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Reproductive Biology of *Coriandrum Sativum* Linn.

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ABSTRACT

In present paper, an attempt has been made to establish the role of insect pollinators in the context of reproductive biology and seed set in coriander. Early morning pattern of flower anthesis with 1460±117 pollen per flower (log value 3.16) were noticed. Pollen-ovule ratio was ca. 730:1. Different insects like *Apis cerana indica*, *A. dorsata*, *Megachile* sp, and members of Diptera and Coleoptera usually visit the flowers for forage. The seed settings were 92.55±8.07%, 60±7.6%, and 29.74±4.8% in control (open pollination), netted flowers, and bagged flowers respectively, indicating the role of insects for increased seed production. SEM studies revealed that morphological features of pollen and stigma corroborate each other for successful fitness with regard to effective pollen-pistil interaction. The best *in vitro* germinating pollen (42±6.74%) with pollen tube length (161±27.56 μm) were obtained in 20% sucrose supplemented with 100 μg/ml boric acid. Salts of Ca, Mg, and K did not show any effect. Stigma receptivity was measured at different time intervals after flower opening in terms of the highest number of *in vivo* germinating pollen grains. Stigmas were receptive maximum (50%) during third day after flower anthesis, showing 51.1% *in vivo* germinating pollen with 94.2μm long pollen tube.

Keywords: Pollen, pollination, insects, seed set, coriander.

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1. INTRODUCTION

Conservation of plant genetic resources and increased seed or fruit yield through pollination management have been globally recognized as a component of an efficient integrated strategy for management of plant gene pools. The cross-pollinated crops species that benefit from insect pollination are difficult to successfully control pollinate using hand pollination methods. Pollinating insects are used for controlling pollination services in crops. The honeybees are efficient in this array. The honeybees may be confined under the cage. Consequently, pollen transfer may be restricted to only those plants grown under the cage. However, much research is needed on the effectiveness of other pollinators such as bumblebees and solitary bee species (Bhowmik, *et. al.* 2017). Honeybees have been selected and managed by man for many centuries (Ranjitha *et. al*., 2019). The quantity and quality of crops grown was found to increase in the presence of honeybees. Honeybees are social insects having a caste system and communal rearing. The procedures used for managing honeybees are well established and are known to pollinate a vast variety of crops. Basic life history parameters, along with information on positive and negative species interactions, need to be known in case-by-case basis so that management decisions will be scientifically informed (Westercamp, 1997).

Knowledge of coriander's reproductive biology can guide measures to increase seed quality, viability, and germination rates. It can also help optimize pollination, seed set, and eventually crop yields. Understanding the reproductive biology of coriander can assist retain and exploit genetic variety, lowering the risk of genetic erosion. It is also essential for creating successful breeding programs that can result in enhanced varieties with desirable features. Strategies to promote pollinator health and lessen the effects of pollinator loss can be informed by the insights it offers on how environmental factors, including temperature, drought, and pollution, affect pollination and seed production as well as the reproductive biology of coriander. Last but not least, researching the reproductive biology of coriander advances our basic knowledge of ecology, evolution, and plant biology. It can also yield insightful comparisons with other plant species, illuminating the variety of reproductive tactics seen in the plant kingdom.

The present investigation has been done in order to know the floral biology, anthesis, pollen out-put, pollen-ovule ratio, pollen and stigma morphology, forage behaviour of insects on flower, pollination mechanisms, *in vitro* and *in vivo* pollen germination, and stigma receptivity of *Coriandrum sativum* Linn. of the family Apiaceae.

2. MATERIALS AND METHODS

At peak flowering period, twenty-five plants in each plot of three different plots were selected and observed. The flower visitors observed to pollinate the flowers of *C. sativum* were collected, preserved, and identified from ZSI, Kolkata. Flower openings were noted following the process of Mathur and Mohan Ram (1986). Pollen anthesis was noticed following the process of Keijzer (1987) by sectioning the almost mature anthers and covering immediately with cover glass. Pollen grains per anther and per flower, and pollen-ovule ration were quantified following the procedure of Dafni (1992). The acetolyzed (Erdtman, 1952) pollen samples were gold coated and observed in a P-SEM-500 scanning electron microscope at low voltage from RSIC, Bose Institute, Kolkata.

In vitro pollen germination was conducted to know the effect of nutrients like sucrose; H_3BO_3; $Ca(NO_3)_2{:}4H_2O$; KNO_3 and $MgSO_4{:}7H_2O$ at various concentrations. Different grades of sucrose and salts were prepared and used individually or in combination. A drop (50µl) of each solution was kept into each groove of grooved slides individually or in combination. The fresh pollen samples were put with platinum needle into nutrient medium that were kept in petridishes lined with moist filter paper, and observed under a Olympus microscope at low magnification (10x X 10x). Results were taken and tabulated following Shivanna and Rangaswamy (1992) and analyzed using standard statistical methods. Fresh stigmas were collected at different times after flower anthesis and washed thoroughly with 0.015 M sodium phosphate buffer (pH 7.2), after which these were fixed in 2% glutaraldehyde for 4 hrs. Following fixation, the stigmas were washed with 0.015 M sodium phosphate buffer (pH 7.2) and dehydrated in an ethanol series (50%, 70%, 80%, 90% v/v and absolute) for 10 minutes in each grade. They were then passed through a mixture of ethanol and amylacetate at different ratio (1:1, 1:2 and 1:3), respectively, for 5 minutes in each grade. Stigmas were then preserved in pure amylacetate solution. After critical point drying (CPD) and gold coating, the morphological details were observed in P-SEM-500 at low voltage from RSIC, Bose Institute, Kolkata. Stigma receptivity and *in vivo* pollen germination were examined by the method of Dafni (1992) first by fixing (with acetic alcohol 1:1), softening (with 4 N NaOH) and staining the stigmas (with aniline blue). Contribution of flower visitors to fruit setting were quantified by comparing fruit set between bagged and unbagged flowers. Bagging and netting of flowers was done at the bud stage, for five different inflorescences per plant using ten different plants of each plot of three different plots.

3. RESULTS AND DISCUSSION

The hermaphrodite, regular flowers open in early morning with consecutive pollen release by transverse mode of anther dehiscence. A single flower produced 1460±117 pollen grains with a log value of 3.16 and c.730 grains per ovule (Table- 1). *Apis* spp., Megachile, and some members of Diptera and Coleoptera, usually visit the flowers after flower opening and act as pollinating agents (Table- 2, Plate 1). The pollen grains are 3-colporate, prolate, P/E ± 40.9 X 18.3 µm, exine ± 3.4 µm thick, surface finely reticulate (Plate – 2) for fitting and suitable landing as well as settling over the stigma surface to fix proper pollen-stigma interaction. The seed setting was high (92.5±9.87%) in open pollination compared to netted (60±7.42%) and bagged (29.7±5.35%) condition reflecting the contribution of insects for high yield of coriander. The individual effects of sucrose and boric acid on *in vitro* pollen germination of coriander were not pronounced at different times after anthesis (Figs. 1 & 2) whereas best *in vitro* germinating pollen (42±6.74%) with pollen tube length (161±27.56µm) were obtained in 20% sucrose supplemented with 100 µg/ml boric acid (Fig. 3). The stigma is minute, with slight depression and notches at the middle portion with long striated cells present, and at the end striation mass of cells with more or less rounded shape and sizes are found (Plate – 3). The intercellular spaces are large, vascular strands continue up to the junction of stigma and style. Frequent spaces between each striation present. Pollen grains are supposed to be landed in the peripheral part of stigma head. The stigma is with continuous pellicle layer and wet type, showing rounded to oval papillae cells (Plate – 4) leaving sufficient spaces for passing of pollen tubes at post pollination phase. Esterases are localized just below stigma head, and the stigmas were found to be receptive maximum (50%) during third day after flower anthesis, showing 51.1% *in vivo* germinating pollen with 94.2µm long pollen tube (Table- 3).

Pollen anthesis took place just after flower anthesis, and the pollen presentation to pollinators is limited by anther/pollen anthesis. Pacini (1992) suggests that if the anther dehisces after flower anthesis, then pollen exposure is limited by anther anthesis. Observation on floral biology and insect behavior showed that the cross pollination appears to be an intricate problem in *Coriandrum* because of small structure of flower, though it offers different floral rewards (Westercamp, 1997). The barriers in reproduction are important features in the study of pollination ecology (Bhowmik et al, 2017) each taxon at any given time is limited by a certain morphogenetic potentiality having effect on gene flow and mode of breeding. In Coriandrum, due to autogamy, homozygosity increases, and also the gene from other lines might be prohibited due to minute size flowers.

Among the different visitors, the honeybees (*Apis* spp.) are the most effective and reliable pollinators in coriander, which may be due to strong correlation between flower structure and position on umbel inflorescence with a provision for alighting place for honeybees. When *Apis* spp. alight on flower, the pollens are deposited on the back of its thorax and abdomen. According to Faegri and vander Pijl (1980) this mode of pollen deposition is known for efficacy and economy in the utilization of pollen. Netting and bagging process confirmed that the insects act as major pollinators, showing high seed yield. The pollen per flower and per ovule is related to fertilization rate (Cruden, 1977). In *C. sativum,* less number of pollen per flower as well as per ovule indicates its autogamous nature, which is genetically inferior. Successful seed setting may depend on the number of pollen produced per flower, leading to pollen competition. The high percentage of seed production in open pollination may be due to less pollen competition over stigma as the number of pollens is less per flower, reflecting autogamy. The insects, especially honeybees, play a vital role in high seed production. With regard to floral structure, forage matter availability, and continuous insect visits, the role of honeybees in high yield might not be neglected. Moreover, the plant shows transition between autogamy and xenogamy as it produces low pollen:ovule ratio (autogamous nature), the necessity of more insect visits, and delayed stigma receptive period (xenogamous nature) respectively. Pollen viability was studied in terms of in vitro pollen germination using sucrose media alone or in combination with boric acid. Viability of pollen has been defined as having the capacity to live, grow, germinate, or develop. It has been reported that pollen viability is so liable that it may differ when pollen is collected at different times of the day (Baez *et al.*, 2002). Pollen collected from flowers in anthesis for one hour show decreased germination (Shivanna and Rangaswamy, 1992). Pollen viability has a genetic component; results may be different depending on the genetic variability of individuals used as donors. Pollen may express genetically based traits during its development (Wierdak, 2013), maturation, and free dispersal phases. Reproductive effort, physiological stress, resource availability may be the factors for variation in pollen viability (Dafni and Firmage, 2000). Populations of out crossing plants are far from being genetically uniform (Heywood, 1991), and may constitute important sources of variability. Regarding germination *in vitro,* the culture is dependent on the quality of pollens (Brewbaker and Kwack, 1963; Heslop-Harrison *et al*., 1984). Temperature has appeared as a critical factor for *in vitro* germination. Receptivity of the stigma is a critical factor for the successful completion of post-pollination events. Receptivity is generally maximal soon after anthesis. The period of receptivity varies from species to species and is influenced by temperature and humidity. Alternation in temperature and humidity drastically reduce the period of stigma receptivity. The duration of stigma receptivity varies from a few minutes to two or three weeks (Dafni, 1992; Dafni and Firmage, 2000).
Although coriander (*Coriandrum sativum* Linn.) is a self-pollinating plant, some research (Westercamp, 1997; Wierdak, 2013; Bhowmik *et al*., 2017 and Ranjitha *et al*., 2019) indicates that cross-pollination may happen, particularly when several plants are cultivated adjacent to one another.

Because it can self-pollinate, it is not very reliant on pollinators. Some pollinators, such as butterflies and bees, might still visit the flowers, though. Coriander has a comparatively low pollination efficiency, which may affect seed set and production. The tiny, unnoticeable blossoms of coriander are grouped in umbels. With both male and female reproductive organs, they have a complex structure. In order to guarantee seed set, it uses a hybrid reproductive system that combines cross-pollination with self-pollination. Factors such as plant density, pollination, and nutrient availability affect how many seeds it produces. The effectiveness of pollination has a direct impact on coriander seed quality. Higher yields and better seed quality can result from improved pollination. Although cross-pollination is possible, coriander is mainly self-pollinated. Breeding strategies can be informed by knowledge of each's relative relevance. Farmers and breeders should concentrate on maximizing pollination by employing strategies including boosting plant density, supporting pollinators, and using additional illumination to increase coriander yields and seed quality. The age of the flower, time of the day, and the presence or absence of stigmatic exudates may attain importance in determining stigma receptivity (Dumas and Gaude, 1983). Thus the present investigation is concomitant to other works by Westercamp (1997); Wierdak (2013); Bhowmik *et. al*. (2017) and Ranjitha *et. al.* (2019).

Table 1. Floral characters of *Coriandrum sativum.*

Floral characters	**Observations**
Flowering period	December-March
Flower type	Hermaphrodite, regular
Flower colour	White/off white-pinkish
Flower odour	Pungent
Flower anthesis pattern and time (hrs.)	Early morning/ 5.00-6.00
Pollen anthesis time (hrs.) and mode	7.00-10.00/ Transverse
Mean number of anthers per flower	c.5
Mean number of pollens/anther	c.292, range 154-356
Mean number of pollens/flower	1460±117
Log pollen per flower	3.16
Pollen-ovule ratio	c. 730:1
Stigma type	Wet-papillate
Mean fruit-set (%) in open condition	92.5±8.07
Mean fruit-set (%) by netting	60±7.6
Mean fruit-set (%) by bagging	29.7±4.8

Table 2. Flower visitors of *Coriandrum sativum.*

Name of the visitors	**Visiting period**	**Forage materials**
Apis cerana indica	Day	Pollen and Nectar
A. mellifera	Day	Pollen and Nectar
A. dorsata	Day	Pollen and Nectar
Megachile sp	Day	Pollen and Nectar
Diptera	Day	Pollen
Coleoptera	Day and Night	Pollen

Table 3. *In vivo* pollen germination of *Coriandrum sativum.*

Period after flower opening	**1 day**	**2 days**	**3 days**	**Drooping**
Total number of stigmas observed	20	20	20	20
Number of stigmas showing germination	-	2	10	8
Stigma receptivity (%)	-	10	50	40
Mean pollen number retained on stigma	50	70	90	70
Mean number of germinated pollen	-	21	46	30
Germinated pollen (%)	-	30	51.1	42.8
Mean pollen tube length (μm)	-	70.2	94.2	117.4

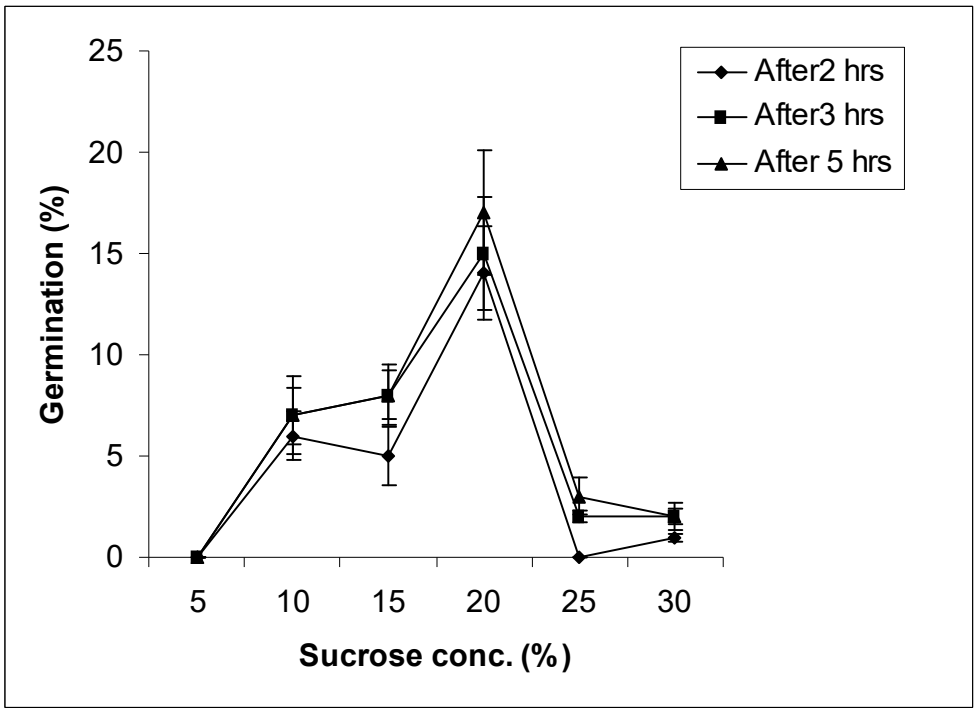

Figure 1. Effect of sucrose on *in vitro* pollen germination of C. *sativum.*

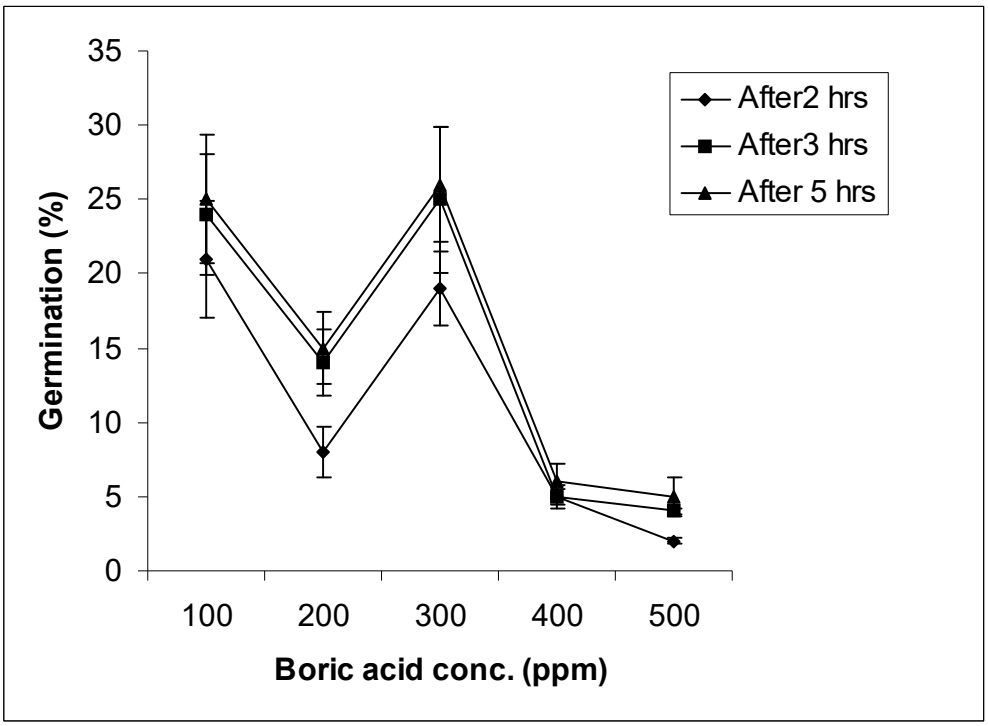

Figure 2. Effect of boric acid on *in vitro* pollen germination of C. *sativum.*

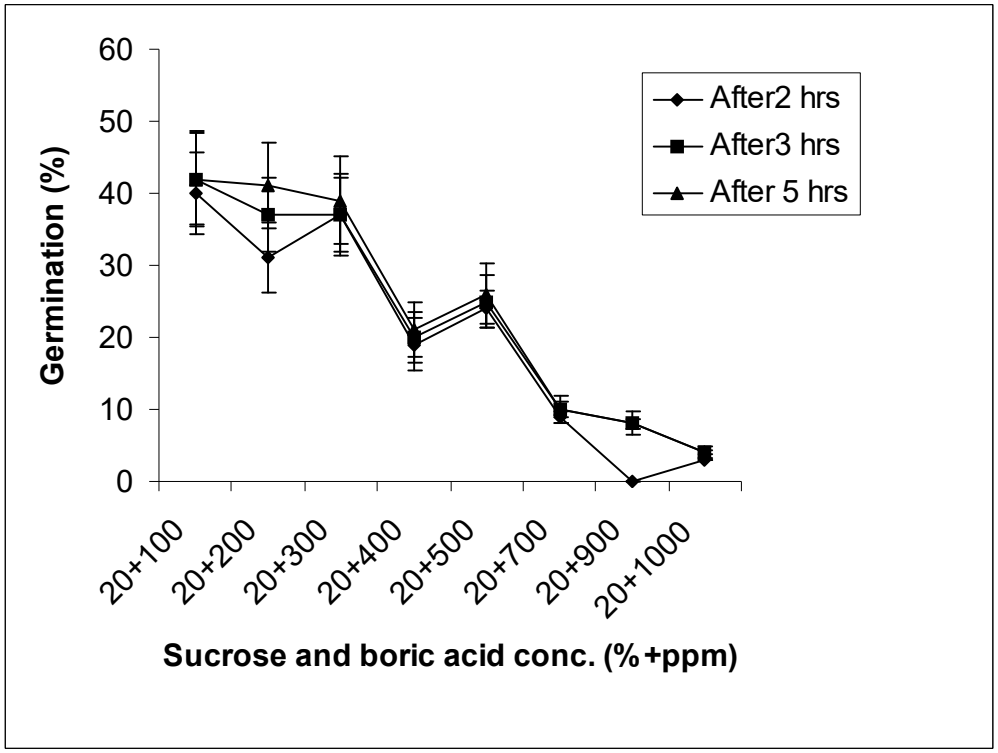

Figure 3. Effect of sucrose and boric acid on *in vitro* pollen germination of C. *sativum.*

Plate 1. Honeybee foraging the flowers of *C. sativum,* causing pollination.

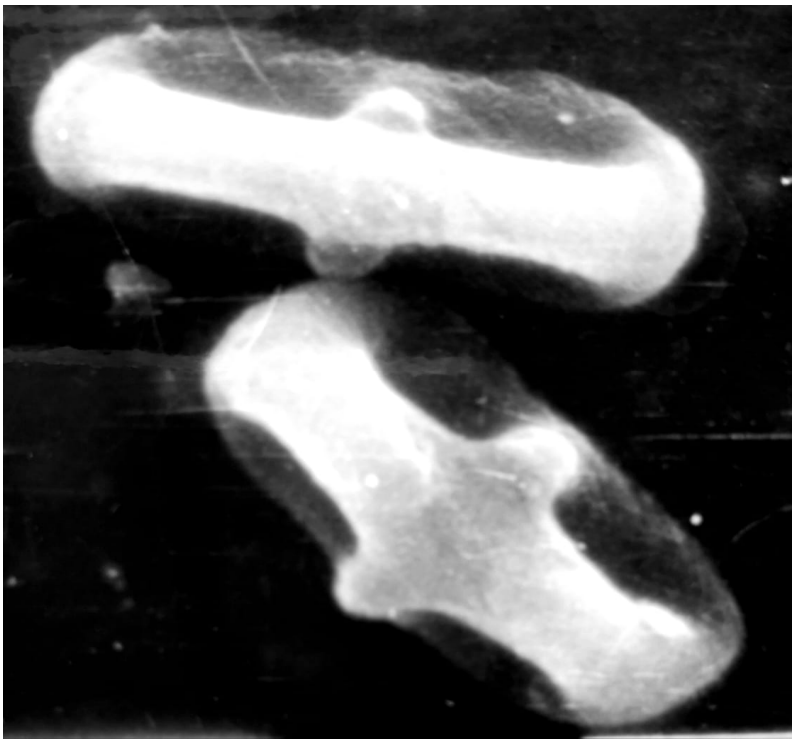

Plate 2. SEM Photomicrograph (X2000) of pollens of *C. sativum.*

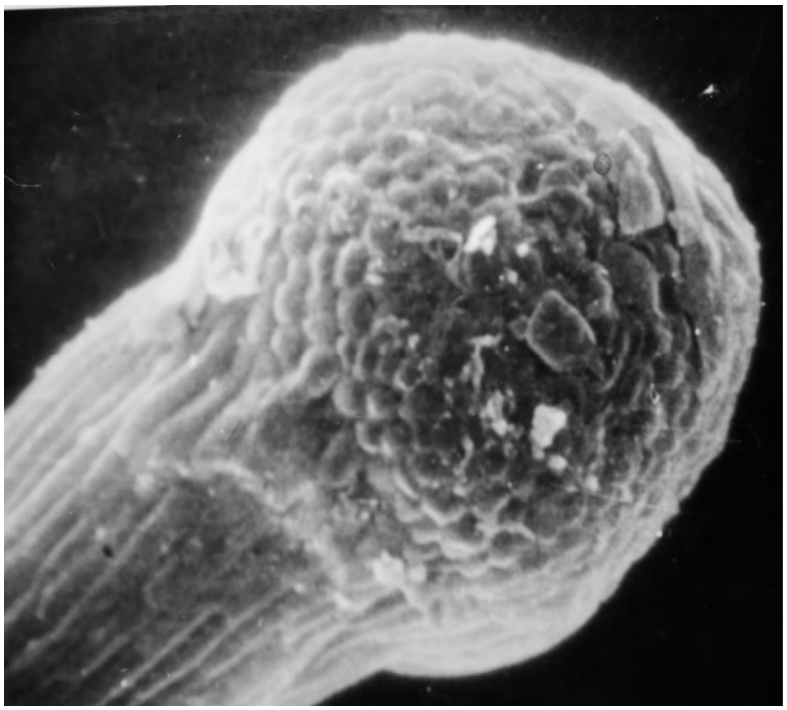

Plate 3. SEM Photomicrograph (X500) of stigma of *C. sativum.*

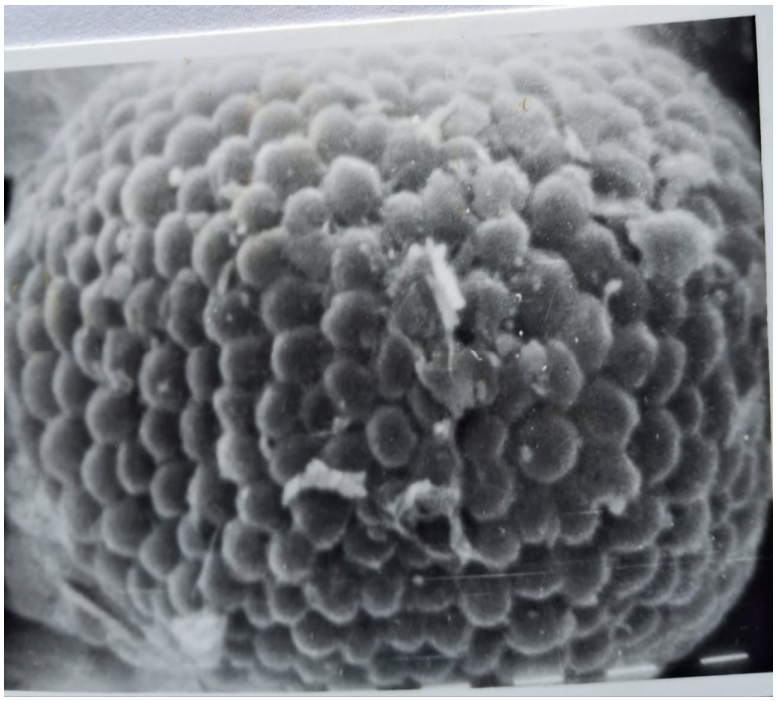

Plate 4. SEM Photomicrograph (X1500) of the tip of stigma of C. *sativum.*

4. CONCLUSIONS

With low number of pollen-ovule ratio per flower, an early morning flower anthesis pattern was seen. Insects such as Diptera and Coleoptera, *A. dorsata*, *Megachile* sp., and *Apis cerana indica* typically visit the blossoms in search of food, and they played a part in increasing seed output. Pollen and stigma morphological characteristics support one another for good fitness in terms of efficient pollen-pistil interactions. Twenty percent sucrose combined with 100 µg/ml boric acid produced the best *in vitro* germinating pollen and tube length. Calcium, magnesium, and potassium salts had no effect. The greatest number of *in vivo* germinating pollen grains was used to determine stigma receptivity at various points after flower opening. Stigmas were at their most responsive on the third day following floral anthesis, exhibiting maximum *in vivo* germinating pollen with a pollen tube measuring 94.2 µm. Farmers and breeders should concentrate on maximizing pollination by employing strategies including boosting plant density, supporting pollinators, and using additional illumination to increase coriander yields and seed quality.

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