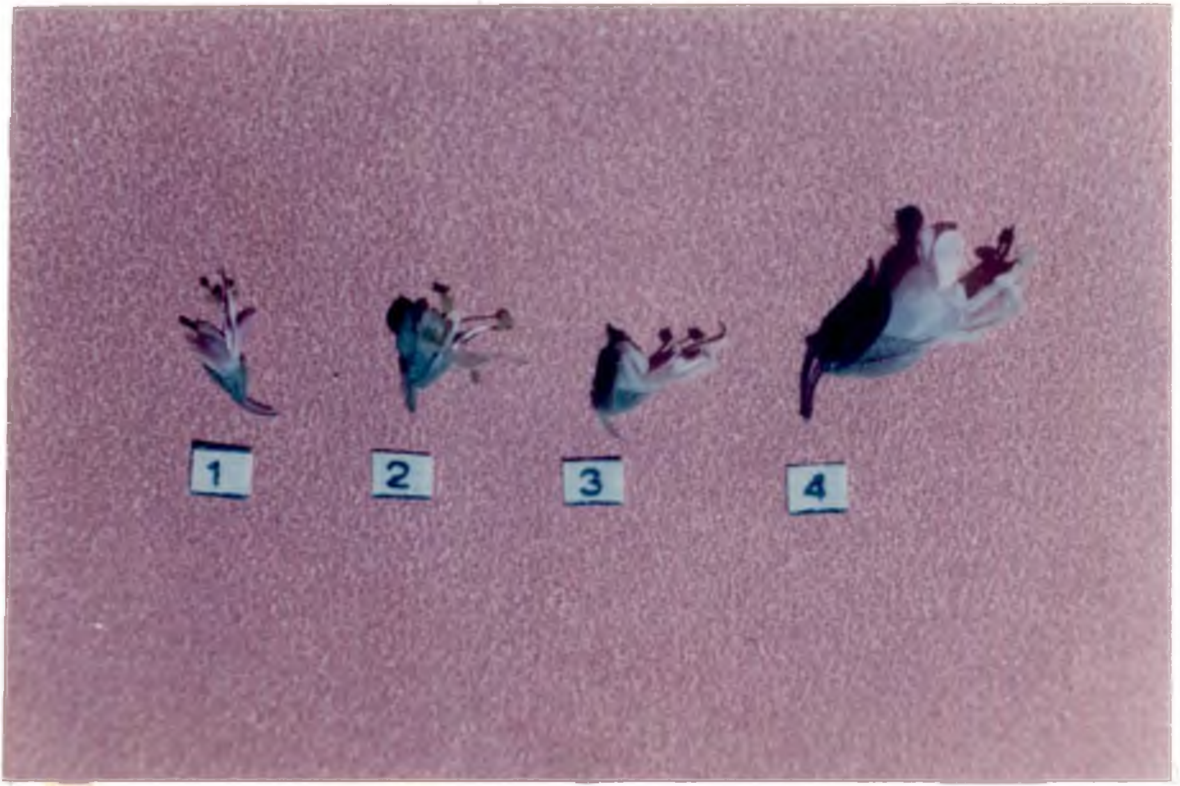
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Plate 6. Corolla of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*

Plate 7. Androecium of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*

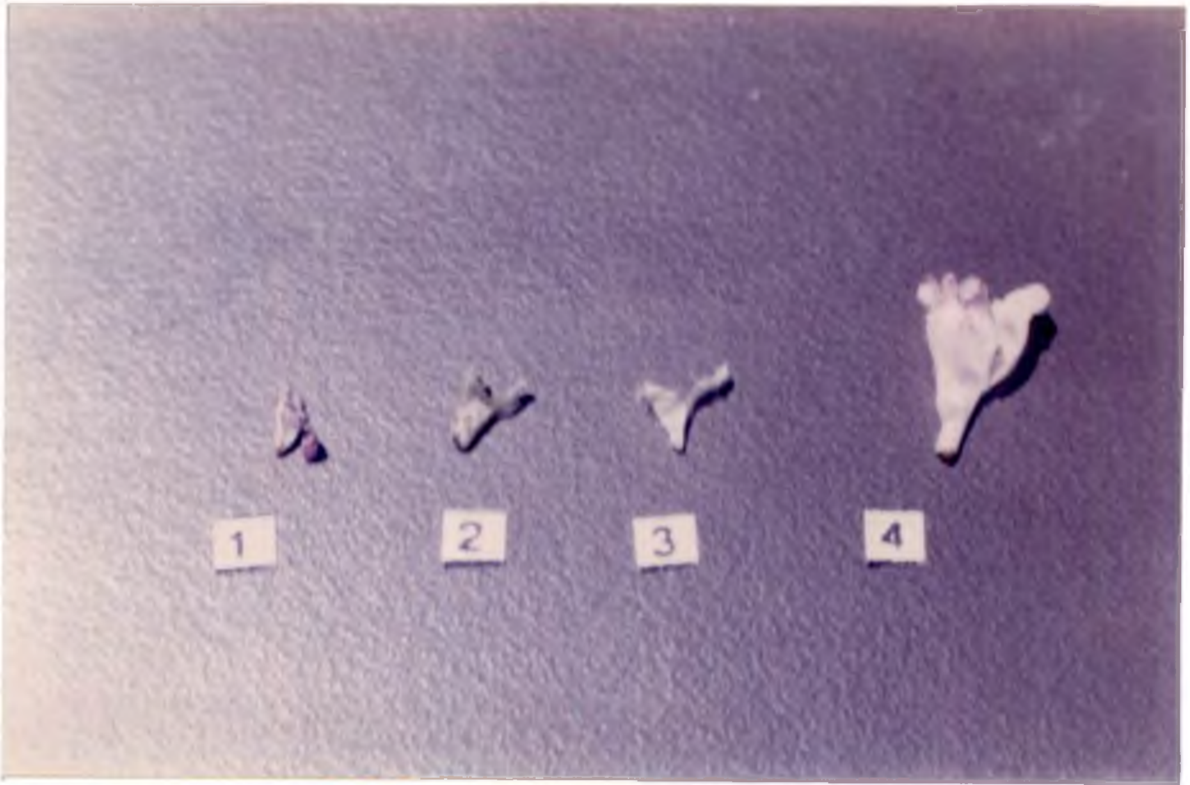
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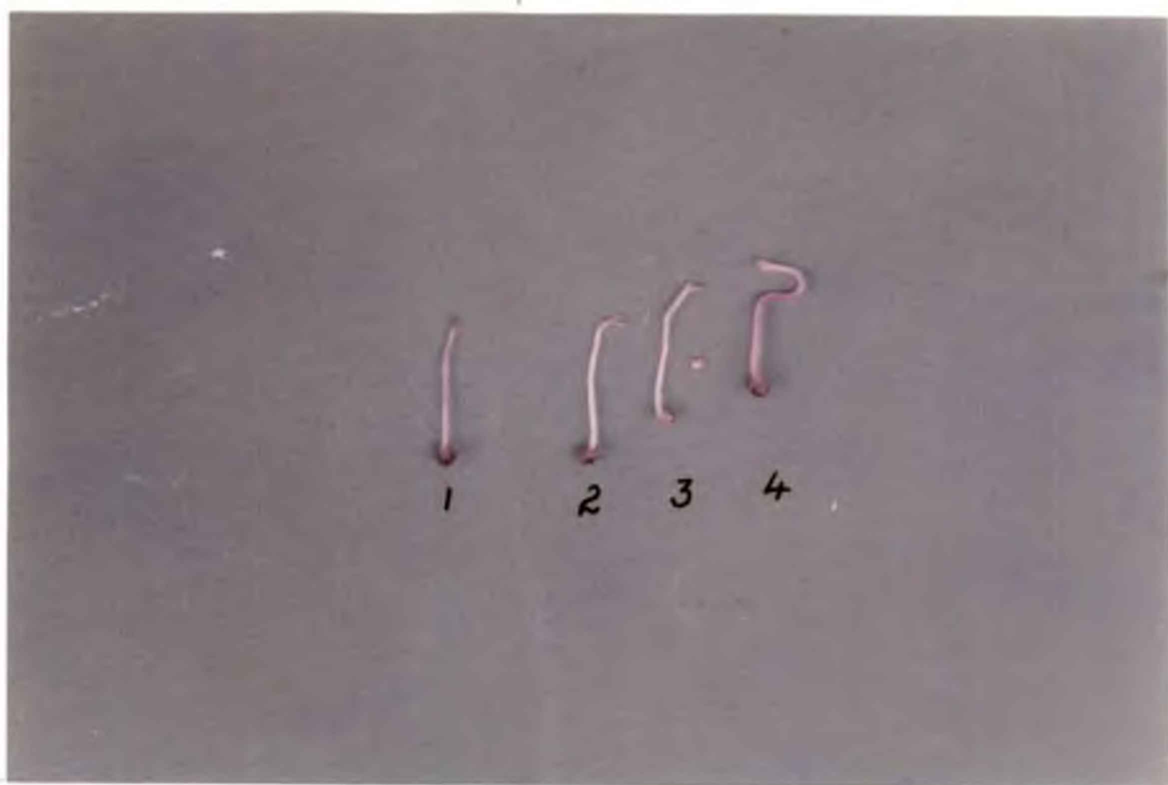
Plate 8. Gynoecium of different species of *Ocimum*

1) *Ocimum tenuiflorum*
2) *Ocimum gratissimum*
3) *Ocimum canum*
4) *Ocimum basilicum*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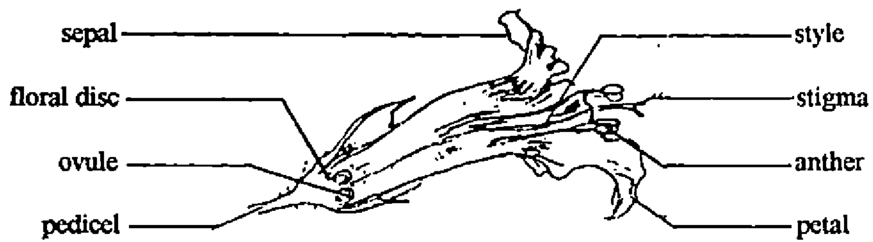
A single flower



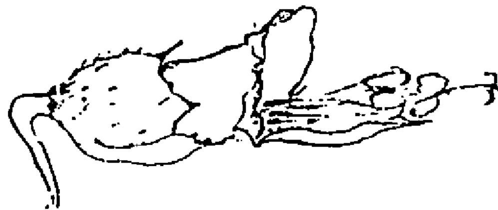
L. S of a single flower

Fig. 1. Floral morphology of *ocimum tenuiflorum*

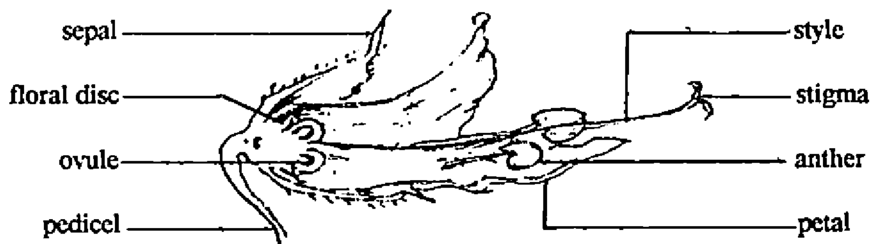
A single flower

L. S of a single flower

Fig. 2. Floral morphology of *ocimum gratissimum*

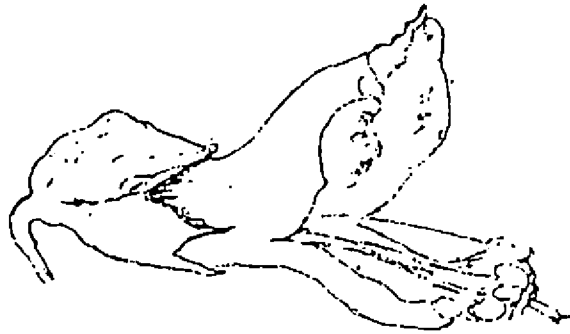


A single flower

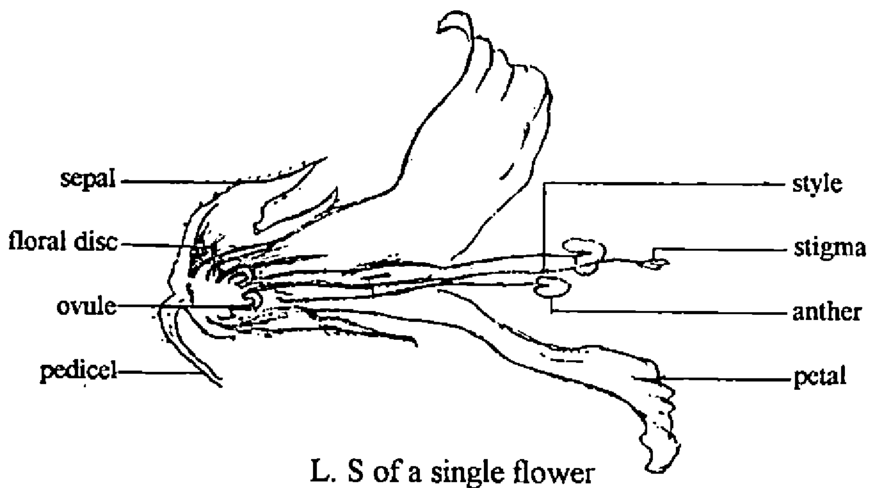


L. S of a single flower

Fig. 3. Floral morphology of *ocimum canum*

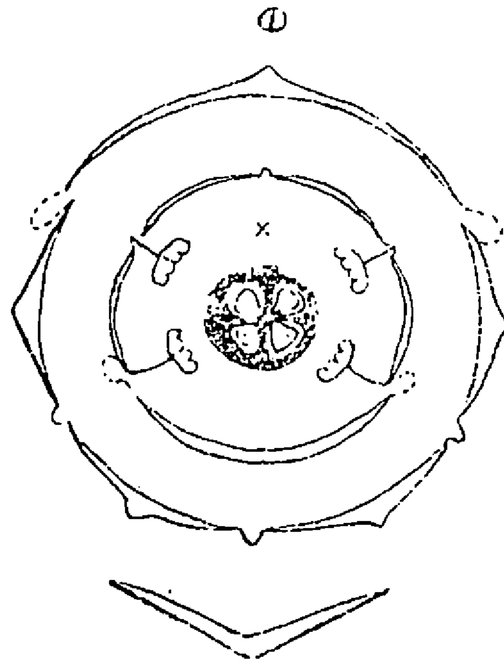


A single flower



L. S of a single flower

Fig. 4. Floral morphology of *ocimum basilicum*



Floral diagram

Ф, ⚥, $K_{(1/4)}$, $\overset{\frown}{C_{(4/1)}, A_{2+2}}$, $\underline{G}_{(2)}$

Floral formula

C. S. of ovary

Fig. 5. Floral diagram, floral formula and C. S. of ovary in *ocimum* species

4.1.4 Anthesis

4.1.4.1 Time of flower opening and peak period of anthesis in an inflorescence

Preliminary study of anthesis revealed that there was no flower opening between 4 pm and 4 am. After the preliminary study observations on flower opening were taken at bihourly intervals. Results of the study conducted at bihourly intervals are presented in Tables 4 and 5. Peak day of anthesis in an inflorescence is given in Table 6 and Fig. 6.

4.1.4.2 Time of anther dehiscence and stigma receptivity

Anther dehiscence was observed in the bud stage in *O. tenuiflorum* and *O. gratissimum* on bright sunny days. During cloudy days the anther dehiscence was delayed in *O. gratissimum* and it occurred only after flower opening. In the other two species *O. canum* and *O. basilicum* anther dehiscence occurred 15-20 minutes after flower opening. In the presence of bee activity the anther dehiscence was found to be earlier i.e., immediately after flower opening in *O. canum* and *O. basilicum*. Details on stigma receptivity in four species are given in Table 7.

4.1.5 Mode of pollination

Results of experiment conducted to find out the role played by different pollinating agents like insects, water and wind in *Ocimum* species are given in Table 8.

4.1.6 Pollen morphology and fertility

Four species of *Ocimum* varied in size as well as shape of pollen grains.

Table 4. Anthesis time in different species of *Ocimum*

Species	Total number of flowers observed	Number of flowers opened						Percentage of flowers opened					
		4-6 am	6-8 am	8-10 am	10-12 noon	12-2 pm	2-4 pm	4-6 am	6-8 am	8-10 am	10-12 noon	12-20 pm	2-4 pm
O. tenuiflorum	568	0	11	181	324	52	0	0	1.94	31.87	57.04	9.15	0
O. gratissimum	447	0	23	152	176	82	14	0	5.15	34.01	39.37	18.34	3.13
O. canum	1575	0	496	660	415	4	0	0	31.49	41.90	26.36	0.25	0
O. basilicum	460	0	121	128	190	21	0	0	26.30	27.83	41.30	4.57	0

Table 5. Peak anthesis time in different species of *Ocimum*

Species	Peak anthesis time	Percentage of flowers opened at peak anthesis time
O. tenuiflorum	10 am - 12 noon	57.04
O. gratissimum	10 am - 12 noon	39.37
O. canum	8 am - 10 am	41.90
O. basilicum	10 am - 12 noon	41.30

Table 6. Peak day of anthesis in an inflorescence in different species of *Ocimum*

Species	Percentage of flowers opened in an inflorescence																								
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day	22nd day	23rd day	24th day	Mean number of days for anthesis/ inflorescence
O. tenuiflorum	17.58	38.74	23.35	9.34	7.97	3.02																			5
O. gratissimum	22.40	22.40	23.49	22.95	3.29	4.92	0.55																		4
O. canum	6.36	9.80	7.95	7.95	8.81	6.50	4.65	5.01	4.77	4.17	4.41	4.90	3.42	3.30	3.92	2.94	2.45	1.84	1.47	1.60	1.71	1.22	0.73	0.12	21
O. basilicum	9.77	8.90	7.04	6.26	8.60	6.65	6.26	5.86	10.16	5.77	5.47	3.13	4.30	3.91	0.00	1.96									12

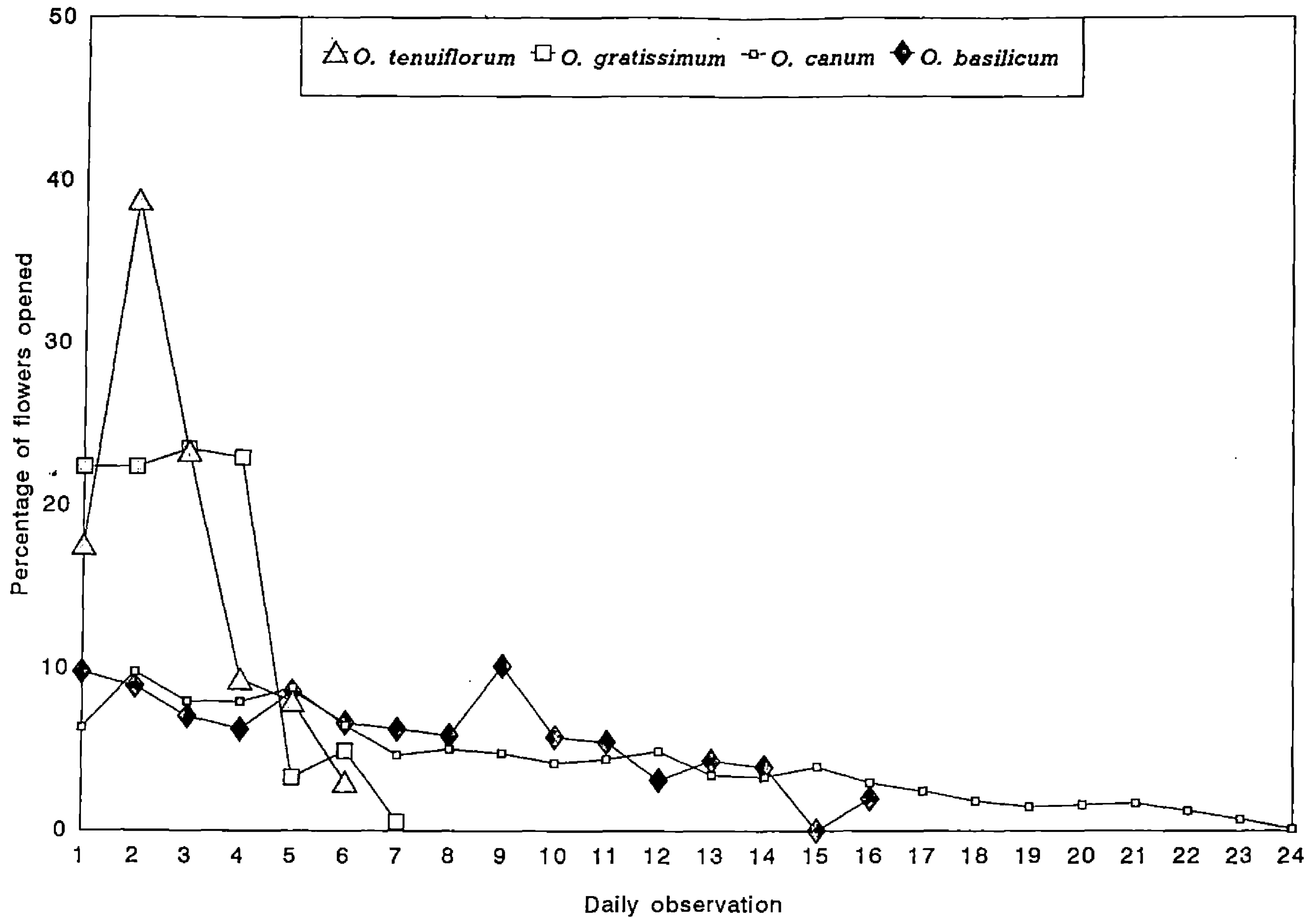


Fig.6. Percentage of daily flower opening in an inflorescence in *Ocimum* species

Table 7. Stigma receptivity in different species of *Ocimum*

Species	Time for starting receptivity after flower opening (minutes)	Duration of receptivity	
		hours	minutes
O. tenuiflorum	30	2	55
O. gratissimum	30	3	55
O. canum	30	3	20
O. basilicum	30	4	30

Table 8. Seed set under different treatments in four *Ocimum* species

Treatments	Mean seed set (%)			
	O. tenuiflorum	*O. gratissimum*	*O. canum*	*O. basilicum*
T_1 - Fully covered	27.24	48.48	23.12	21.78
T_2 - Fully covered and sprayed with water at anthesis time	24.86	55.72	10.56	6.94
T_3 - Kept uncovered only at anthesis time	90.79	94.99	91.97	92.14
T_4 - Fully uncovered	89.11	94.91	92.49	91.10
SEm±	4.83	3.12	3.98	3.24
CD (0.05)	14.48	9.35	11.93	9.71

Morphological features of pollen grains of different species under study are presented in Table 9 and Plates 9-12.

The results of pollen fertility analysis by acetocarmine staining technique are given in Table 10.

4.2 Seed characters

4.2.1 Seed moisture content (%)

Moisture content of freshly harvested seeds was examined by gravimetric method and results are presented in Table 11.

4.2.2 1000-seed weight

The 1000-seed weight of four species of *Ocimum* are given in Table 12.

4.2.3 Seed density

Observations on seed density of the four species are given in Table 13.

4.2.4 Dormancy and germination percentage

Germination test was conducted on the day of harvest and repeated at ten days interval until the germinability exceeded 80 per cent. The data pertaining to this are presented in Table 14.

4.3 Biochemical studies

4.3.1 Total phenol

Total phenol content of seeds of four different species of *Ocimum* are presented in Table 15.

Table 9. Morphological features of pollen grains of different species of *Ocimum*

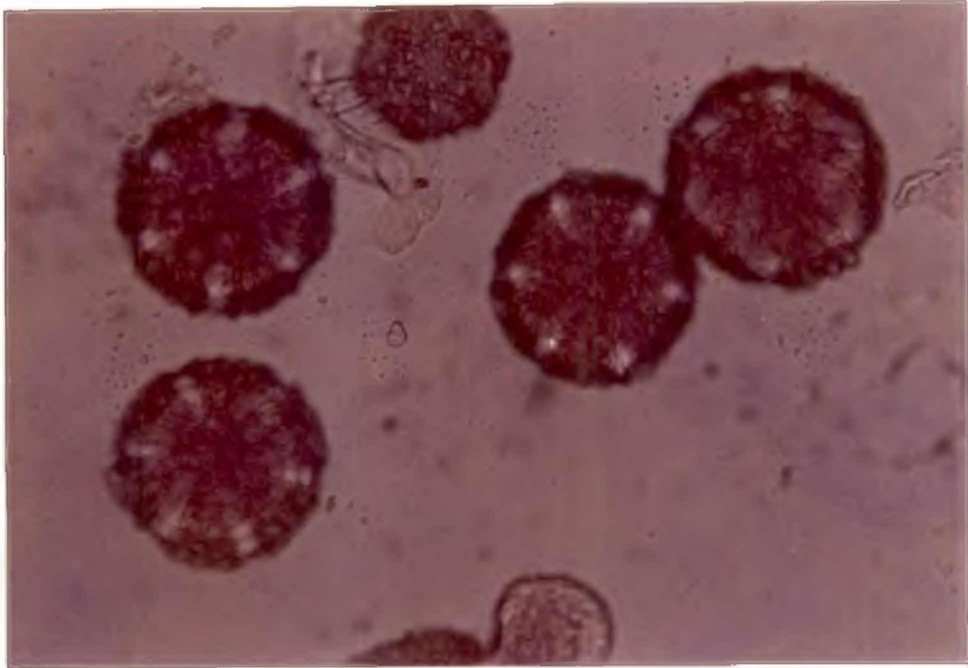
Species	Colour as appeared to naked eye	Type	Mean size (microns)	Shape
O. tenuiflorum	Yellow	Hexacolpate and reticulate	37.8	Round
O. gratissimum	Yellow	Hexacolpate and reticulate	38.25 x 54.25	Oval
O. canum	White	Hexacolpate and reticulate	52.2	Round
O. basilicum	White	Hexacolpate and reticulate	62.1	Round

Table 10. Pollen fertility in different species of *Ocimum*

Species	Mean fertility (%)	Range
O. tenuiflorum	92.50	83.33-100
O. gratissimum	77.24	75.75-78.94
O. canum	93.59	80.00-100
O. basilicum	80.70	75.00-88.00
CD(0.05)	4.11	

Plate 9. Pollen grains of *Ocimum tenuiflorum* (x 1000)

Plate 10. Pollen grains of *Ocimum gratissimum* (x 400)



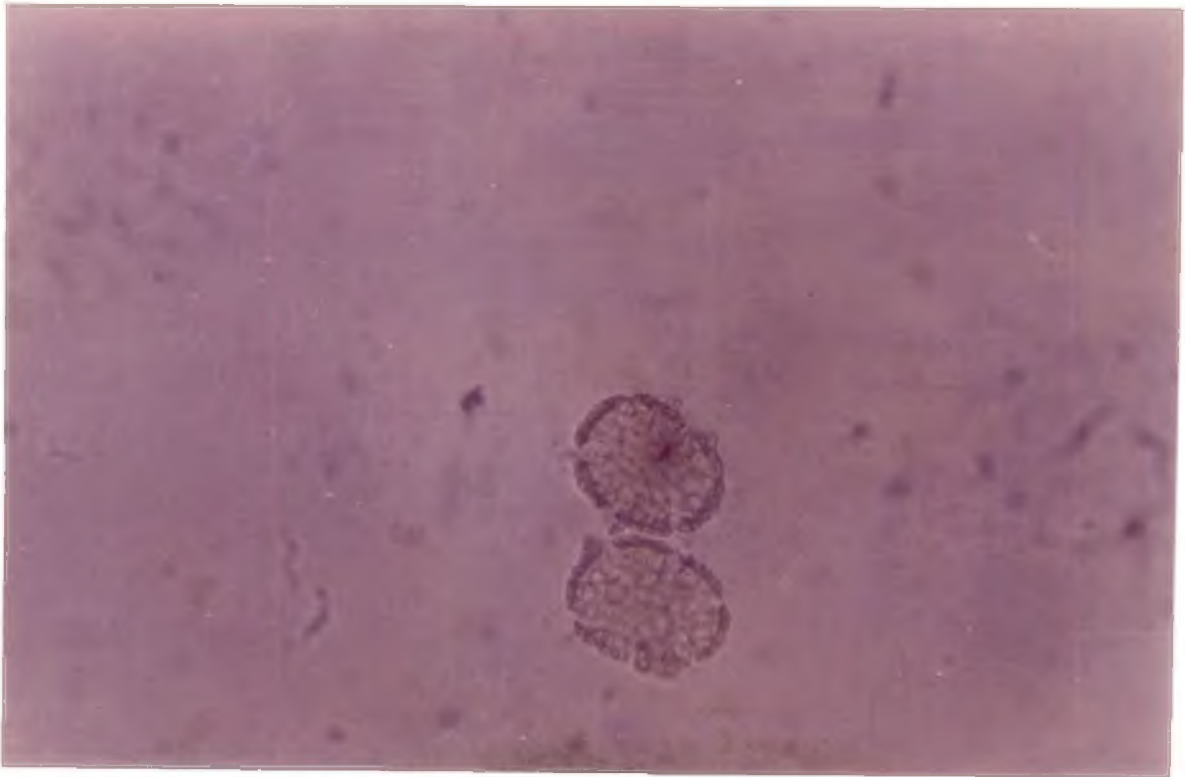
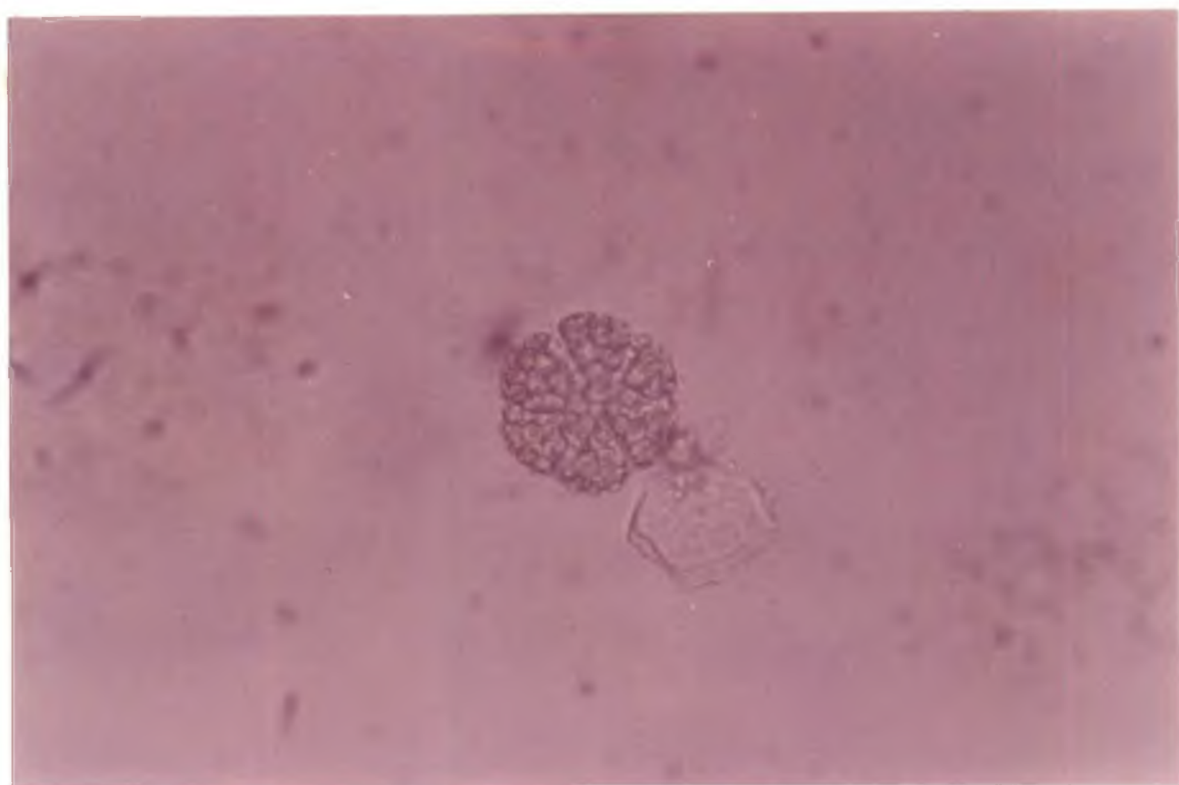


Plate 11. Pollen grain of *Ocimum canum* (x 400)

Plate 12. Pollen grains of *Ocimum basilicum* (x 400)



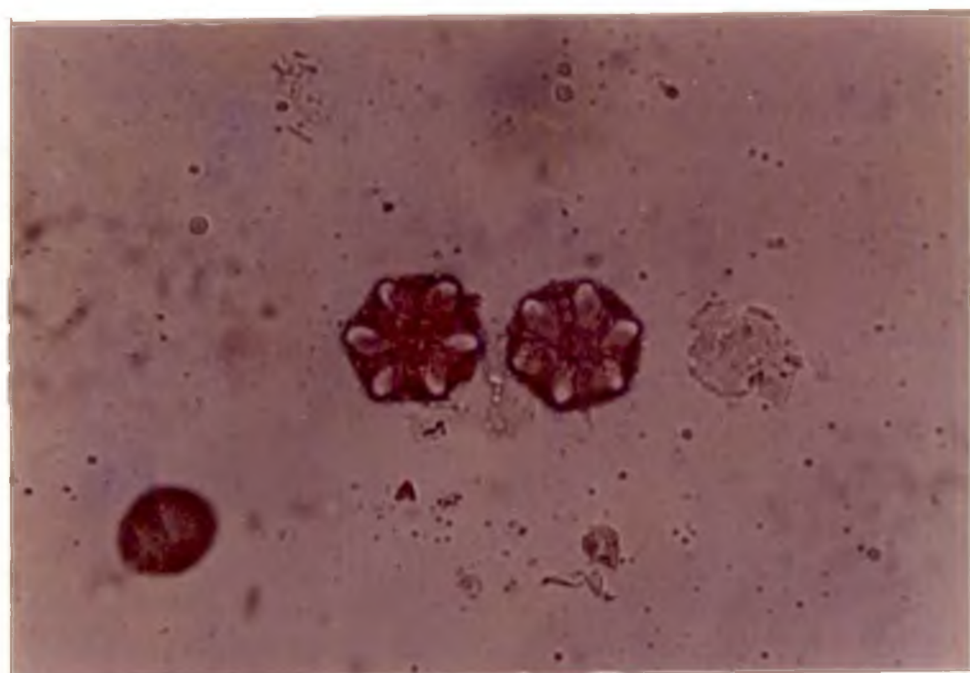


Table 11. Moisture content of freshly harvested seeds of different species of *Ocimum*

Species	Mean value (%)
O. tenuiflorum	11.67
O. gratissimum	7.83
O. canum	7.88
O. basilicum	8.45
SEm±	0.56
CD(0.05)	1.73

Table 12. 1000-seed weight in four species of *Ocimum*

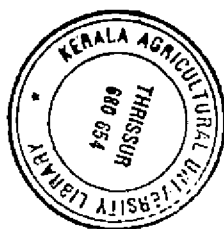
Species	Mean value (g)
O. tenuiflorum	0.2035
O. gratissimum	1.1562
O. canum	0.3605
O. basilicum	1.2681
SEm±	0.03
CD(0.05)	0.09

Table 13. Seed density in four species of *Ocimum*

Species	Mean value
O. tenuiflorum	0.5726
O. gratissimum	0.6582
O. canum	0.4468
O. basilicum	0.6432
SEm±	0.02
CD(0.05)	0.06

Table 14. The germination percentage and period of dormancy in different species of *Ocimum*

Species	At harvest	Days after harvest												Dormancy at harvest (%)	Dormancy period
		10	20	30	40	50	60	70	80	90	100	110	120		
O. tenuiflorum	0	0	0	0	0	0	0	0	0	1.0	42	79	84	100	120
O. gratissimum	0	13	60	95	-	-	-	-	-	-	-	-	-	100	30
O. canum	0	20	32	40	40	45	50	55	85	-	-	-	-	100	80
O. basilium	25	25	30	34	55	65	85	-	-	-	-	-	-	75	60



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4.3.2 Phosphorylase enzyme activity

Experiment was started according to the method described by Linskens *et al.* (1964). But no activity was observed. Assuming that the phosphorylase form is different, the method was modified by taking higher quantity of extract directly and raising the temperature to 35°C during activity study. Still activity was not observed. In order to avoid the inhibitors and muscilage activated charcoal was used and to find out the possibility of using co-factor, AMP was added. Again there was no activity. Finaly an extraction buffer was prepared instead of citrate buffer in the method described by Linskens *et al.* (1964) and OD value recorded at 650 nm. The composition of extraction buffer used is given below:

Hydroxy methyl (Tris)	- 21.1995 g	A
Citric acid	- 2.62675 g	
Vitamin C	- 0.52839 g	
Cysteine HCl	- 0.52689	

The above formulation A made up to 500 ml. From this 1 ml of A made upto 100 ml and pH adjusted to 7 to make the extraction buffer.

Phosphorylase enzyme activity of selected *Ocimum* species are given in Table 15.

Relation of phosphorylase enzyme activity and phenol content in the seeds are presented in Fig. 7.

Table 15. Total phenol content and phosphorylase enzyme activity of different species of *Ocimum*

Species	Total phenol (mg/g of seeds)	Activity for 1 g of sample (OD value)
O. tenuiflorum	1.15	0.498
O. gratissimum	1.67	0.199
O. canum	2.39	0.121
O. basilicum	1.22	0.346

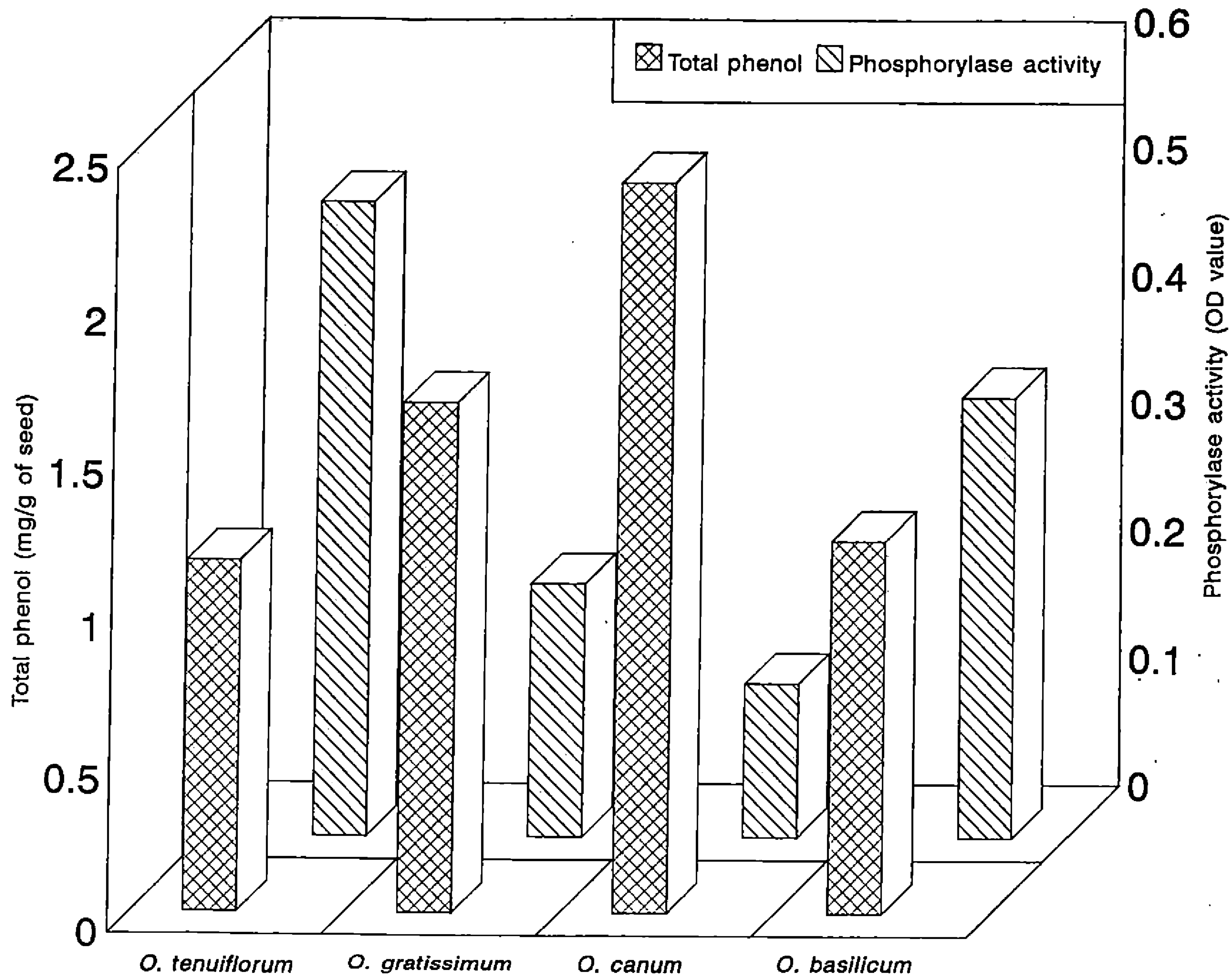

Fig.7. Relation of phosphorylase activity and total phenol content in *Ocimum* species

Discussion

DISCUSSION

Based on the observations on the different aspects of inflorescence characters, anthesis, mode of pollination and seed characters the results are disscussed.

5.1 Reproductive biology

5.1.1 Time taken for inflorescence development

Analysis on the species level variations in the number of days required for inflorescence development showed significant difference among the four species of *Ocimum* - *O. tenuiflorum, O. gratissimum, O. canum* and *O. basilicum* (Table 1).

The time taken for inflorescence development showed a variation ranging from 6 days in *O. canum* to 21 days in *O. gratissimum* (Table 1). *O. gratissimum* and *O. tenuiflorum* differed significantly from *O. canum* and *O. basilicum*. Hence it can be concluded that Sanctum group which includes *O. tenuiflorum* and *O. gratissimum* require more number of days for inflorescence development than Basilicum group which includes *O. canum* and *O. basilicum*.

5.1.2 Inflorescence characters

Inflorescence was a verticillaster produced on current seasons growth and was terminal in position in all the four species studied. There was similarity in the basic features of inflorescence in all of them. But they significantly differed in length of inflorescence, flowers/inflorescence and distance between verticils (Table 2). All the four species followed the same sequence of flower opening and it

started from the base and progressed towards the tip. The uniformity in the acropetal succession of flower opening observed in the four different species belonging to two groups may be due to identity in basic features. An acropetal sequence of flower opening has also been reported by Sobti and Pushpangadan (1982) in *Ocimum*. But the reports of Putievsky (1983) did not agree the present result.

The mean length of inflorescence varied from 7.5 cm in *O. tenuiflorum* to 11.6 cm in *O. gratissimum* (Table 2). The mean length of fully developed inflorescence of *O. tenuiflorum* (7.5 cm) observed in the present study deviated notably from mean length of 15.3 cm reported by Singh and Sharma (1980).

Significant variation was observed among the species in total number of flowers per inflorescence and distance between verticils. *O. tenuiflorum* and *O. basilicum* did not show significant difference in number of flowers per inflorescence (47 and 42). But they were significantly different from *O. gratissimum* and *O. canum* (67 and 69) which were found to be on par (Table 2). The distance between verticils was the highest in *O. basilicum* (12.5 mm) and differed significantly from the other species. *O. tenuiflorum* exhibited the lowest value (6.5 mm) among the four species.

O. gratissimum and *O. basilicum* did not differ in inflorescence length, but significant difference was observed in the total number of flowers per inflorescence. This can be attributed to the greater distance between adjacent verticils of *O. basilicum*. Verticil to verticil distance was comparatively lower in Sanctum group than Basilicum group (Table 2). It can be seen that the mean length of inflorescence was reduced from 11.6 cm in *O. gratissimum* to 7.5 cm in *O. tenuiflorum* in Sanctum group. The reduction in length of inflorescence in

O. tenuiflorum is compensated by increased flower production per unit length of inflorescence. Similar is the case with *O. canum* of Basilicum group (Table 2). In *O. canum* the mean length of inflorescence is only 9.6 cm. The reduction in inflorescence length is compensated by the presence of more number of flowers per unit length.

5.1.3 Floral morphology

Detailed study on the floral morphology revealed that the basic floral structure of all the four species were identical. The flowers were bracteate, pedicellate, complete, zygomorphic, hermaphrodite, pentamerous, hypogynous and bilabiate.

Calyx consisted of five sepals, gamosepalous, bilabiate and persistent. Corolla consisted of five petals, gamopetalous and bilipped. Stamens four in number, epipetalous, didynamous and dorsifixed. Gynoecium was bicarpellary, tetralocular with one ovule in each locule, placentation axile, style gynobasic and stigma bifid (Table 3). The flowers of all the species were short lived and withered away within 3-5 hrs of flower opening. Flowers of Sanctum group were less conspicous with sessile bracts and flowers of Basilicum group were more conspicous with pedicellate bracts.

The reports on floral morphology of *O. sanctum* by Singh and Sharma (1980) and *O. basilicum* by Bendre and Kumar (1975) are in agreement with the above results.

5.1.4 Anthesis

5.1.4.1 Anthesis time and peak period of anthesis in an inflorescence

During preliminary study mature buds remained unopened between 4 pm and 4 am. Detailed study on anthesis, conducted at bihourly intervals, revealed that there was a steady increase in the per cent of flower opening from 6 am to 12 noon in *O. tenuiflorum, O. gratissimum* and *O. basilicum* and from 6 to 10 am in *O. canum* followed by a decline (Table 4). It is also evident that flower opening was maximum between 8 am to 12 noon and reached the peak between 10 am and 12 noon in *O. tenuiflorum, O. gratissimum* and *O. basilicum*. Above results agree with the reports of Singh and Sharma (1980) in tulsi. In *O. canum* maximum flowers opened between 6 and 10 am with the peak period from 8 to 10 am. There was uniformity in the peak time of anthesis of three species under study with slight variation in *O. canum*.

Eventhough the peak time of anthesis remained the same in *O. tenuiflorum, O. gratissimum* and *O. basilicum* the percentage of flower opening varied.

The mean number of days for completion of anthesis in an inflorescence varied from 4 in *O. gratissimum* to 21 in *O. canum* (Table 6). The highest percentage of flower opening was recorded on the second day in *O. tenuiflorum* and *O. canum*, third day in *O. gratissimum* and 9th day in *O. basilicum* (Table 6). After reaching the peak, the per cent of flower opening gradually decreased on the subsequent days in *O. tenuiflorum, O. gratissimum* and *O. canum*. However irregularity was observed in *O. basilicum*.

From Table 6 it is clear that there is variation in the pattern of anthesis in Sanctum group and Basilicum group. Anthesis in an inflorescence was completed in a period of 4-5 days in Sanctum group. This can be due to the fact that the proportion of flower opening on the initial days of anthesis was high (> 15%). The proportion of flower opening on each day of anthesis remained low (< 15%) in Basilicum group and hence the period of anthesis per inflorescence ranged from 12-21 days. The longest anthesis period of 21 days in *O. canum* recorded in the present study can also be attributed to the presence of significantly higher number of flowers per inflorescence.

5.1.4.2 Anther dehiscence and stigma receptivity

Anther dehiscence was found to occur in bud stage in *O. gratissimum* and *O. tenuiflorum* which belonged to Sanctum group. Similar results were observed by Singh and Sharma (1980). According to them a partial dehiscence of anthers occurred in the bud of tulsi.

In other two species *O. basilicum* and *O. canum*, dehiscence of anthers occurred 15-20 minutes after flower opening. But, Raju (1989) found that in *O. basilicum* anther dehiscence occurred in the bud. This is quite contradictory to the present finding.

In all the four species studied the stigma became receptive 30 minutes after flower opening and remained so for varying periods depending on the species. The duration of stigma receptivity varied from 2 hrs 55 min in *O. tenuiflorum* to 4 hrs 30 min in *O. basilicum* (Table 7).

Thus it can be concluded that flowers of *Ocimum* are protandrous and the anther dehiscence was influenced by factors like bee activity, weather conditions etc. Cloudy days delayed the dehiscence of anthers and bright sunny days and high bee activity promoted early dehiscence of anthers.

5.1.5 Mode of pollination

Observation on mode of pollination revealed that there was fruit set even in completely bagged inflorescence. The percentage of fruit set varied from 21.78 per cent in *O. basilicum* to 48.48 per cent in *O. gratissimum*. This points to the fact that there is no self-incompatibility mechanism operating in the species.

Among the four species fruit set was higher under selfed conditions in *O. tenuiflorum* and *O. gratissimum* compared to other two species. Good seed set noticed by Sobti and Pushpangadan (1982) in species belonging to Sanctum group is in agreement with the present finding.

The data presented in Table 8 revealed that fruit set in water sprayed inflorescence varied from 6.94 per cent in *O. basilicum* to 55.72 per cent in *O. gratissimum*. No significant difference was noticed in seed set in water sprayed inflorescence compared to fully covered inflorescence in *O. gratissimum* and *O. tenuiflorum*. In *O. canum* and *O. basilicum* percentage fruit set was lower in watered inflorescence when compared to fully covered inflorescence. This may be due to the decay of flowers in Basilicum group when sprayed with water, as they were more delicate than that of Sanctum group.

Significantly higher fruit set was observed in case of inflorescences fully uncovered and uncovered only at the anthesis time. These two treatments were on par with each other also. From these points it is evident that *Ocimum* species is more adapted to cross pollination. The morphological feature of the flower and protandrous nature favours cross pollination in *Ocimum*. The pollination has to be effected through agents like wind and insects. It also appears that water has no role in pollination mechanism of *Ocimum*. It was also observed that insects like ants, bees, flies and other hymenopterans visited the flowers at anthesis time. They may be acting as pollinators. This observation was in line with the findings of Darrah (1974), Krishnan (1981) and Raju (1989). It can be concluded that *Ocimum* is adapted to cross pollination and day flying insects are playing a major role in effecting pollination.

5.1.6 Pollen morphology and fertility

Pollen grains of all the four species were hexacolpate and reticulate. The findings of Husain and Heywood (1982) in *Origanum* and allied genera and Gill and Chinnappa (1982) on West-Himalayan Labiatae supported the above results.

Pollen grains appeared as yellow powdery mass to the naked eye in Sanctum group and white powdery mass in Basilicum group. Species level difference was observed in the size as well as shape of pollen grains. Except in *O. gratissimum* pollen grains were spherical in shape. In *O. gratissimum* pollen grains were ovoid in shape. The mean diameter of pollen grains ranged from 37.8 to 62.1 microns among the four species (Table 9). The highest mean diameter was recorded in *O. basilicum* followed by *O. canum*. Reports of Singh and Sharma (1980) that pollen grains were

round and size ranged from 36.40 to 48.55 microns in tulsi supported the present result. Reports of Parry (1969) and Gill and Chinnappa (1982) are also in agreement with the present finding. Kumar *et al.* (1994) observed that in marjoram, a member of Labiatae, pollen grains were oval with size ranging from 27.70 to 43.74 microns. Similar results were obtained for *O. gratissimum* in the present study.

Estimation of pollen fertility by acetocarmine staining technique revealed significant variation among the species. The mean pollen fertility varied from 77.24 per cent in *O. gratissimum* to 93.5 per cent in *O. canum* (Table 10). Singh and Sharma (1980) observed that pollen fertility of *O. sanctum* ranged from 83.60 to 94.15 per cent. This supports the findings of the present study. Ryding (1993) reported that pollen fertility of *O. basilicum* was 98 per cent. It is not in conformity with the reports of the present investigation.

5.2 Seed characters

5.2.1 Moisture content

Among the four species *O. tenuiflorum* differed significantly in moisture content from the other three species which were found to be on par (Table 11). Highest moisture content was for *O. tenuiflorum* (11.67%) and lowest for *O. gratissimum* (7.83%). *O. tenuiflorum* belonged to high moisture content group. *O. canum* and *O. gratissimum* belonged to low moisture content group. *O. basilicum* had a medium moisture content at harvest.

5.2.2 1000-seed weight

There was significant differences among the four species with respect to 1000-seed weight (Table 12). The highest 1000 seed weight was for *O. basilicum*

(1.27 g). This was followed by *O. gratissimum* (1.16 g), *O. canum* (0.36 g) and *O. tenuiflorum* (0.20 g). These results are in agreement with the reports of Putievsky (1983) in *O. basilicum* and Dey and Choudhuri (1982) in *O. sanctum*. *O. gratissimum* and *O. basilicum* fell into high seed weight group.

5.2.3 Seed density

The four species under study differed significantly in seed density (Table 13): It varied from 0.4468 in *O. canum* to 0.6582 in *O. gratissimum*. *O. gratissium* and *O. basilicum* seed weight was found to be high. Higher seed density observed in these two species can be attributed to higher seed weight.

5.2.4 Dormancy

There was no uniformity in the breaking of dormancy and it was achieved by different species in different periods (Table 14). *O. gratissimum* belonged to low dormancy period group and *O. tenuiflorum* to prolonged dormancy period group. Medium dormancy period group included *O. canum* and *O. basilicum.*

O. basilicum alone recorded an initial sprouting value of 25 per cent. All the other species recorded initial sprouting value of zero leading to cent per cent dormancy at harvest (Table 14).

The results also revealed that there was a progressive increase in the per cent of germination in successive tests after ten days. However, the pattern of increase observed was not the same in all the species. A sudden increase of germination per cent (40% or more between successive tests) was observed in

O. tenuiflorum and *O. gratissimum* both belonging to Sanctum group. Basilicum group which included *O. canum* and *O. basilicum* exhibited a slow increase in germination per cent. Hence it can be concluded that dormancy breakage was sudden in Sanctum group and gradual in Basilicum group.

5.3 Biochemical studies

5.3.1 Total phenol

Four species of *Ocimum* showed variation in the total phenol content of seeds (Table 15). Among the four species *O. canum* had the highest and *O. tenuiflorum* the lowest phenol content. Dey and Choudhuri (1982) reported that the polyphenol content in *O. tenuiflorum* seed was influenced by plant age and it varied from 0.2 to 0.6 mg/g of seed. Same species analysed in the present study showed a higher value (1.15 mg/g). This variation in the phenol content may be due to the influence of other factors including soil conditions.

5.3.2 Phosphorylase enzyme activity

Phosphorylase is the first glycolytic enzyme necessary for the release of energy needed for growth and development and it is having a major role in breaking dormancy.

Present study revealed that phosphorylase form in *Ocimum* was independent in nature for different media and conditions. In *Ocimum* spp. phosphorylase activity was obtained in an extraction buffer having a combination of Hydroxy methyl (Tris), citric acid, vitamin C and cysteine at pH 7. Citrate buffer extraction showed no activity as reported by Linskens *et al.* (1964). It may be due to the difference in the phosphorylase form either in structure or configuration.

Based on seed weight, phosphorylase activity and total phenol content *Ocimum* spp. can be grouped into three (Table 16).

(1) Species with low seed weight, high enzyme activity and low phenol content included *O. tenuiflorum*.

(2) Species with high seed weight, medium/high phosphorylase enzyme activity and medium/low phenol content included *O. gratissimum* and *O. basilicum*.

(3) Species with low seed weight, low phosphorylase activity and high phenol content included *O. canum*.

In the first group which included *O. tenuiflorum* dormancy period was high due to complete depletion of stored carbohydrate by phosphorylase activity. Here phenol interference was low. This is in agreement with the findings of Dey and Choudhuri (1982) that low phenol content delayed germination.

The second group included *O. gratissimum* and *O. basilicum* where the seed weight was high and the phosphorylase activity medium/high. Stored food was not in any way depleted in the course of germination due to high seed weight. Total phenol content did not affect germination. This group represented low/medium dormancy period.

The third group which included *O. canum* had low phosphorylase activity, due to high phenol content and this helped in the slow depletion of carbohydrate stored in the seed, and showed medium dormancy period.

Table 16. Comparative biochemical evaluation of seeds of four *Ocimum* species

Character	*O. tenuiflorum*	*O. gratissimum*	*O. canum*	*O. basilicum*
Phosphorylase activity	High	Medium	Low	High
Phenol content	Low	Medium	High	Low
Seed weight	Low	High	Low	High
Moisture content	High	Low	Low	Medium
Dormancy period	High	Low	Medium	Medium

Results of present investigation showed that, moisture content was positively related to phosphorylase enzyme activity. At the same time phenol was negatively related to both moisture content and phosphorylase enzyme activity of the seed.

Summary

SUMMARY

Investigations on "Reproductive biology and enzyme studies in *Ocimum* spp." were carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the year 1994-1996. Four different species of *Ocimum* viz. *O. tenuiflorum, O. gratissimum, O. canum* and *O. basilicum* were selected for the study. Out of these two belonged to Sanctum group (*O. tenuiflorum* and *O. gratissimum*) and two belonged to Basilicum group (*O. basilicum* and *O. canum*). The main objectives of this study were to investigate and compare the floral biology, anthesis and mode of pollination of the four selected species and to make a comparative evaluation of the physiological attributes and phosphorylase enzyme activity of the seeds. The salient findings of the study are summarised below.

1. The time taken for inflorescence development varied from 6 days in *O. canum* to 21 days in *O. gratissimum*. Species coming under Sanctum group (*O. tenuiflorum* and *O. gratissimum*) required longer period for inflorescence development than Basilicum group (*O. canum* and *O. basilicum*).

2. Significant variation was observed among the four species in mean length of inflorescence, number of flowers in an inflorescence and distance between verticils. Verticils were closer in Sanctum group than in Basilicum group. The sequence of flower opening was acropetal in all the four species.

3. The four species studied resembled in the basic floral structure. Flowers of Sanctum group were found to be less conspicous with sessile bracts and that of Basilicum group were more conspicous with pedicellate bracts.

4. The time taken for completion of anthesis in an inflorescence ranged from 4 days in *O. gratissimum* to 21 days in *O. canum*. Basilicum group required longer period for the completion of anthesis in an inflorescence when compared to Sanctum group. The peak anthesis time was between 10 am and 12 noon in *O. tenuiflorum, O. gratissimum* and *O. basilicum*. In *O. canum* it was found to be earlier ie. between 8 am and 10 am.

5. Anther dehiscence was found to occur in bud stage in *O. tenuiflorum* and *O. gratissimum* and 15-20 minutes after flower opening in *O. canum* and *O. basilicum*. Flowers were protandrous and the anther dehiscence was influenced by factors like bee activity, weather conditions etc. Bright sunny days and high bee activity promoted early dehiscence of anthers. In all the four species stigma became receptive 30 minutes after flower opening and remained so for varying periods depending on species and environmental factors.

6. No self incompatibility mechanism was observed in any of the species studied.

7. The flowers of *Ocimum* species were found to be adapted to cross pollination. The morphological features and protandrous nature of the flowers favoured cross pollination. Pollination was mainly effected by day flying insects like bees, flies and ants.

8. Pollen grains were observed to be hexacolpate, reticulate and size varied with the species. Except in *O. gratissimum* in all other cases pollen grains were spherical. Pollen fertility studies by acetocarmine staining technique revealed that there was significant difference among the species. Fertility percentage ranged from 77.24 in *O. gratissimum* to 93.59 in *O. canum*.

9. Seed moisture content showed significant difference among the four species. It was the highest in *O. tenuiflorum* and the lowest in *O. gratissimum*.

10. The four species studied differed significantly in 1000-seed weight. 1000-seed weight was the highest for *O. basilicum* and the lowest for *O. tenuiflorum*.

11. Seed density varied significantly with the species. Values ranged from 0.4468 in *O. canum* to 0.6582 in *O. gratissimum*.

12. The four species of *Ocimum* exhibited varying periods of seed dormancy ranging from 30 days in *O. gratissimum* to 120 days in *O. tenuiflorum*. The intensity of dormancy was cent per cent in *O. tenuiflorum, O. gratissimum* and *O. canum* at harvest. The dormancy break was sudden in Sanctum group and slow in Basilicum group.

13. The four species under study differed significantly in the total phenol content and phosphorylase enzyme activity of the seeds. Based on seed weight, phosphorylase activity and total phenol content, *Ocimum* spp. can be grouped into three (1) Species with low seed weight, high enzyme activity and low phenol content included *O. tenuiflorum*. (2) Species with high seed weight, medium/high phosphorylase enzyme activity and medium/low phenol content included *O. gratissimum* and *O. basilicum*. (3) Species with low seed weight, low phosphorylase activity and high pehnol content included *O. canum*.

14. Results of present investigation also showed that, moisture content of seeds was positively related to phosphorylase enzyme activity. At the same time phenol content was negatively related to both moisture content and phosphorylase enzyme activity of the seed.

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REPRODUCTIVE BIOLOGY AND ENZYME STUDIES IN *OCIMUM* Spp.

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

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KERALA, INDIA.

1997

ABSTRACT

The present study on "Reproductive biology and enzyme studies in *Ocimum* spp." was undertaken at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period 1994-1996 with a view to make a comparative evaluation of the reproductive biology and phosphorylase enzyme activity of different species of *Ocimum*. The four species of *Ocimum* viz. *O. tenuiflorum*, *O. gratissimum*, *O. canum* and *O. basilicum* collected and maintained in the Department were used for the study.

The different species of *Ocimum* varied significantly in the time taken for inflorescence development, completion of anthesis in an inflorescence, number of flowers per inflorescence and distance between verticils. Species level variations were also observed in seed moisture content, seed density, 1000-seed weight and seed dormancy period.

Flowers of the four selected species were identical in basic structure although there existed variation in size, colour, hairyness and shape of floral parts. Anthesis occurred earlier in *O. canum* than the other three species. Pollen grains of *Ocimum* were hexacolpate and reticulate. However, species level difference existed in the size, shape and fertility of pollen grains. No self incompatibility mechanism existed in the four species studied. The floral morphology and protandrous nature makes the species adapted to cross pollination. Insects and ants are the main agents of pollination. Comparison of Sanctum and Basilicum groups revealed that Sanctum group which includes *O. tenuiflorum* and *O. gratissimum* required longer time for inflorescence development than Basilicum group which includes *O. canum* and

O. basilicum. Verticils were closer in the inflorescence of Sanctum group than Basilicum group. Sanctum group produced less conspicous flowers with sessile bracts and yellow pollen grains. In this group anther dehiscence occurred in bud stage. Basilicum group produced conspicous flowers with pedicellate bracts and white pollen grains. Anther dehiscence was after flower opening in this group. Dormancy break was sudden in Sanctum group and gradual in Basilicum group.

Moisture content of *Ocimum* seeds was positively related to phosphorylase enzyme activity. Phenol content was negatively related to both moisture content and phosphorylase enzyme activity of the seeds.

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