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Floral Morphology and Floral Phenology of *Piper nigrum* L.

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ABSTRACT: The research project "Floral morphology and floral phenology of *Piper nigrum* L. was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvanthapuram during 2017-2019. The floral morphology revealed light green (145 C) and dark green flower colour (140 A) as per the Royal Horticulture Society Colour Charts. The odour of the flower was fresh floral fruity odour as per the fragrance wheel and the odour was slightly minty. Presence of nectar was noticed on the black pepper flowers. The flowers in the inflorescence complete opening within 9 days from the opening of the first flower and flower opening starts from 4-5pm. The flower size on third day and fourth day of stigma receptivity was 1.51 × 1.32 mm. 48 to 98 flowers in a spike depending on the length of spike. The stigma was 4 lobed, wet type and papillate. The duration of stigma receptivity was there for 7 days from the time of anthesis of flower. The peak stigma receptivity was on fifth day of anthesis. The duration of pollen availability in an inflorescence varied from 9 days in small inflorescences (6 cm) to 12 days (9cm) in long inflorescences. The longevity of flowers varied from 14-15 days. Flower emergence duration ranged from 19 to 20 days. Flowering frequency in black pepper was maximum in the month of july. The flowering intensity was maximum in the fifth day of anthesis. The anthesis period in an inflorescence varied from 9 to 12 days. The maximum anthesis period in a plant was maximum in the month of July and minimum in January and February. The mean days from the emergence of spike till the full emergence of flowers in the spike (duration of spiking) was 26.84 days in a plant. The mean duration of spiking in a plant population was 259.5 days. The fruit is drupe and the period taken from fertilization to maturity varied from 150-175 days. Limited work in breeding as well as hybridization may results of lacking of fundamental information. Hybridization is considered a good breeding methodology to create new variety that is able to resist to *Phytophthora capsici* and/or nematodes. Successful hybridization is resulted from understanding of flower biology which is further useful for the crop improvement programme.

Keywords: Phenology, Fertilization, Inflorescences, Pollination, Anthesis.

INTRODUCTION

Black pepper (*Piper nigrum* L.) the 'king of spices' and one of the oldest spices known to mankind belongs to the family Piperaceae. Black pepper originated in the tropical evergreen forests of the Western Ghats of India and is presently largely cultivated in India, Brazil, Indonesia, Malaysia, Sri Lanka, Vietnam and China (Ravindran, 2006). Black pepper is valued for its pungency contributed by the alkaloid piperine (Ravindran, 2006; Nybe *et al.*, 2007) and has numerous reported physiological and drug-like actions (Ravindran, 2006; Nybe *et al.*, 2007). Black pepper is cultivated for its matured dried fruits which is the most widely used spice in the world (Ravindran, 2006).

Black pepper is a plant that cannot tolerate excessive heat and dryness (Vijayakumar *et al.*, 1984). Black pepper grows successfully between 20° North to 20° South of equator and from sea level up to 1500 m above

MSL (Ravindran, 2006; Nybe *et al.,* 2007). It is a plant of humid tropics, requiring 2000 - 3000 mm of rainfall, tropical temperature and high relative humidity with little variation in day length throughout the year.

Floral biology is often correlated with the pollinator mechanisms like nectar and pollen rewards, temporal separation of male and female phases and the arrangement of floral parts which may influence pollen deposition and carry over (Endler, 1979). Floral morphology is factor closely related to breeding system since autogamy only occurs in hermaphrodite or monoecious plants while dioecious plants are always out crossed (Loveless and Hamrick, 1984). Each part of flower may have a special role in one or more events during production and dispersal of gametes and seeds. It is usually assumed that every floral organ has a more or less definite role in pollination but quite often replacement functions are also known (Galen 1999; Dafni and Firmage, 2000). A basic understanding of floral structure, phenology and pollination systems is thus a pre requisite for studies on reproductive biology (Dafni and Firmage, 2000). Study on reproductive biology is essential for developing effective strategies for both *in situ* and *ex situ* conservation of species (Moza and Bhatnagar, 2007).

Floral longevity, the length of time a flower remains open and functional, varies among plant species. It is important in understanding pollination ecology (Ashman and Schoen, 1994). Flowers must remain open to contribute to plant fitness through ovule fertilization and pollen dissemination, when they require resources for respiratory maintenance and pollinator attraction (Ashman and Schoen, 1994).

Phenology is the timing of biological events and their relationship to seasonal climatic changes (Austin, 1972). Therefore, the detailed information regarding the phenology is a pre-requisite for the studies on the floral biology and breeding system (Bawa *et al.,* 1990). Temperature, moisture and photoperiod are the three known factors that affect the phenology (growth and reproduction) of both plants and their pollinators (Sun *et al*., 2009). Timing of flowering helps in maintaining reproductive isolation and in reducing competition for pollinators. Hence, in any pollination ecology study, it is important to record observations on flowering phenology of the crop (Belavadi, and Ganeshaiah 2013). Floral phenology has to be recorded frequently in time 1-4 h depending on the species for several days (Shivanna and Tandon 2014).

Rainfall of 70 mm received in 20 days during May – June is required for triggering off flushing and flowering processes in the plant, but once the process is set off there should be continuous shower until fruit ripening. Any dry spell even for a few days, within this critical period of 16 weeks (flowering to fruit ripening) results in low yield (Pillay *et al.,* 1988). The study of phenological aspects of plants involves the observation, recording and interpretation of the timing of their life history events and many studies on flowering time stress the role of interactions between plant species which share pollinators or predators (Zhang *et al,* 2015). Plant phenological study has great significance because it not only provides knowledge about plant growth pattern but it also provides the idea on the effects of environment and selective pressure on flowering and fruiting behaviour (Zhang *et al,* 2015). The variation in flowering and fruiting patterns may affect the degree of genetic variability in the ensuring offspring of each species therefore, observing phenological event as a crucial step in reproductive biological studies. For plants, recurrent biological events include vegetative processes such as leaf flushing and shedding as well as reproductive events such as bud formation, flowering and production of fruits (Francis *et al.,* 2007).

MATERIALS AND METHODS

Experiment was be carried out in black pepper variety Panniyur-1 in the field grown pepper plants and bush pepper plants maintained in pots. Twenty-five plants of black pepper variety, Panniyur 1 of uniform age grown in the Instructional Farm, College of Agriculture, Vellayani was selected and marked for the study. The plants were observed from March 2018 to March 2019 for studying the floral initiation, floral morphology and floral phenology. Instructional Farm in College of Agriculture, Vellayani is located at 8.5° North latitude and 76.9° East latitude at an altitude of 29 m above MSL. Field plants of black pepper maintained at Instruction Farm and bush pepper plants in pots maintained in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani were used as study material.

Study on floral morphology and floral phenology of black pepper. The time of emergence of first flower to last flower in an inflorescence was recorded in twenty-five black pepper plants in field during the flowering period. Fifty inflorescences altogether were sampled to record the floral morphology.

Flower colour. The observation on flower colour were made for 100 flowers of fifty inflorescences from first day of anthesis to last day of anthesis by comparing them with Royal Horticultural Society colour chart.

Flower odour. Fifty flowers were collected from first day of opening of stigma and placed in glass vials and closed for 5 min, 30 min and 6 hrs to allow the accumulation of floral scent and was smelled. (Braunschmid *et al*., 2017)**.**

Presence of nectar. Twenty-five inflorescences were taken and using insulin syringe of 1 ml capacity and nectar was drawn from thirty-five flowers from each inflorescence on third day of stigma opening when there was maximum accumulation (Morrant *et al*., 2009).

Flower opening time. The pepper flowers are devoid of corolla and calyx and hence emergence of stigma was recorded as the flower opening time. Observation on flower opening time was recorded in 50

inflorescences at random on 25 plants in the field at intervals of 6 h from 6 am to 12 noon, 12 noon to 6pm and 6 pm to 12 midnight and 12 midnight to 6 am from June to July when flowering intensity was maximum. Based on the result, the period of flower opening was reduced between 12 noon to 12 midnight on hourly basis to record the time when maximum flower opening occurs.

Flower size. The length and breadth of flower at full emergence of the stigma (third day to fifth day of stigma emergence) were measured from six flowers of five inflorescences taken under the microscope Leica MZ95 with scale inserted and measured the length and breadth of flower in millimeter.

Number of flowers per spike. Fifty inflorescences from selected twenty-five plants in the field were selected and tagged. The observation was recorded by counting number of flowers in a spike after the completion of anthesis.

Number of anthers/flowers. Fifty flowers were selected from twenty-five inflorescence and were tagged. Each flower was observed for anther emergence and counted the number of anthers per flower.

Anther dehiscence time. Fifty inflorescences were selected and tagged from twenty-five plants. Observation regarding time of anthesis were recorded from the time of anther emergence till it dehisce and loose completely. The data were recorded from 10 am to 4 pm at one hour interval and the dehisced anthers were removed from the spike after each count, to avoid recounting.

Anther dehiscence mode. Fifty flowers were selected from fifty inflorescence and tagged. Each flower were observed for anther emergence to the dehiscence of anther and the mode of dehiscence was found out.

Stigma type. Twenty-five stigmas of fifty inflorescences of different plants were selected. The observation was made by taking out the stigma by using the sharp needle from the flower and pressing gently the stigma with fingers on the petridish lined with filter paper to understand the type of stigma whether it is dry or wet type.

Stigma receptivity. Two flowers each from five inflorescences of black pepper plants grown in the field which were observed for 8 days from the emergence of stigma. The stigma receptivity were observed by hydrogen peroxide method and different stages of stigma development were observed through stereomicroscope and photographed.

Duration of stigma receptivity. The observation was made for 8 days from the opening of stigma. Bubbling in presence of hydrogen peroxide was considered as a positive result (Osborn *et al*. 1988) when the stigmas were analyzed with H_2O_2 method (Dafni 1992). The different development stages of stigma emergence were described.

Flower longevity. Flower longevity was recorded based on the length of time the flower remained open and functional. Flower was judged to be open when the perianth appeared to be fresh, with the stamens presenting pollen, or when the stigma appeared fresh. A flower was considered to be senescent when the corolla fell apart or was discolored and wilted, or when the stamens were wilted and empty of pollen and the stigma discolored (Primack, 1985). Since black pepper does not have perianth the longevity can be assessed by stigma remaining fresh and pollen being present. Black pepper plant being protogynous the longevity of the flower can be considered when the pollen being present and remain fresh. The data was expressed in days.

Anatomical observation of floral morphology. Morphology of the flower and flower parts were studied by using a hand lens and a compound microscope.

Flower emergence duration. Fifty flowers were observed from the time of full emergence of spike to the bud initiation at two days interval until the convex shape is formed and then fixed in a solution of formalin, acetic acid and alcohol under a stereoscopic microscope (Marafon *et al*., 2010).

Flowering frequency. Twenty-five plants were recorded for observation. These plants were tracked with time and from date of first flowering of any plant, number of plants in flowering was recorded each day till all of them stop flowering. From this frequency or proportion of plants that are in flowering (*Pi*) on any given day was arrived as *Pid*. The *Pid* may be plotted on a graph along days.

Flowering intensity. Five plants were selected and five inflorescences from each plant was marked for observation. The number of opened flowers in each inflorescence was observed till all flowers were opened in an inflorescence. The intensity of flowering was computed as the average number of flowers opening per inflorescence each day (*fid*). This data was plotted against days.

Anthesis period in an inflorescence. Fifty inflorescences were selected and tagged from twenty-five plants. Observations were recorded from the first day of opening of the flower in an inflorescence to the last day of opening of the last flower in the inflorescence.

Anthesis period in a plant. Five plants were selected and tagged. The time taken from the opening of the first formed flower till the day when the last flower in the last formed inflorescence is recorded as the anthesis period in a plant.

Duration of spiking in the plant. Fifty inflorescences were selected and tagged. The observation was made by counting the days from the emergence of first spike till the full emergence of flowers in the spike. The observations were recorded for March 2018 to March 2019.

Duration of spiking in the plant population. The observation was recorded for March 2018 to March 2019. The observation was made by counting the emergence of first spike to last spike in the five plants.

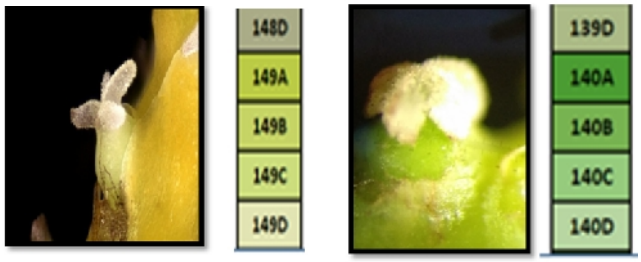
Fruit maturation period from fertilization. Fifty inflorescences were selected and tagged from twenty-

five plants. Artificial pollination was carried out in 8 flowers in an inflorescence and the number of days taken from fertilization to fruit maturity was recorded by color change of one or two fruits from green to red. The data was measured in number of days.

RESULTS AND DISCUSSION

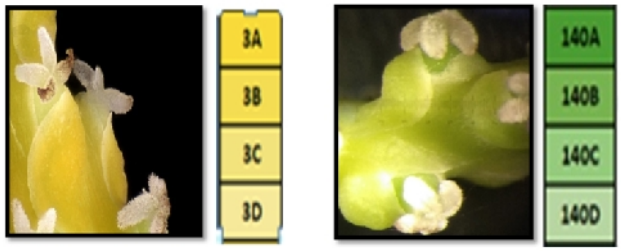
Flower colour. The flower colour noticed in hundred flowers collected from twenty-five inflorescences of twenty-five field grown black pepper plants revealed Light green (149 C) to Dark green (140 A) colour as per the Royal Horticulture Society colour charts. Out of hundred flowers observed fifty-two were Light green (149 C) and 48 flowers were Dark green (140 A) has been shown in Fig. 1. The bract colour varied from Yellow (3 B) to Light green (140 A) as per the Royal Horticultural Society colour charts. The flower colour of black pepper observed were Light green,149C (52%) and were Dark green,140A (48%). According to Figuerido and Sazima (2000) flower colour of *Piper nigrum* was creamy, yellowish or whitish. Ravindran (2006) reported that when spike is young it was green or whitish green, or light purple and when mature it was green, pale purple or pale yellow.

The presence of fresh floral fruity odour was noticed as per the fragrance wheel. The odour of fifty black pepper flowers kept in closed glass container was slightly minty during the first five minutes and increased to strong minty after 30 minutes and started losing the odour and became light minty odour six hours after keeping in closed containers as revealed by ten panel members. Tebbs (1989) reported lemon or lime odour in some Mesoamerican Piperaceae species and was probably reported as the most widespread odour in the family. Sweet lemon or lime odour in *P. amalago P. arboreum P. crassinervium P. gaudichaudianum P. glabratum P. macedoi , P. mikanianum P. mollicomum P. regnelli, P. xylosteoides, Ottoniamartiana, Ottoniapropinqua, Pothomorphe umbellate* except for *P. aduncum* whose flowers were scentless as reported by Figuerido and Sazima (2000).



Light green (Code: 149 B) Dark green (Code:140 A)

Fig. 1. Flower colour of *Piper nigrum* L.

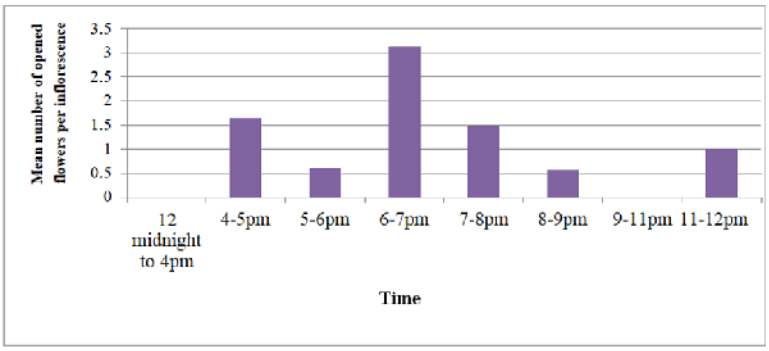


Yellow(Code: 3 B) Light green (Code:140 B)

Fig. 2. Bract colour of *Piper nigrum* L.

Presence of nectar. Presence of nectar was noticed in the flower between the bract and stigma and the amount of nectar varied from 23 to 25 µl as observed from thirty-five stigma of one inflorescence. The mean of nectar content from thirty-five stigma of fifty inflorescences were 24.08 µl. The mean nectar content of one flower is 1.45µl. Nectar was not discernible in the flowers, as observed for other *Piper* species according to Fleming (1985).

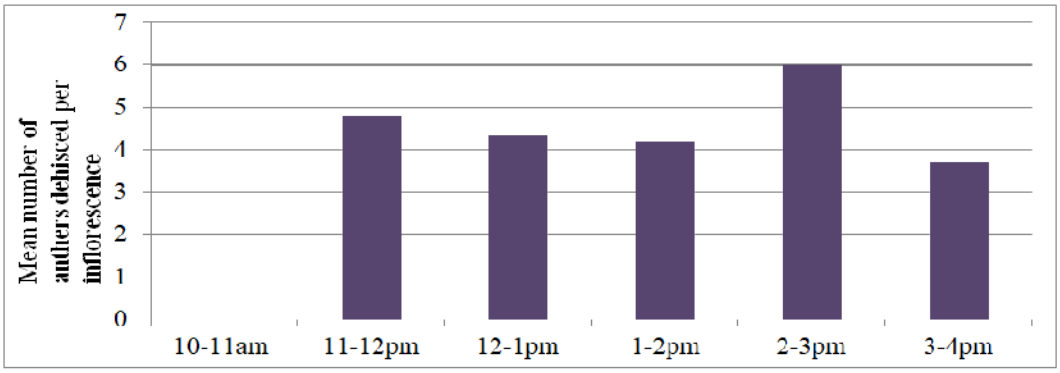
Flower opening time. The flower opening time was taken from fifty inflorescences of twenty-five black pepper plants grown in the field for eight days and maximum flower opening was observed at 6-7 pm followed by 4-5pm and 7-8 pm (Graph 1). According to Kanakamany (1982) anthesis of black pepper was from 7.30-8.30 pm. According to Ravindran (2006) anthesis in black pepper took place during 4 pm. Nybe *et al.* (2007) reported anthesis in black pepper as from 6-6.30pm.



Graph 1. Flower opening time of *Piper nigrum* L.

Anther dehiscence mode. Anther dehiscence mode was obtained from fifty flowers of fifty inflorescences taken from twenty-five black pepper plants grown in the field. All black pepper flowers showed longitudinal dehiscence. Stereomicroscopic observation of anther dehiscence opening revealed longitudinal splitting of anther to release the pollen.

Anther dehiscence time. The anther dehiscence of fifty inflorescences from twenty-five black pepper plants grown in the field were taken from first day of anther dehiscence to ninth day. The mean anther dehiscence time in a spike is given in table 8. Anther dehiscence occurred from 11 am and continued till 4 pm and was maximum at 2-3 pm (Graph 2). According to Nybe *et al*. (2007) anther dehiscence took place between 14.30-15.30 hrs (2.30-3.30 pm).



Graph 2. Anther dehiscence time in *Piper nigrum* L.

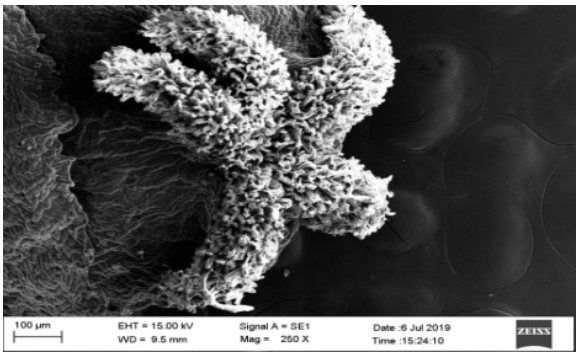
Flower size of black pepper (*Piper nigrum* L.). The length and breadth of thirty flower was taken on third day, fourth day and fifth day. On third day and fourth day the mean length of flower was 1.51 mm and mean breadth of flowers was 1.32 mm. The flower size was maximum (1.53 × 1.34 mm) on the fifth day of stigma receptivity.

Number of flowers per spike. The average number of flowers per spike was carried out from fifty inflorescences of twenty-five black pepper plants grown in the field. The number of flowers per spike varied from 49-98 flowers as shown in the Fig. 3 and it depends on the length of the spike.

Stigma type. Twenty-five stigmas of fifty inflorescences taken from twenty-five black pepper plants grown in the field revealed the presence of four lobes in each stigma.

Fig. 3. Number of flowers per spike in *Piper nigrum* L.

Stigma was wet type and papillate. Scanning Electron Microscopic image of stigma is shown in Fig. 4. Ravindran (2006) observed the stigma of black pepper as 3-5 lobed and papillate.



Scale bar -100µm

Fig. 4. Scanning Electron Microscopic image of stigma of *Piper nigrum* L.

Stigma receptivity. Two flowers each were taken from five inflorescences of black pepper plants grown in the field were observed for stigma receptivity for 8 days from the emergence of stigma. The stigma receptivity by hydrogen peroxide test revealed stigma receptivity for 7 days. Maximum bubble was counted on fifth day of emergence of stigma. Stereomicroscopic image of stigma through hydrogen peroxide is shown in Fig. 5. According to Chen *et al*. (2018) the stigma was more receptive from 2-6 days in black pepper. Stigmatic receptivity restricts the successful pollination in a plant. Stigma receptivity is the ability of stigma to support the viable and compatible pollen to generate. The receptivity of stigmas can be characterized by assaying the activity of several enzymes such as peroxidase, esterase, alcohol dehydrogenase and acid phosphatase (Shivanna and Sastri, 1981).

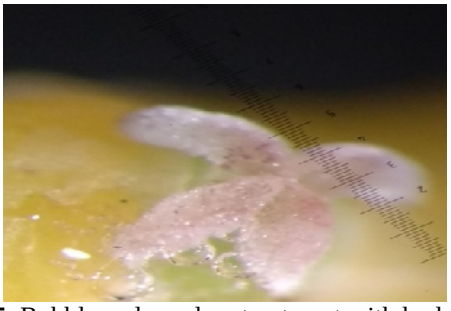


Fig. 5. Bubbles released on treatment with hydrogen peroxide.

In the present study peroxidase enzyme was checked and peroxidase enzyme of stigma released the oxygen bubbles with hydrogen peroxide treatment which was counted. On the eighth day, no bubbles were seen and the stigma was dried up.

Duration of stigma receptivity. The stigma receptivity was observed for 8 days. The duration of stigma receptivity was for seven days as revealed from hydrogen peroxide test. The different developmental stages of stigma observed through stereomicroscope can be described as follows in Fig. 6. According to Ravindran, 2006 the period of receptivity of stigma varies from 3–9 days. According to Kalinganire *et al*. (2000) it was for 10 days and Nybe *et al.* (2007) reported 7 days. Stigma remains receptive from 4 days to 6 days and up to 10 days (Quyen *et al.,* 2019).

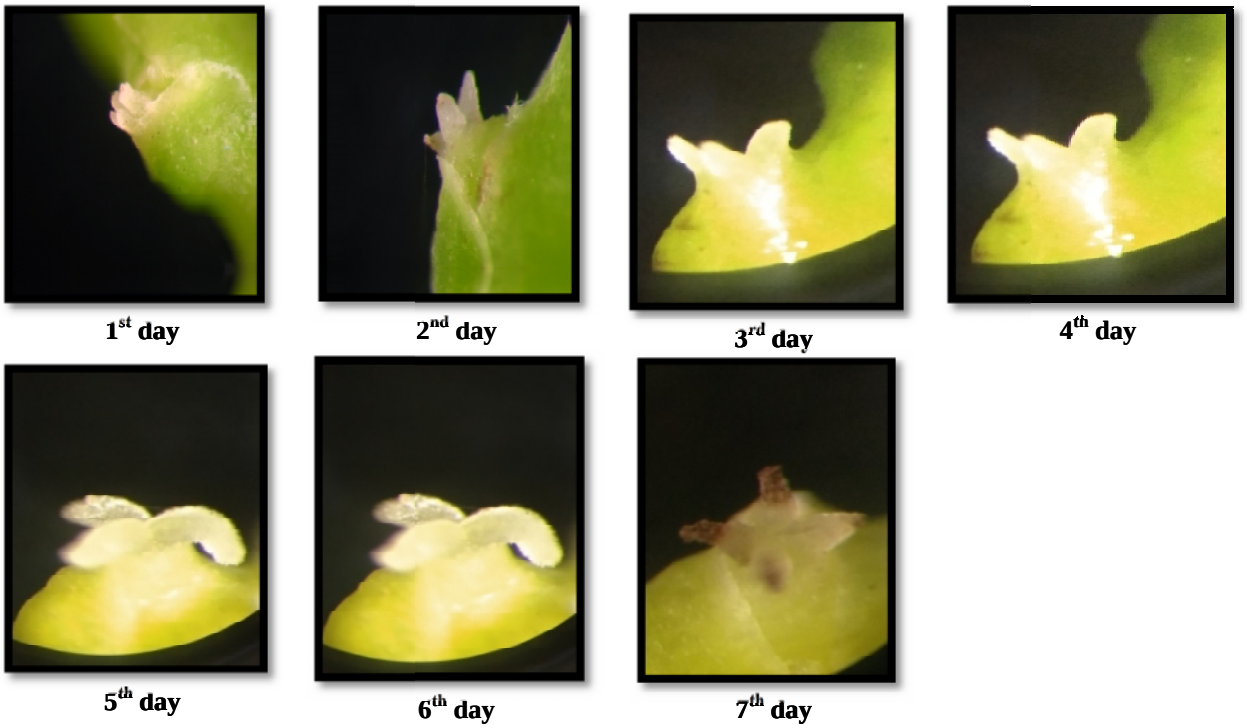


Fig. 6. Stereomicroscopic image of different stages of stigma receptivity of *Piper nigrum* L.

Flower longevity. Pollen being present and remain fresh in a flower after 14 -15 days of stigma emergence and is considered as the longevity of the flower.

Anatomical observation of floral morphology. The cross and longitudinal section of the immature spikes revealed the rudimentary structures of ovary and stamens under stereomicroscope and is shown in the Fig. 7 and 8. Through these tests it was possible to observe the germination of pollen tube.

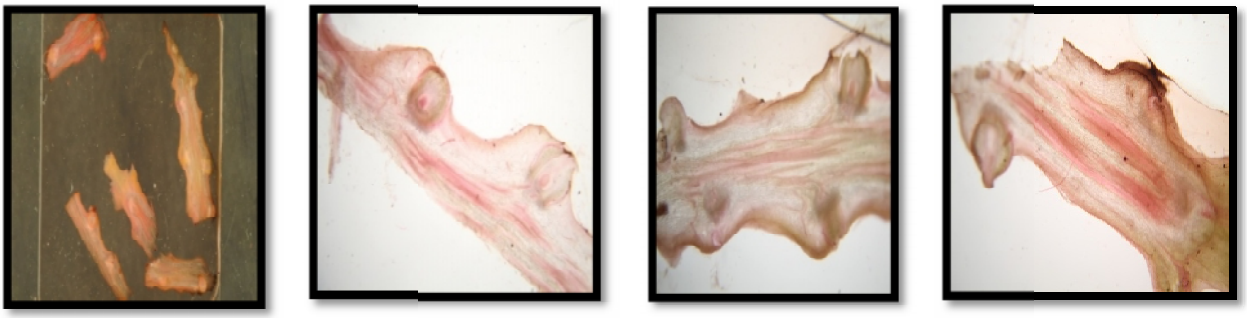


Fig. 7. Anatomical observation of floral morphology (longitudinal section).

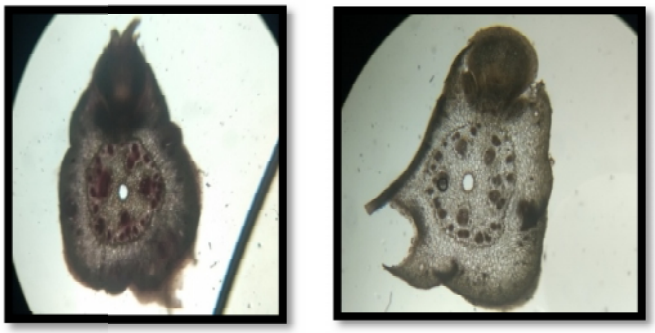
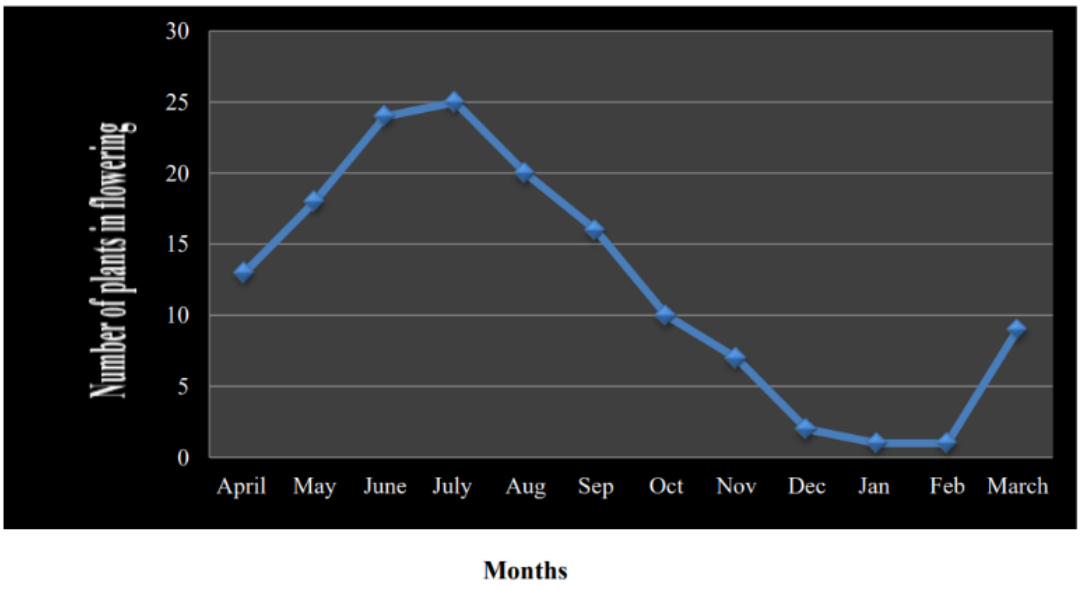


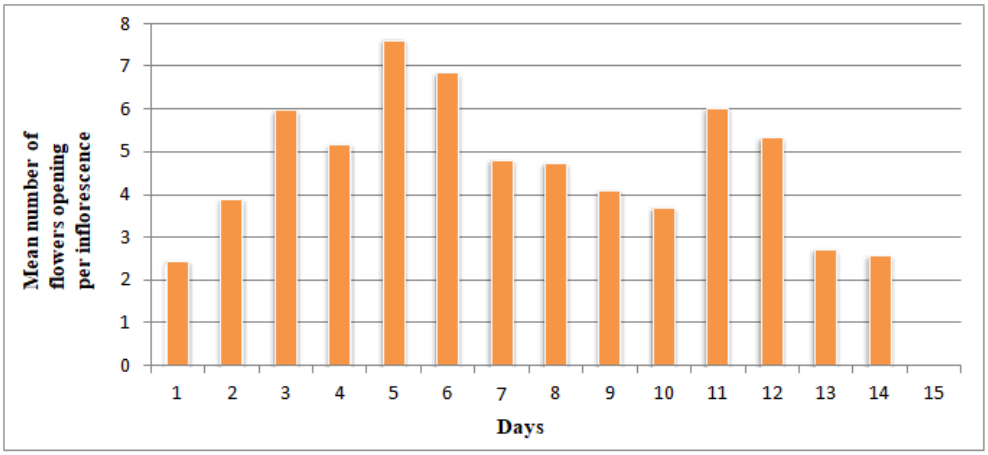
Fig. 8. Anatomical observation of floral morphology (cross sectio).

Flower emergence duration. The time period taken from bud initiation of individual flower to full emergence of flower is 19-20 days.

Flowering frequency. The Flowering frequency in black pepper was maximum in the month of July and minimum in January and February and shown in Graph 3. **Flowering intensity.** The average number of flowers opened per inflorescence each day represented as flowering intensity was maximum on the fifth day of anthesis (Graph 4).



Graph 3. Flowering frequency in *Piper nigrum* L.



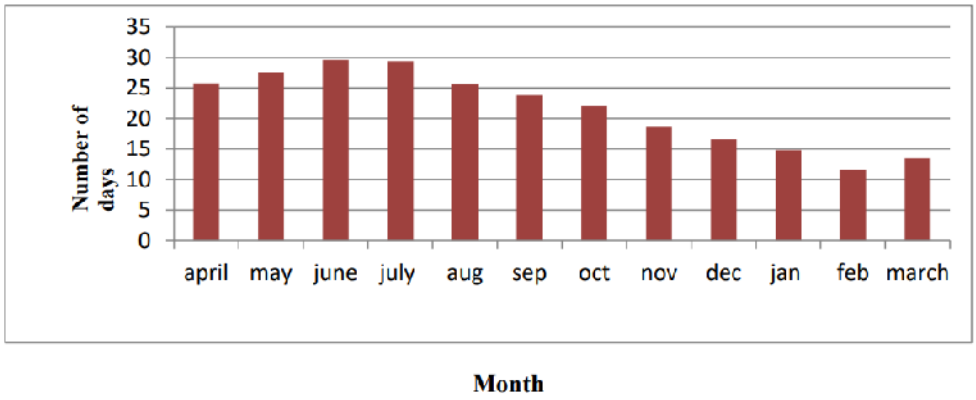
Graph 4. Flowering intensity in *Piper nigrum* L.

Anthesis period in an inflorescence. The anthesis period in inflorescence varied from 9 days (6 cm) in small inflorescence and 12 days (9 cm) in long inflorescence. The duration of pollen availability in an inflorescence varied from 9 days in small inflorescence (6 cm) to 12 days (9 cm) in long inflorescence (Graph 4). The pollen release was complete in 9 days in small inflorescence which means that pollen release occurred during the time of stigma receptive period itself, substantiating self-pollination. Four anthers are there and are released at different time and hence the duration of availability of pollen per inflorescence is more.

Anthesis period in the plant. The anthesis period in a plant was maximum in the month of July and minimum in the month of February and January.

Duration of spiking in the plant. The emergence of spike till the full emergence in a spike varied from 23-30 days. The mean duration of spiking was 26.84 days in a plant.

Duration of spiking in the plant population. The observation was recorded for 1 year from emergence of first spike to last spike in five plants. The mean duration of spiking in a plant population was maximum in the month of June – July and minimum in the month of February (Graph 5). In a black pepper plant the pollen availability varied from 27 days in the month of July to 0 in the month of March 2018. Throughout the year except in the month of March, pollen was available due to the presence of spike formed. The data on mean rainfall during the period from March 2018 to March 2019 substantiates the spike formation (Appendix 11). However, the maximum pollen availability was in the month of June– July which corresponds to the maximum production of spikes during these periods (Fig. 5).



Graph 5. Duration of spiking in a plant population of *Piper nigrum* L.

Fruit maturation period from fertilization. Fifty inflorescences taken from twenty-five black pepper plants grown in the field and the artificial pollination was carried out in 8 flowers in an inflorescence and the number of days taken from fertilization to fruit maturity was recorded by color change of one or two fruits from green. The fruit is a drupe and the period taken from fertilization to maturity varied from 150-175 days. Pepper fruit takes 6–8 months for full maturity from flowering, depending upon variety and the average being 7 months (Ravindran, 2006).

CONCLUSION

The floral morphology was light green (145 C) and dark green flower colour (140 A). The odour of the flower was fresh floral fruity odour and the odour was slightly minty. Presence of nectar was noticed on the black pepper flowers. The flowers in the inflorescence complete opening within 9 days from the opening of the first flower and flower opening starts from 4-5pm. 48 to 98 flowers in a spike depending on the length of spike. The stigma was 4 lobed, wet type and papillate. The duration of stigma receptivity was there for 7 days from the time of anthesis of flower. The duration of pollen availability in an inflorescence varied from 9 days in small inflorescences (6 cm) to 12 days (9cm) in long inflorescences. The longevity of flowers varied from 14-15 days. Flower emergence duration ranged from 19 to 20 days. Flowering frequency in black pepper was maximum in the month of July. The flowering intensity was maximum in the fifth day of anthesis. The anthesis period in an inflorescence varied from 9 to 12 days. The maximum anthesis period in a plant was maximum in the month of July and minimum in January and February. The fruit is drupe and the period taken from fertilization to maturity varied from 150-175 days.

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Conflict of interest. None.

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