POLLINATION BIOLOGY OF BLACK PEPPER

(*Piper nigrum* L.)

by

POOJA S.

(2017-12-025)

THESIS

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DECLARATION

I, hereby declare that this thesis, entitled "**POLLINATION BIOLOGY OF BLACK PEPPER (*Piper nigrum* L.)**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or society.

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Pooja S.

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LIST OF ABBREVIATIONS

IKI	Iodine Potassium Iodide
TTC	Triphenyl Tetrazolium Chloride
DAB	Diaminobenzidine
ABF	Aniline Blue Fluorescence
et al.	Co-workers/ Co authors
CD	Critical difference at 5% level
CV	Coefficent of variation
mm	Millimeter
S	Significant
%	Percent
°C	Degree celcius
M^2	Square meter
No.	Number
cvs.	Cultivars
rpm	Rotation per minute
cm^2	Centimeter square

Introduction

1. INTRODUCTION

Black pepper (*Piper nigrum L.*) one among the distinguished spices known for its pungency and medicinal properties from the family Piperaceae is rightly called the 'King of spices' in view of the legendary position it held in international trade from centuries ago (Ravindran, 2006). Wet evergreen forests in the hill ranges of Western Ghats in South India, now declared as one among the "global hot spots of biodiversity" is the primary center of origin of this valued spice crop (Ravindran, 2006). India is known as the home of spices and Indian spices are most sought after globally, due to their exquisite aroma, texture and taste (Ravindran 2006). Black pepper is having several medicinalproperties such as defensive role against infection by insects, animals and microbes (Nybe *et al.*, 2007) treating digestive disorders, gastric problems, diarrhea, ingestion and respiratory problems (Ravindran, 2007). India produced with 57,000 metric tonnes of black pepper from an area of 1, 34280 ha in the year 2016-17. Apart from India, pepper is produced in Brazil, Sri Lanka and Vietnam. Vietnam is the world's largest producer and exporter of pepper (Spices Board, 2018). Kerala, Karnataka and Tamil Nadu are the major black pepper producing states in India.

In black pepper spike shedding and poor berry setting is noticed in many pepper plants. Geetha and Nair (1989) attributed spike shedding to inadequate pollination, low moisture and low N and K in shoots. Nybe *et al.* (2007) reported that spike shedding occurred in almost all months from spike emergence to harvest. Inadequate pollination might be one of the factors leading to spike shedding which can affect the yield. Barring a few reports there are very little studies on the pollination biology of black pepper. Understanding on the floral morphology and phenology is essential to understand the pollination biology of the plant. More over studies conducted till now could not give a conclusivereport on the agents involved in the pollination of black pepper though the role of rain water, wind. insect, dew and apomixis has been viewed by many early researchers. Thus, for understanding the agent/agents in pollination biology of black pepper, floral morphology, floral phenology and breeding system need to be investigated. Keeping this in view, the present study is proposed with the objective to study the floral morphology, floral phenology, agent/agents of pollination and breeding system in black pepper.

Review of Literature

2. REVIEW OF LITERATURE

Black pepper (*Piper nigrum*L.) the 'king of spices' and one of the oldest spices known to mankind (Babu *et al*., 2006) 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.

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.

It has been suggested that black pepper originated as a natural hybrid between either diploids or tetraploids (Ravindran, 1991). Black pepper *(Piper nigrum* L.) is a predominantly self-fertilized perennial vine (Sasikumar *et. al*., 1992).

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).

2.1 Botany of *Piper nigrum* L.

Black pepper is perennial climber, climbing on support trees with the help of aerial clinging shoots. Black pepper exhibit dimorphic branching producing orthotropic branches (main stem) and plagiotropic branches (fruiting stem). The orthotropic branches are straight, upward growing with monopodial growth habit. The nodes are swollen with 10-15 short adventitious roots and a leaf per each node. At the axil leaf of the orthotropic branches lies an axillary bud which develops into a plagiotropic branch. The plagiotropic branches are without aerial roots that grows laterally with sympodial growth habit, and produce flowers and fruit. As the shoot grows, the terminal bud gets modified into the spike and growth is confined by axillary bud. The orthotropic shoots occasionally produce axillary branches that exhibit monopodial growth habit. They do not undergo branching as the orthotropic shoot, and do not usually produce any clinging roots on the nodes. They are positively geotropic, grow downward and hence called hanging shoots Pepper plants also produce adventitious runner shoots from the base especially during the rainy season or when the soil moisture availability is high. They have prominent aerial root initials on the nodes and on coming into contact with soil, strike root quickly and are the common planting materials used extensively for clonal propagation.

Leaves are simple, alternate, cordate, varying in breadth, broadly ovate and glabrous (Ravindran, 2000). Pepper leaves are variable in size and shape and are the distinguishing features of the cultivars. Leaves have prominent veins from the base to apex (Ravindran, 2006). Spikes in black pepper can be straight or curved (Parthasarathy *et al*., 2007).

Descriptors of black pepper has been put forwarded by Bioversity International (1995) in which different spikes form are described likespike orientation as erect and prostrate, spike shape as filiform, cylindrical, globular, conical and others. Bract type as sessile oblong and adnate to the rachis, peltateorbicular, cupular with decurrent base, fleshy, connate, transformed into a cup, deeply copular with decurrent base and others.

Black pepper is mostly is dioecious in wild form (Krishnamurthi, 1969), but in the cultivated types, the plants are mostly gynomonoecious (i.e., bearing female and bisexual flowers) or trimonoecious (i.e., bearing female, male and bisexual flowers), and are fertilized by self-pollination (Thangaselvabai *et al*., 2008). Ravindran (2006) reported that pepper flower is sessile, bracteate, achlamydeous, unisexual or bisexual. Cultivated pepper has bisexual flowers. Bracts oblong, decurrent with free upper margin, develop into a shallow cup in female spikes. Stamens 2, dithecous, carpel single, ovary spherical, style absent, stigma 3–5 lobed, papillate.The berry like fruit is a drupe, each containing a single seed, and when dried it is called peppercorn (Chaveerch *et al*., 2006; Ravindran, 2006). Flowers are sessile. White small flowers on inflorescence vary in number from 25 to 100, arranged in four to five rows (Parthasarathy *et al*., 2007). The flower bud differentiation in black pepper was influenced considerably by rainfall and relative humidity. The peak flower bud differentiation was from middle June to end of July (Ravindran, 2006; Nybe *et al.,* 2007). The flowering starts usually in June-July following the monsoon rain (Ravindran, 2006).

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, 1994).

2.2 Phenology

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 (Brearley *et al.*, 2007).

2.3 Pollination biology

Pollination biology in India started mostly as a descriptive science, aiming to understand plant morphology and anatomy in relation to pollination. Most of the early work on pollination biology was restricted to documenting the kind, number and time of floral visitors on various plant species (Vasudeva and Lokesha, 1993). Lopez *et al*., 1999 stated that the combination of androecium, style and stigma types reflects important adaptations related to pollination. Therefore, flower structure, phenology and the evolutionary ecology of pollination partnerships are interwoven, so much so that systematists rely on floral structure for identification and phylogenetic studies (Kevan, 1979).

Pollination biology in India started mostly as a descriptive science aiming at understanding plant morphology and anatomy in relation to pollination. Pollination also has to be considered over an evolutionary timescale, but the extent of recent anthropogenic change in global ecosystems means that the field has pressing implications for the conservation of species and sustainability of food production (Randal *et al*., 2009).

2.4 Floral morphology and phenology

Tebbs (1989) reported pink and violet flowers in the inflorescences of *Piper* sp. According to Figuerido and Sazima (2000) flower colour of *Piper nigrum* is creamy, yellowish or whitish. Ravindran (2006) reported that when spike is young it is green or whitish green, or light purple and when mature it is green, pale purple or pale yellow.

Tebbs (1989) reported lemon or lime odour in some Mesoamerican Piperaceae species and is probably the most widespread odour in the family. Nectar is not discernible in the flowers, as has been observed for other Piper species (Fleming, 1985). Sweet lemon lime odour in *P. amalago, P. arboretum, P. crassinervium, P. gaudichaudianum, P. glabratum, P. macedoi, P. mikanianum, P. mollicomum, P. regnelli, P. xylosteoides, Ottonia martiana, Ottonia propinqua, Pothomorphe umbellate* except for *P. aduncum* whose flowers are scentless was reported by Figuerido and Sazima (2000).

According to Kanakamany (1982) anthesis of black pepper is from 7.30-8.30 pm. According to Ravindran (2006) anthesis in black pepper takes place during 4 pm. Nybe *et al*. (2007) reported anthesis in black pepper as from 6-6.30pm.

Anther dehiscence in black pepper is controlled to a very great extent by temperature and relative humidity (Iljas 1960, Martin and Gregory 1962). Waard and Zevan (1969) found that in Sarawak the opening usually took place between 12.00 and 14.00 h on days when relative humidity of 60 per cent was attained and at a temperature of 32°C, combined with bright sunshine. He also mentioned that the mass of pollen may spill freely over adjacent stigmas and other parts of the spike. Pollen dispersal under field conditions can be erratic over time and dependent on relative humidity and temperature in pecan (Yates and Darrel, 1993). Nybe *et al*., (2007) reported that anther dehiscence takes place between 14.30-15.30 hrs (2.30-3.30 pm) and Chen *et al*., 2018 reported the anther dehiscence at 23.00-24.00 hrs (11.00-12.00 pm).

Pollen grains are metabolically dormant and highly desiccated when released from the anthers (Harrison, 1979, Buitink *et al*., 2000). Pollen grains are reduced male gametophytes which, upon pollination, produce pollen tubes that grow through the tissues of the pistil to effect fertilization and seed set.

Iljas (1960) and Martin and Gregory (1962) reported that pollen grains of black pepper are small, the mean diameter being approximately 10 μm irrespective of cultivars. The species and cultivars of *Piper* examined were difficult to distinguish on the basis of plant morphology which makes development of an intrageneric classification challenging. However, the variation in pollen morphology between varieties offers another avenue for demarcating the taxa. The pollen grains of the *Piper* species like *Piper nigrum, Piper mullesua, Piper hymenophyllum, Piper galeatum, Piper colubrinum, Piper chaba, Piper barberi, Piper argyrophyllum* and cultivars of Panniyur 1 to 7 were monosulcate and exine ornamentation pattrn was either tuberculate or granulate with redundant features (Divya *et al.*, 2015).

Piper spp. exhibit much uniformity in pollen morphology, except for the variation in size. Pollen grains are small, the diameter along the equatorial axis ranges from 8.0–18.0 μm and along the polar axis, between 6.5–15.0 μm. Grains are monosulcate, the sulcus extending upto the lateral extremities. Very rarely the grains are non aperturate or porate (Rahiman, 1981). The sulcus is in the form of a narrow slit with thick borders all along the periphery. The exine surface is reticulate, the bronchi being large and irregular on the distal polar surface. In *P. nigrum* the pollen grains measure along the equatorial axis from 9.5–13.0 μm, the mean being 11.0 μm; and along the polar axis 7.0–10.5 μm having a mean of 8.84 μm (Rahiman, 1981). The grains are spheroid to suboblate, rarely pyramidal, sulcus measures 7.0–11.0 μm in length and 1.0–2.0 μm in breadth.

Piper nigrum exhibits much uniformity in pollen morphology (Ravindran *et al.*, 2000). Chen *et al.*, (2018) reported that SEM observation showed pollen grain size of black pepper was about <10 μm in diameter, categorised under myosotis, spherical shaped, radially symmetrical and with irregular pinulose sculpturing.

Pollen counts are an essential feature of research on pollination like estimation of male reproductive output, comparison of functional gender of different plant morphs, quantification of pollen removed by pollinator visits and estimation of airborne pollen levels causing allergy (Kearns and Inouye, 1993). The total amount of pollen per anther varies with the cultivar. Martin and Gregory (1962) estimated 100,000–300,000 pollen grains per spike.

Assessment of pollen viability is a pre-requisite and also important in studies on pollen storage, reproductive biology and hybridization (Harrison *et al.*, 1984). Pollen viability is a critical factor, which is considered as an important parameter of pollen quality (Dafni and Firmage, 2000).

Sucrose is the best carbohydrate source for pollen germination, as it maintains the osmotic pressure of the medium and acts as a substrate for pollen metabolism (Shivanna and Johri 1985). Germination percentages were significantly low in higher concentration of sucrose medium. According to Shivanna and Johri (1985), the optimum concentration of sucrose varies from species to species.

In vitro pollen germination test indicated that highest percentage of pollen germination and tube elongation was observed in Brewbakers medium. It contains sucrose which acts as a nutritive material for pollen germination (Johri and Vasil, 1961) and helps in maintaining osmotic balance between the germination media and pollen cytoplasm. *In vitro* germination has been extensively used since pollen grains of large number of species readily germinate *in vitro* on a simple reaction with a carbohydrate, source, boron and calcium (Shivanna and Rangaswamy 1992).

Pollen germination and growth of pollen tubes are important research materials for physiological, biochemical, biotechnological, ecological, evolutional and molecular biological studies (Ottavio *et al*., 1992). Pollen grain can remain viable even upto 5 days after the opening of the flowers in black pepper (Sasikumar *et al*,. 1992). Another study found that pollen collected after 20 hrs showed relatively low germination percentage and short pollen tube elongation. The pollen collected from this stage may be non-viable, even though the pollen is able to achieve satisfactory high germination percentage and pollen tube elongation. According to Harrison (1979), non-viable pollen grains may hydrate to the same extent as living pollen grains, swell, and even develop short tubes before the tubes eventually rupture. In the pollen viability study, results suggest that black pepper pollen were more viable between five and 10 hrs after anther dehiscence (Chen *et al*., 2018).

Pollen fertility can be assessed by acetocarmine and glycerin staining technique (Radford *et al.,* 1974). The stained pollen grains were treated as fertile and unstained pollens were counted as sterile.

The adhesion of pollens on the stigma is a primary requirement for successful pollination. After falling on the stigmatic surface, pollen grains were subjected to hydration and then pollen wall proteins were released on to the stigmatic surface (Harrison *et al.,* 1975). Generally receptivity reaches a maximum soon after anthesis (Shivanna and Johri, 1985). In most plants, the stigma is receptive to pollination over a wide range of floral developmental stages (Amy and Rosanna, 2010; Chen *et al*., 2013).

Stigmas remain receptive for ten days with peak receptivity 3-5 days after exertion in black pepper (Martin and Gregory, 1962). Nambiar *et al*., (1978) reported that distal flowers in inflorescence of black pepper lost their receptivity in fewer days which is indicated by a viscous condition. According to Chen *et al*. (2018), the peak receptivity of stigma is 3-5 days in black pepper. Stigma receptivity could be evaluated by the arrival of peroxidase on stigmatic surfaces of the black pepper flower and the presence of peroxidase is indicated by the appearance of a blue or greenish colour (Dafni and Maues, 1998). Chen *et al.*, 2018 revealed a study on receptivity via a hydrogen peroxide test, showing no significant difference at any stages of stigma, including the early emergence stage. According to Kalinganire *et al.* (2000) stigma receptivity of black is for 10 days while according to Nybe *et al.* (2007) stigma receptivity lasted for 7 days.

2.5 Pollination agent and Breeding system

Pollination is the transfer of pollen grains from the anther which is the male part of the flower to the stigma which is on the female part. Barber (1906) and Anandan (1924) attributed pollination in black pepper to splashing of rain, the latter reported that rain drops help in scattering pollen grains in different directions, either wash down the pollens to lower spikes or carry them to neighbouring vines. He ruled out the role of insects in pollination. It appeard that water is a medium for pollen distribution. Heavy rains often have an adverse effect on pollination. Similarly lack of rain during the flowering period result in poor fruit set (Anandan 1924; Govinda and Venkateswaran 1929).

According to Menon (1949) and Martin and Gregory (1962) pollination in piperaceae occurs by wind or rain because of the similarity of its minutely flowered spicate inflorescence. Studies have shown that pollen transportation by wind is negligible under Indonesian situation in black pepper (Iljas, 1960) and practically absent under Indian condition. But Martin and Gregory (1962) based on their study in Puerto Rico, indicated that 32–64 per cent of the pollen on the spike may be dispersed to the air within 24 h after exposure and they recorded anemophily only for *Piper nigrum* in Panama. In Sarawak, in the cv. *Kuching,* fresh pollen appeared in glutinous clusters dispersable in water (Waard and Zevan 1969).The other Central American *Piper* species except *Piper nigrum* are not wind pollinated (Semple, 1974 and Fleming, 1985). The majority of *Piper* species grown at the edge of the forest, was at appropriate place for pollen transport by wind according to Arruda and Sazima, (1988), but *Piper arboreum, P. gaudichaudianum, P. mikanianum, P. regnelli, O. martiana*and *O. propinqua* are also grown in the forest under-storey and are effectively pollinated by wind.

Bawa *et al.* (1985) also recorded some an emophilous under storey species within the Moraceae. Species of Piperaceae exhibit some classic traits of anemophilous plants, such as the increase of individuals flowering in windy months (from September to December), the small and pulverulent pollen grains and the protogynous flowers (Lloyd and Webb, 1986; Honig *et al.*, 1992).

Pepper flowers are not adapted for insect pollination. However presence of insects have been reported by Martin and Gregory (1962) and Semple (1974) suggestive of their role in pollination. Semple (1974) found that in Costa Rica several species of *Trigona* bees are the most common visitors on *Piper* spp. No direct evidence for insect pollination came from other sources. However studies conducted in Kerala, the centre of diversity for black pepper, rule out the role of insects in pollination. Insect activity is rare in pepper plants except for ants and certain other crawling insects and insect pests such as pollu beetle.

Accumulation of dew may cause the disintegration of the pollen lumps (Martin and Gregory, 1962). Drops collected from the spikes were reported to contain considerable quantities of pollen (Waard and Zeven, 1969).

Many piper species had minute odorous and nectarless flowers visited by insects (Semple, 1974; Fleming, 1985). Phenology, floral biology and breeding system were described for 14 piperaceae species at two sites covered by semideciduous forest in South Eastern Brazil. Five of them showed a substantial degree of self-compatibility and one was andromonoecious. Of thisseven species were wind pollinated and three were exclusively pollinated by insects. *Piper amalago, P. crassinervium* and *P. glabratum* may be exclusively insect-pollinated

Flower visitors were mainly hover flies and bees. Central American *Piper* species were pollinated mainly by stingless bees of the genus *Trigona*. Flowering occurred continuously throughout the year, with a peak during the windy months. Hoverflies and bees visit Piperaceae flowers for pollen. Moving along the inflorescence, flies touch the open anthers with the label and gather pollen with the pulvilli and arolia. In some instances, hoverflies also touch the bracts with the labell, presumably collecting pollen grains or some other substance. The bees move faster along the inflorescence, collecting pollen with their legs. Flies visit several inflorescences before leaving the plant, whereas bees visit only two to four inflorescences, both of them moving from the proximal part of the inflorescence to the distal part. Species of Coleoptera and Hemiptera were also found in Piperaceae inflorescences, but they rarely moved between flowers and behaved as herbivores, chewing and sucking flowers and fruits. Thomisidaespiders' prey upon bees and flies visiting the inflorescences of *Piper crassinervium* and *P. regnelli* (Figueiredo and Sazima,2000).

Breeding system has been identified as a major factor influencing genetic structure (Braker, 1959). Breeding system in plants is used as a tool to regulate the component of fecundity and other commercial traits for selection and domestication (Frankel and Galun, 1997). The most obvious way to discover the breeding system of plants starts with the morphological examination of the flowers (Silvertown and Charlesworth, 2001). The breeding system of a given taxon is reflected in its floral structure, advertisement and reward. Plants can be predominately out-breeding, inbreeding or some mixture of the two. In many flowering plants, specific mechanisms have been evolved to promote any one of these systems.

Out breeding is also known as out crossing where allogamy or xenogamy involves the transfer of gametes from one individual to another which is genetically different. Out crossing can be promoted by genetically determined self in compatibility mechanisms. Self-incompatibilty refers to the inability for the fertilization between gametes derived from an individual genotype (Simpson, 2006). Inbreeding is referred as selfing and is the union of gametes derived from single individual. In flowering plants, inbreeding may occur either within a single flower (autogamy) or between the flowers of the same plant (geitonogamy). In self-compatible species the tendency of pollinators to visit several flowers in sequence on a single plant also increases the opportunity for geitonogamy and a resulting increase in the selfing rate.

Gentry (1955) suggested agamospermy in *Piper nigrum*. The breeding systems of piperaceae are known for only very few species, the paleotropical *Piper nigrum* and the neotropical *P. Arieianum* which are self-compatible (Martin and Gregory, 1962; Sasikumar *et al*., 1992), and the paleotropical *P. methysticum* which is self- incompatible (Prakash *et al*., 1994). *Piper aduncum* and *Pothomorphe umbellata* may be agamospermous, though tests for agamospermy were not carried out, since all inflorescences of these species developed fruits (Figueiredo and Sazima, 2000).

Geitonogamy is the self pollination mechanism involving gravitational descending of pollen grains combined with action of rain water or dew drops. This is reported as effective mechanism in plants having long pendent inflorescences like black pepper. While heavy rains have an adverse effect on pollination, lack of rain during the flowering period also result in poor fruit set. (Anandan 1924, Govinda and Venkateswaran, 1929). Iljas (1960) on the other hand reported the presence of dry, powder like pollen in the cv. *Bangka* and suggested the possibility of direct gravitational distribution (geitonogamy). He reported geitonogamy from Indonesia suggesting that, the distal flowers of a raceme were pollinated by more proximal flowers. He found that free hanging spikes isolated inside polyethylene bags displayed good fruit set, irrespective of insects or rain water. Pepper is adapted for such a mode of pollination. Pendent hanging spikes, spiral arrangement of flower, sequential ripening of stigmas and prolonged receptivity of stigmas are all favourable to geitonogamy. About 86-95 per cent selfing (self-pollination) was observed in the varieties when the spikes of the varieties were protected from water or insects by enclosing them in polyethylene bags (Sasikumar *et al.,* 2001).

Hybridisation ensures a wide variety among the cultivars of black pepper ensuring quality attributes and yield. In India, two achievements through conventional breeding have been reported so far, that is, Panniyur 1 and Panniyur 3, both from crosses between Uthiarankotta and Cheriyakaniyakadan (Ravindran *et al.*, 1981). Limited achievement of conventional black pepper breeding is possibly due to lack of fundamental information on the nature of the reproductive biology, particularly on pollen viability and stigma receptivity (Chen *et al.*, 2018)

Artificial pollination procedures for black pepper were reported by Ravindran *et al.* (1981), Sim (1985) and Chen (2011). In general, the artificial hybridisation was carried out by inseminating the pollen suspension of the male plant onto the stigma of female plant. However, no specification regarding the viable stage of pollen for collection and the receptive stage of the stigma has been reported. So doubt exists regarding the reliability of the procedure and the technique of artificial pollination. In addition, intricacy in artificial pollination of black pepper is perhaps due to the catkin type of inflorescences, minute sized flower and lack of uniformity in emergence of anther and time of anthesis. Hence, a fundamental study in floral biology of black pepper is needed to ensure efficiency of conventional breeding (Chen *et al.*, 2018)

Intervarietal hybridization is an extremely important approach for crop improvement and enhancing crop yield. This involves pollen transfer from the pollen parent to the stigmatic lobes of the female parent. Iljas (1960) suggested using suction pump for emasculation. Martin and Gregory (1962) described two techniques of pollination in pepper. In one technique ripe anthers were opened using scalpel and pollen was scooped up and applied on the stigma of selected spikes while in other technique spikes from male and female parents were brought together and brushed with a camel hair brush. Emasculation was tried by alcohol, hot water and excision. Warrd (1969) developed a method of hand pollination which made use of protogyny in the cv. *Kuching*. Before cross pollination all spikes present on the female parent were removed to prevent geitonogamy. Portion of spike having freshly opened anthers were cut off and placed on the end of a long pin and the entire pollen cluster was gently brought into contact with the young stigma and the method gave 50–75 per cent success. Ravindran *et al.* (1981) developed an efficient crossing technique making use of the protogyny.

Apomixis in black pepper was first reported by Gentry (1955) on male sterile cv. *Uthirankotta*. Fruiting in female plants of *P. longum* and *P. chaba* growing in the germplasm conservatory of IISR has been reported. In the Western Ghat forests, small populations of either male or female plants are met with quite frequently and in such female populations good fruiting was noticed. All evidences tend to point to the presence of apomixes in the South Indian species of *Piper* (Ravindran, 2006). Apomixis seems to disappear or at least gets suppressed to a great extent with the gain of bisexuality (Ravindran, 2006). However Chen (2013) reported that occurrence of apomixes in *P. nigrum*, was not proved and hence apomixis do not contribute to fruit set in pepper.

In a study conducted by Sasikumar *et al.* (1992) the highest fruit setting was observed under open pollination in the three most popular cultivars, Karimunda, Panniyur1 and Aimpiriyan. Spike setting was complete (100%) in all the three cultivars under selfing, bagging, hand pollination and open pollination. Self fertilization was the highest in cv. Aimpiriyan followed by *cvs*. Panniyur1 and Karimunda. Water free pollination produced the highest spike and fruit set in cv.

*Panniyur*1 followed by Karimunda and Aimpirian. In all the three cultivars fruit set was less under water free condition than under open pollination. The extent of autogamy in three cultivars were 86 per cent in *Karimunda,* 92 per cent in *Panniyur*1 and 95 per cent in *Aimpiriyan* and the percentage of self compatibility was 84 per cent, 91 per cent and 86.5 per cent respectively. These results indicated that selfing is the predominant form of fertilization in cultivated bisexual pepper. Though protogyny occurs, it appears ineffective to prevent selfing as the pendent spike is abundantly assured of pollen from upper flowers.

Materials and Methods

3. MATERIALS AND METHODS

The research project "Pollination biology of black pepper (*Piper nigrum* L.) was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvanthapuram during 2017-2019. The details of the materials used and methods adopted during the course of investigation of the researchproject are presented below.

3.1. EXPERIMENTAL SITE

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, floral phenology, agents of pollination and breeding system. Fifty numbers of two years old bush pepper plants of two year old maintained in the Department of Plantation Crops and Spices in pots were used to study the role of wind, insect and rain water, in pollination as well as for studying the breeding system in black pepper. The role of dew will also be looked into.

3.2. CLIMATE

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. The data on the meteorological parameters of the area are presented in the Appendix I.

3.3. 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.

3.3.1. 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.

3.3.2. Flower odour

Fifty flowers were collected from first day of opening of stigma and placed in glass vials (Plate 1) and closed for 5 min, 30 min and 6 hrs to allow the accumulation of floral scent and was smelled. The method used was a modification of the method used by Braunschmid *et al.,* 2017.

3.3.3. 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 is (Morrant *et al.*, 2009).

3.3.4. 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.

3.3.5. 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.

3.3.6. 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.

3.3.7. Number of anthers/flower

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

3.3.8. 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.

3.3.9. 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.

3.3.10. Number of pollen grains/ flower

The pollen grains were collected from matured anthers just before dehiscence and transferred on to a cavity slide with a drop of water and crushed well with a spatula to get all the pollen grains out to the water. The contents are then transferred on to a glass vial and the volume was made upto10ml using 70% ethyl alcohol. From this, a known amount, about 1ml is transferred to a cavity slide and the pollen grains were counted using haemocytometer (Plate 2). The total pollen grains in the vial was determined using the counts. From this the number of pollen grains per anther was calculated and multiplied by four. Ten observations were taken.

3.3.11. Pollen characteristics

For pollen morphology studies, the anther at the time of maturity were collected and preserved immediately in 70% ethanol. Slide preparation for pollen morphology studies were made by the acetolysis method proposed by Erdtman (1952). The preserved material of anthers were transferred to a centrifuge tube and crushed with a glass rod. The dispersion was sieved through a brass mesh of 48 divisions cm^{-2} and was collected in a glass centrifuge tube. After centrifugation in centrifuge of 2000 rpm for 5 minutes, the supernatant was decanted and the pollen grains after washing in glacial acetic acid were treated with acetolysis mixture consisting of acetic anhydride and concentrated sulphuric acid (9:1) in the centrifuge tube. A glass rod was placed in each tube and was transferred to a water bath at 70-100° C for three to five minutes, till the medium became brown in colour. Centrifugation of this mixture was carried out in centrifuge of 2000 rpm for five min, the supernatant was decanted off and glacial acetic acid was added to the sediment and again centrifuged and acid was decanted. The permanent slides of acetolysed pollen grains were made by mounting them in glycerin jelly of 2 drops and the edges were sealed with paraffin wax. The prepared slide was taken for observation of pollen shape, pollen size, viable and nonviable pollen using Scanning Electron Microscope of Sophisticated Instrumentation and Computation Centre, University of Kerala.

Plate 1. Flowers of *Piper nigrum* L. kept in glass vials for finding flower odour

Plate 2. Counting of pollen grains per flower in *Piper nigrum* L. using haemocytometer.

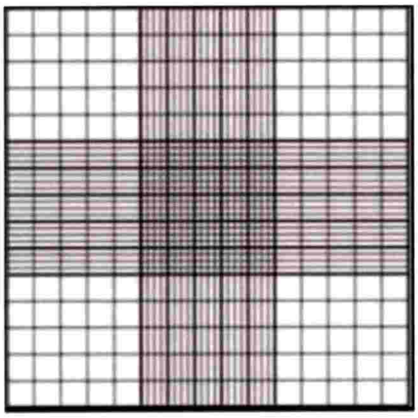


Plate 2a. Counting grid of haemocytometer.

3.3.12. Stigma type

Twenty five stigma of fifty inflorescences of different plants were selected. The observation were 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.

3.3.13. 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.

3.3.14. Duration of pollen availability/inflorescence

Fifty inflorescences of twenty five plants were observed for 15 days in each inflorescence from the period of full maturity of anther till all anther is dehisced and pollen is lost.

3.3.15. Duration of pollen availability/plant

Five plants were observed for a period till the pollen is available from the last maturated inflorescence. The observation were recorded from anther emergence to dehiscence in a selected plant.

3.3.16. Duration of stigma receptivity

The observation were 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.

3.3.17. 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.

3.3.18. Pollen fertility

Pollen stainability as an index of fertility was determined by staining pollen grains by acetocarmine glycerin staining technique. A modification of the technique followed by Marks (1954) was used for the study. Matured anthers were collected, kept in clean slide. To this one drop of acetocarmine was added and macerated to release the pollen grains. Debris were removed and one drop of glycerine was added over this and mixed once again using needle and covered by cover slip. After 10 minutes the slides were examined under stereo microscope. The number of stained and unstained pollen grains were counted. The stained pollen grains were considered as fertile whereas the unstained, undersized, partially stained and shriveled pollen grains were counted as sterile. The pollen fertility was calculated as

$$\text{Pollen fertility percentage} = \frac{\text{Number of fully stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

The mean percentage of fertile pollen was calculated from six samples with each containing about 59 to 85 number of pollen grains.

3.3.19. Pollen viability

3.3.19.1. Staining technique

3.3.19.1.1. Iodine Potassium iodide test

Potassium iodide (1g) and 0.5 g iodine were dissolved in 100 ml distilled water for preparing IKI solution. Pollen grains were placed in IKI solution for 10 minutes and viability was assessed by counting the dark brown or red coloured pollen grains (Sulisoglu and Cavusoglu, 2014). The test was carried out in six samples.

$$\text{Pollen viability percentage} = \frac{\text{Number of fully stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.3.19.1.2. Triphenyl Tetrazolium Chloride test

1%TTC (0.2 g TTC and 12 g sucrose in 20 ml distilled water) was used and a drop of the mixture was dropped on a microscope slide and the pollen was spread with a slim brush and covered with a cover slip. Pollen viability counts were made after 10 min. Pollen grains stained orange or bright red colour were counted as viable (Sulisoglu and Cavusoglu, 2014).The test was carried out in six samples

$$\text{Pollen viability percentage} = \frac{\text{Number of fully stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.3.19.2. Pollen germination tests

3.3.19.2.1. In vitro pollen germination

In vitro pollen germination was conducted to determine the effect of different nutrients like sucrose, boron and calcium nitrate at various concentrations. Different graded of sucrose (5, 10, 20, 30, 40%) were prepared and used individually and also in combination. In addition to this, Brewbaker - Kwack medium (Brewbaker- Kwack, 1963) was also used for *in vitro* germination test. One or two drops of each medium were placed separately on a clean glass slide.

Freshly dehisced pollen grains were added into the solution and spread thoroughly. The slide were incubated in petridish lined with moist filter paper for 24 h. After incubation, a drop of cotton blue was added to it and allowed to disperse. Pollen grains which had produced pollen tubes longer than the diameter of the pollen were recorded at a magnification of 40X (Belavadi and Ganeshaiah, 2013).

3.3.19.2.2. In vivo germination

Ten flower buds from five inflorescences were selected and remaining flower buds were removed. Each inflorescence was covered with polythene cover. Pollen grains were collected from other flowers after anther dehiscence and placed on the stigma of each flower. *In vivo* pollen germination was investigated by observing pollen germination and pollen tube entry into the stigma in these manually pollinated black pepper flowers.

3.3.19.2.2.1. DAB (Diaminobenzidine) test

Pollinated stigma were collected after 6 h and kept in moist chamber for 2 h and Stained stigmas were placed on a clean slide and added a drop of glycerin. Mounted stigmas were observed under microscope Leica MZ95 and were photomicrographed.

3.3.19.2.2.2. ABF (Aniline Blue Flourescence) method

Fix the pollinated stigma for 24 h and store them in 70% ethanol. Then transfer the fixed stigma to 4N NaOH (delicate material) for clearing. The temperature and the period of clearing depend on the size and texture of the pistil. For the most materials overnight clearing at laboratory temperature is sufficient. The period of clearing can be decreased by increasing the temperature to 60°C. Transfer the fixed stigma to water in a petridish / beaker and rinse them carefully. The mount the water rinsed in a 1:1 mixture of aniline blue and glycerine; alternatively, leave the softened and rinsed stigma in aniline blue overnight and then mount them in a drop of glycerine. Gentle pressure was applied on the coverglass to achieve required degree of spreading of the tissue. Then observed under fluorescent microscope.

3.3.20. Anatomical observation of floral morphology

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

3.3.21. 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).

3.3.22. 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.

3.3.23. 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.

3.3.24. 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.

3.3.25. 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.

3.3.26. Duration of spiking in the plant

Fifty inflorescence 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.

3.3.27. Duration of spiking in the plant population

The observation were 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.

3.3.28. Fruit maturation period from fertilization

Fifty inflorescence 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.

3.4. EXPERIMENTS ON AGENTS OF POLLINATION

The role of wind, insect, rain and dew has been proposed by many researchers in the pollination of plants belonging to family Piperaceae. Hence the role of wind, insect, rain water and dew was investigated.

Treatments – 6

T_1- Wind

FW_1 - Single inflorescence in the field grown black pepper plants were selected. A few fully developed flowers were retained and remaining part of spike was cut. The stamens on emergence were emasculated and covered by nylon net cover (Plate 3a) so that fertilization of stigma would happen only by pollens brought by wind from outside.

FW_2 - Three-five inflorescence were selected and covered by nylon net cover (Plate 3b). A few fully developed flowers on the lower spike were retained and the remaining part of the spike was cut off. The stamens at emergence on the lower spike were emasculated and rebagged. On this the fruit set will be decided by the pollen from the above spikes and those brought by the wind.

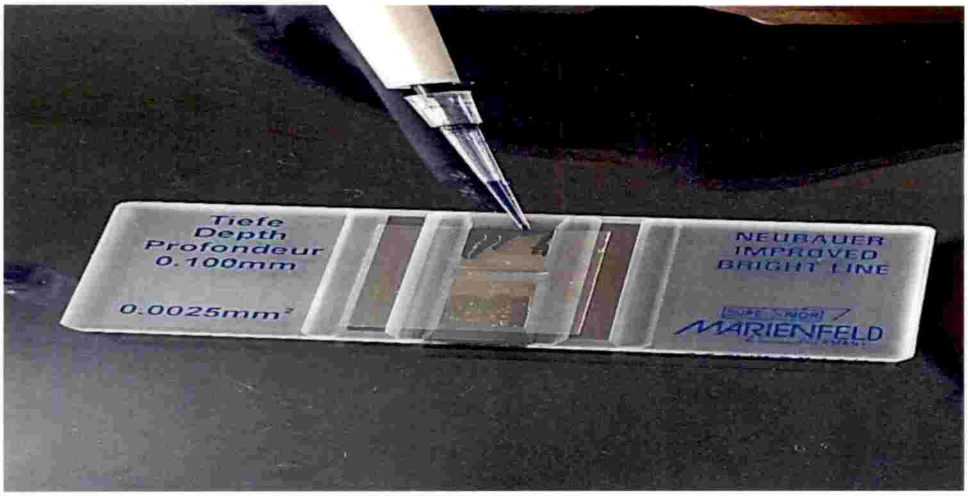
FC_2– Twenty five inflorescences in the field grown pepper plants were selected. A few fully developed flowers before the start of anther emergence were marked and the remaining portion of the spike were cut off. The stamens on emergence were emasculated immediately and covered by polythene cover excluding wind, insects, rain from pollination.

PW_1 - Twenty five inflorescence of bush pepper plants in pots kept under polyhouse were selected. The inflorescence was cut at the lower portion retaining only a few developed flowers at the base. The stamens on emergence were emasculated on the selected inflorescences and single inflorescence was covered by polythene cover (Plate 4a). The cover was removed and manual fanning was given for 10 minutes at 1 hour interval from the stigma receptivity period .

PW_2 - Three-five inflorescence together were selected from bush pepper plants kept in polyhouse. A few developed flowers were kept and the remaining portion of the spike was cut. The stamens on emergence were removed from the lower spike and covered by polythene cover (Plate 4b).

5

Plate3. Filling of counting chamber with grains in known volume of pollen suspension.



Experiments on agents of pollination

Plate 4. Black pepper spike covered by nylon net permitting wind pollination.

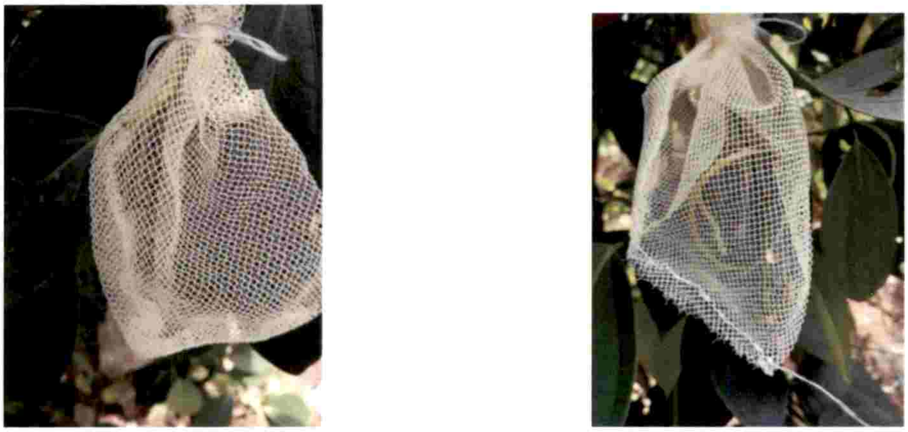


Plate 4. Treatments on wind pollination

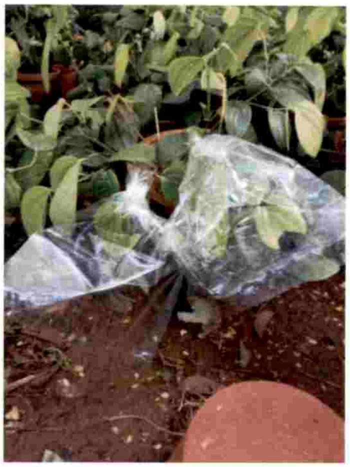


Plate 4a. Treatment (PW1)

5. Experiments on wind pollination

Plate 5a. Slide coated with vaseline to collect pollen of *Piper nigrum* L.

PC_2 -Twenty five inflorescence of pot grown bush pepper were selected. A few developed flowers were kept and the remaining portion of the spike was cut. The stamens on emergence were removed and covered by polythene cover to exclude wind, insects and rain.

Fruit set was checked 20 -25 days after flowering and percentage fruit set was worked out and used as an index of successful pollination.

In wind pollinated plants, pollen grains are light weight, powdery and large quantity of pollen grains are seen. The characteristics of the pollen of black pepper was studied and compared with the characteristics of wind pollinated pollen. Fresh microscopic slides covered with Vaseline (Plate 5a) was placed immediately below and between spike within a plant population (Plate 5b, Plate 5c, Plate 5d). The slides were taken after anther dehiscence, stained and checked under optical microscope to see the presence of pollen.

T_2 – Insect

Twenty five inflorescences in the field and twenty five in the pot grown was observed from the anthesis period to see whether any insect is constantly associating with the spike.

Visitation rates of insects was determined by counting the number of insect visits to flowers on an inflorescence for 10 min every 2 h between 6.00 am –8 .00 pm and further two times at 12.00 night and at 4. 00 am (Belvadi and Ganeshaiah 2013) in bush pepper plants. The behaviour of insect species was monitored by field observations and the presence of pollen on their body parts was checked using a stereoscopic microscope. The foraging behaviour of insects were recorded and captured using an insect net and were identified with the help of entomologists. Pollination efficiency of pollinators could not be checked since insects were not found to be the agents. Further experiments for foraging was not needed since the role of insects could not be found. However an attempt was undertaken to collect ants and allowed to move over the collected pollen and these

Plate 5b. Petriplate coated with vaseline kept in between two blackpepper plants at a distance of 4.5m apart from the base of the pepper plant

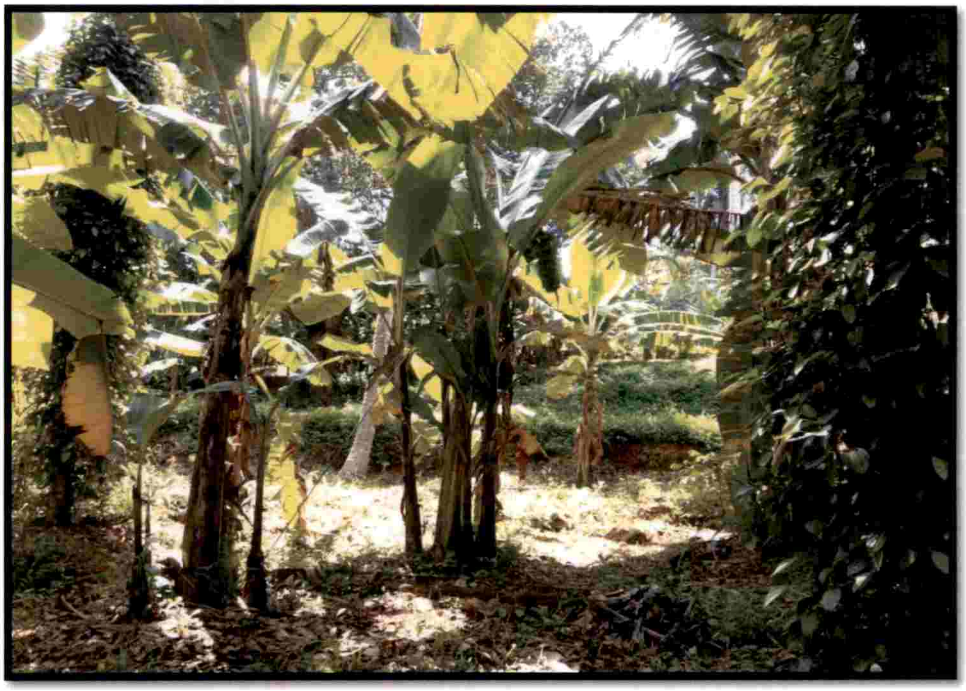


Plate 5c. Petriplate coated with vaseline kept at a distance of 1.5m from the base of the pepper plant

Plate 5c. Petriplate coated with vaseline kept at a distance of 2m from base of the pepper plant

Plate 6. Treatments on rain water

Plate 6a.Treatment (PR1)

Plate 6b.Treatment (PR2)

ants were then released to the inflorescence. But further results could not be obtained due to the ferocious movement after the release of the ant.

T_3 - Rain water

The role of rainwater in pollination was studied by the following treatments.

FR_1 - Twenty five inflorescences of black pepper grown in field were covered by polythene cover and at the time of natural raining the inflorescence was exposed and after the rain it was covered once again (Plate 6a).

FC_2 - Twenty five inflorescences of black pepper grown in the field were selected. The inflorescence was cut keeping only a few fully developed flowers and were emasculated at the emergence of stamen and covered by polythene cover excluding wind, insects and rain. These were taken as control.

PR_1 - The rain water was imitated as splashing on other ten bush pepper plants kept in pots as a whole at time of anthesis and whole plant was covered (Plate 6b) excluding wind and insects. Ten bush pepper plants were kept in poly house and only basin irrigation was given.

PC_2 -Twenty five inflorescence of pot grown bush pepper were selected. A few developed flowers were kept and the remaining portion of the spike was cut. The stamens on emergence were removed and covered by polythene cover to exclude wind, insects and rain

Fruit set was checked 20 -25 days after flowering and percentage fruit set was worked out and used as an index of successful pollination.

T_4 - Dew

Twenty five spikes of black pepper plants were observed regularly in the morning during anthesis in the field as well as in the pot grown bush pepper to see whether any dew accumulated in the spike. The dew accumulated was collected and stained and observed for the presence of pollen.

3.5. EXPERIMENT ON BREEDING SYSTEM

In order to understand the breeding system, different pollination systems like autogamy, geitonogamy, xenogamy, open pollination and apomixes were carried out in the bush pepper. The pollination experiments were carried out on selected plants at the time of maximum stigma receptivity. A minimum of twenty five inflorescences of pot grown bush pepper were taken for each experiment.

Design of the experiment - CRD

Treatments - 10

B_1 - Twenty five inflorescences of pot grown bush pepper plants were selected, marked and tagged at the time of anthesis and kept open for pollination and after 20-25 days fruit set was observed.

B_2 -Three flowers from top, middle and base were selected and covered with cloth bag (Plate 7a).

B_3 -Single flower was selected and covered with cloth bag (Plate 13).

B_4 - Four – five mature inflorescences in the pot grown black pepper plants were covered by a single cloth bag (Plate 7b). A few developed flowers in the lower spike were kept and the remaining part of the inflorescence was cut. Emasculation of observational lower spike was done during the period of emergence of stamen.

B_5 - Twenty five inflorescences in the pot grown bush pepper plants were selected. The inflorescence was cut after keeping a few well developed flowers. On the emergence of stamen in these inflorescences emasculation were carried and self-pollinated with pollens collected from the same plant and rebagged (Plate 7c).

7. Experiments on breeding system

7a. Treatment B2

plate 7b. Treatment B3

Plate7c. Treatment B4

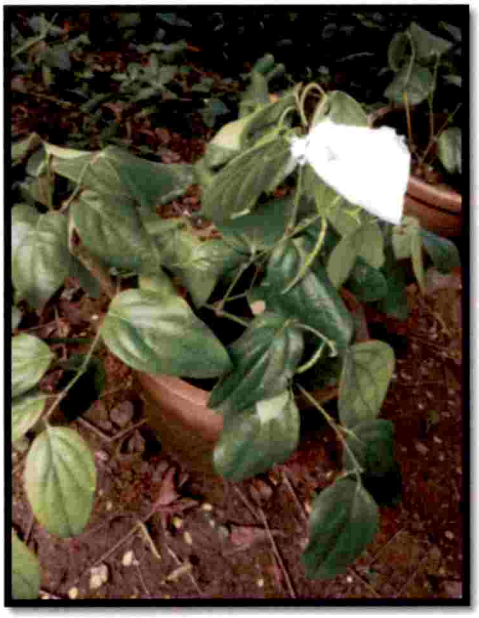


Plate 7d. Treatment B5

Plate 7e. Treatment B6

Plate 11f. Treatment B7

Plate11g.Treatment B8

Plate11f.Treatment B9

Plate11i. Treatement B 10

B_6 - Four to five mature inflorescences in the pot grown black pepper plants were covered by a polythene bag (Plate 7d). The inflorescence in the lower portion was cut after keeping a few developed flowers and emasculation of observational lower spike during the emergence of stamen was carried out. Water was sprayed to imitate rain to carry pollen.

B_7 - Twenty five inflorescences of pot grown pepper plants were selected. The lower portion of the inflorescence was cut after keeping only a few developed flowers and covered (Plate 7e). The flowers were emasculated on emergence of stamen tagged and bagged, the buds upon opening was hand cross-pollinated with pollen collected from other plant using brush and then re bagged .

B_8 – Three to four inflorescences of pot grown pepper plants were selected and after keeping a few developed flowers the lower portion of inflorescences were cut and emasculated. Another pot containing inflorescence will be kept near and both the inflorescences were covered (Plate 7f) together and allowed to pollinate.

B_9 – Twenty five mature inflorescences of bush pepper plants were emasculated and bagged (Plate 7g) without pollination (Richards, 1986).

B_{10} - Twenty five inflorescences of pot grown bush pepper were covered by cloth bag (Plate 7h) and kept as control.

Fruit set was checked 20 -25 days after flowering and percentage fruit set was worked out and used as an index of successful pollination.

3.6. INSECT

Observation on floral visitors and their foraging behavior were carefully made during entire flowering period at regular intervals throughout the day. Experiments were carried out to understand the mechanisms of pollination, nature and the interaction between the plant and pollinators (Faegri and pijil 1979). Number of insects visiting the flower was recorded. The number of visits per inflorescence per two hour during the entire period of 24 hours was recorded and the visits per inflorescence per hour was calculated as visitation rates (Lobo *et al.*, 2016) and the average time spent by an insect on the inflorescence was also recorded as foraging time with the help of stop watch. Insects visiting the flowers were anaesthetized with the help of choloform and collected for identification.

Results

4. RESULTS

The research project "Pollination biology of black pepper (*Piper nigrum* L.) was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvanthapuram during 2017-2019. The data collected from field and pot grown bush pepper experiment were statistically analyzed and the results are presented in this chapter.

4.1. Floral morphology and floral phenology of black pepper

4.1.1. 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 (Plate 8) as per the Royal Horticulture Society colour charts and represented in Appendix II. Out of hundred flowers observed fifty two were Light green (149 C) and 48 flowers were Dark green (140 A) has been shown in Plate 21. The bract colour varied from Yellow (3 B) to Light green (140 A) as per the Royal Horticultural Society colour charts and it is shown in Plate 9.

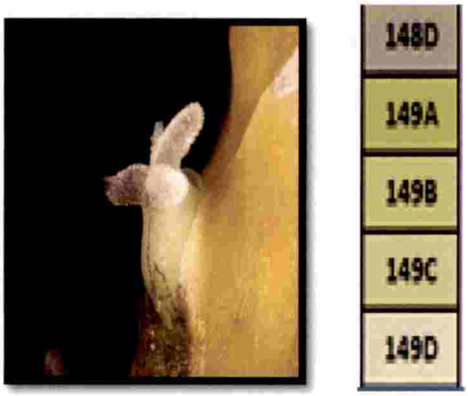
4.1.2. Flower odour

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 loosing the odour and became light minty odour six hours shown after keeping in closed containers as revealed by ten panel member as it is in shown in Appendix III.

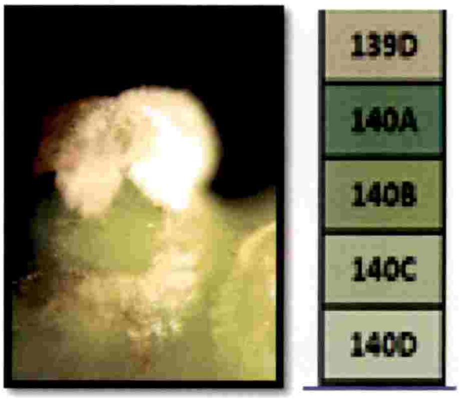
4.1.3. Presence of nectar

Presence of nectar was noticed in the flower between the bract and stigma (Plate 10a) and the amount of nectar varied from 23 to 25 μl as observed from thirty five stigma of one inflorescence as shown in Plate 10c. The mean of nectar

Plate 8. Flower colour of *Piper nigrum* L.



Light green (Code: 149 B)



Dark green (Code:140 A)

Plate 9. Bract colour of *Piper nigrum* L.

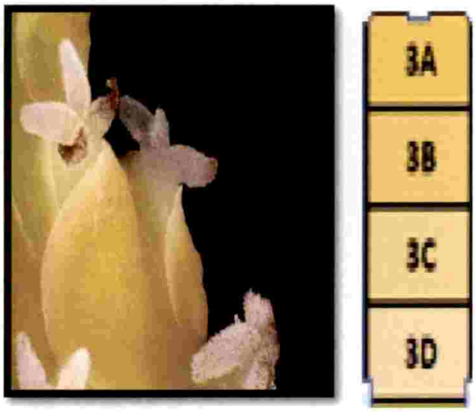


Plate 9 – Yellow(Code: 3 B)

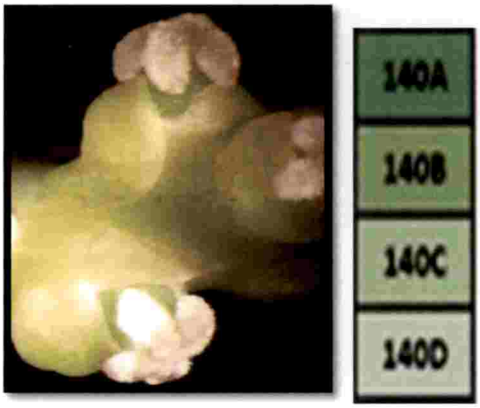


Plate 9 - Light green (Code:140 B)

Plate 10. Presence of nector in *Piper nigrum* L.

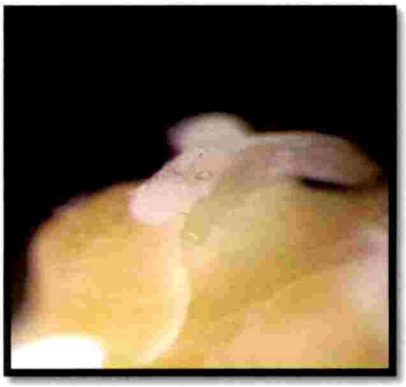


Plate 10a - Presence of nectar in flower

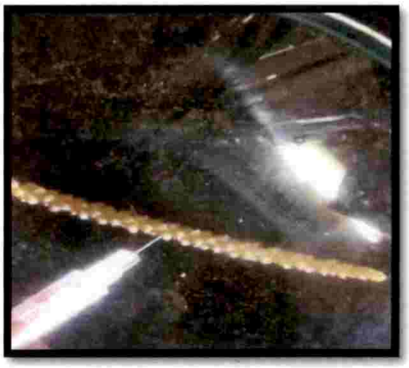


Plate 10b - Nectar drawn from the stigma through insulin syringe

Plate 10c – Nectar collected in the insulin syringe

Plate 10d – finding out brix content of ncetor Refractometer

content from thirty five stigma of fifty inflorescences were 24.08µl as shown in Appendix IV. The mean nectar content of one flower is 1.45µl.

4.1.4. 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. The details of individual observation is shown in appendix V.

Table 1. Mean flower opening time in black pepper (*Piper nigrum* L.).

No. of days	Time period								
	12 mid night to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm
1	0	1.56	0.7	2.46	1.5	0.58	0	0	1.1
2	0	1.48	0.56	2.44	1.48	0.58	0	0	1.02
3	0	1.46	0.56	3.48	1.48	0.58	0	0	0.96
4	0	1.62	0.56	4.96	1.48	0.58	0	0	0.96
5	0	2.48	0.56	6	1.48	0.58	0	0	1.18
6	0	1.56	0.7	2.48	1.5	0.58	0	0	1.08
7	0	1.56	0.7	1.58	1.48	0.58	0	0	0.84
8	0	1.48	0.56	1.56	1.48	0.58	0	0	0.92
Mean	**0**	**1.65**	**0.613**	**3.12**	**1.485**	**0.58**	**0**	**0**	**1.008**

4.1.5. Flower size of black pepper (*Piper nigrum* L.)

The data on flower size (mm) are given in the Appendix VI. The length and breadth of thirty flower was taken on third day, fourth day and fifth day. The mean presented in Table 2. 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 x 1.34 mm) on the fifth day of stigma receptivity.

Table 2. Mean length and breadth of the flowers on third, fourth and fifth day of *Piper nigrum* L.

Inflorescence	Third day		Fourth day		Fifth day	
	Mean length (mm)	**Mean breadth (mm)**	**Mean length (mm)**	**Mean breadth (mm)**	**Mean length (mm)**	**Mean breadth (mm)**
R1	1.51	1.32	1.51	1.32	1.53	1.34
R2	1.51	1.32	1.51	1.32	1.53	1.34
R3	1.51	1.32	1.51	1.32	1.53	1.34
R4	1.51	1.32	1.51	1.32	1.53	1.34
R5	1.51	1.32	1.51	1.32	1.53	1.34
Mean	**1.51mm**	**1.32 mm**	**1.51 mm**	**1.32mm**	**1.53mm**	**1.34mm**

4.1.6. 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 Plate 11 and it depends on the length of the spike (Appendix VII).

4.1.7. Number of anthers per flower

The number of anther in each flower was four and was uniform in all fifty flowers (Appendix VII) and Stereomicroscope and Scanning Electron Microscopic image are shown Plate 12.

4.1.8. 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 is presented in Appendix IX. The mean anther dehiscence time in a spike is given in Table 3. Anther dehiscence occurred from 11 am and continued till 4 pm and was maximum at 2-3 pm.

Table 3. Mean anther dehiscence time in *Piper nigrum* L.

No. of days	Time period					
	10am-11am	**11am-12pm**	**12-1pm**	**1-2pm**	**2-3pm**	**3-4pm**
1	0	5.7	5.12	4.86	6.86	4.56
2	0	5.42	4.8	4.76	6.82	4.2
3	0	4.9	4.68	4.58	6.62	3.92
4	0	5.7	5.12	4.86	6.86	4.56
5	0	5.24	4.8	4.72	6.74	4.08
6	0	4.9	4.68	4.58	6.62	3.92
7	0	5.7	5.12	4.86	6.86	4.52
8	0	2.58	2.44	2.38	3.32	1.84
9	0	2.78	2.34	2.1	3.36	1.78
Mean	**0**	**4.769**	**4.344**	**4.189**	**6.007**	**3.709**

4.1.9. 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 (Appendix X). Stereomicroscopic observation of anther dehiscence opening revealed longitudinal splitting of anther to release the pollen (Plate 14) and Scanning Electron Microscopic image of anther (Plate 13)

Plate 11. Number of flowers per spike in *Piper nigrum* L.

Plate 12–Anthers in *Piper nigrum* L.

Plate 13 - Scanning Electron microscopic image of anthers (Scale bar 30µm)

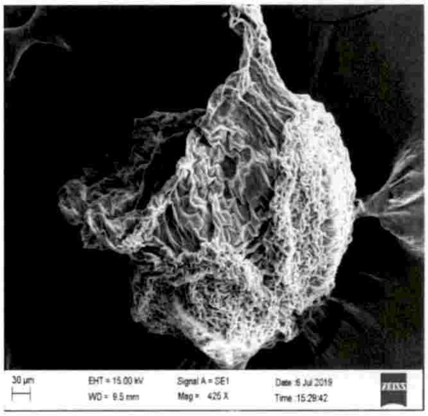


Plate 14. Stereomicroscopic view of anther dehiscence mode of *Piper nigrum* L.

4.1.10. Number of pollen grains per flower

The number of pollen grains per inflorescence was calculated by haemocytometer and it varied from 5,01,500-7,00,000 pollen grains (Table 4) and it depends on the number of flowers in a spike. The number of pollen grains per flower varied from 8,300 - 10,000 (Plate 15).

Table 4. Number of pollen grains per flower in *Piper nigrum* L.

Sl. No.	Number of flower	Number of pollen grains per flower (four anthers)	Number of pollen grains per inflorescence
1	70	9,900	6,93,000
2	61	8,300	5,06,300
3	65	8,700	5,65,500
4	76	9,100	6,91,600
5	70	10,000	7,00,000
6	68	9,700	6,59,200
7	73	9,000	6,57,000
8	59	8,500	5,01,500
9	66	9,200	6,07,000
10	63	8,600	5,41,800
Mean	**67.1**	**9,100**	**6,12,290**

4.1.11. Pollen characteristics

The pollen morphology studied using Scanning Electron Microscope revealed monosulcate (Plate 16) pollen grains with the mean polar diameter of pollen grain was 10.414 µm and the mean equatorial diameter was 6.309 µm (Plate 17) having an exine thickness of 924.8 nm (Plate 18) are shown in the table 5.

Plate 15. Number of pollen grains in 1mm^2

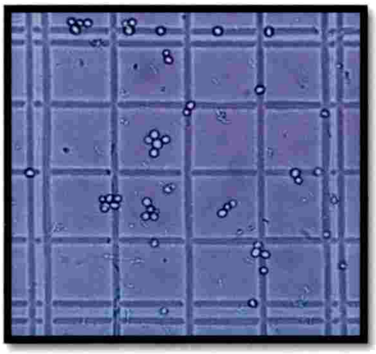
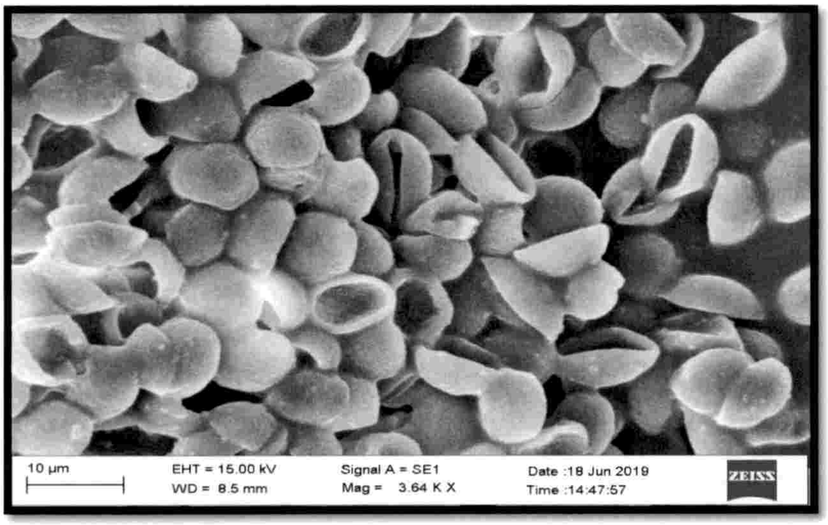


Plate 16-Scanning Electron Microscopic image of pollen grains of *Piper nigrum* L.



Scale bar 10 µm

Table 5. Polar and Equatorial axis diameter of pollen grains of *Piper nigrum* L.

Sl No.	Polar axis diameter (µm)	Equatorial axis diameter (µm)
1	10.60	9.0
2	8.622	8.3
3	12.55	6.687
4	10.4	8.496
5	9.898	6.533
Mean	**10.414**	**7.803**

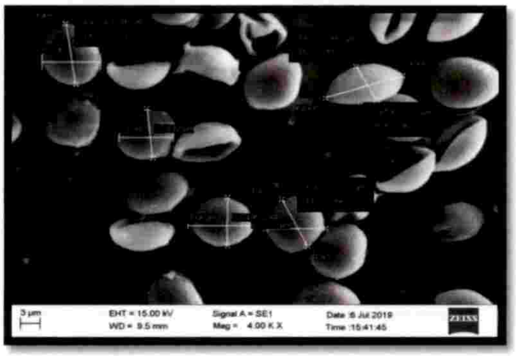
4.1.12. Stigma type

Twenty five stigma of fifty inflorescences taken from twenty five black pepper plants grown in the field revealed the presence of four lobes in each stigma. Stigma was wet type and papillate (Appendix XI). Scanning Electron Microscopic image of stigma is shown in Plate 19.

4.1.13. Stigma receptivity

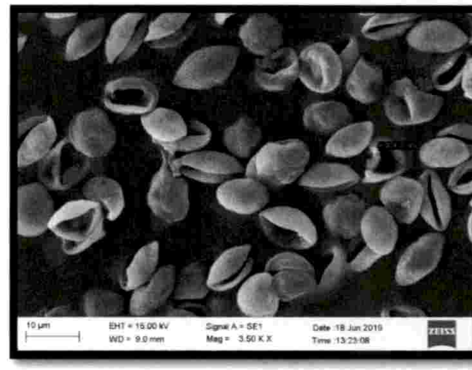
Two flowers each were taken from five inflorescences of black pepper plants grown in the field were observed for stigma receptivity for 8 days from the emergence of stigma and is presented in Table 6. The stigma receptivity by hydrogen peroxide test revealed stigma receptivity for 7 days. Maximum bubbles were counted on fifth day of emergence of stigma. Stereomicroscopic image of stigma through hydrogen peroxide is shown in Plate 21.

Plate 17. Equatorial and Polar diameter Of pollen grains of *Piper nigrum* L.



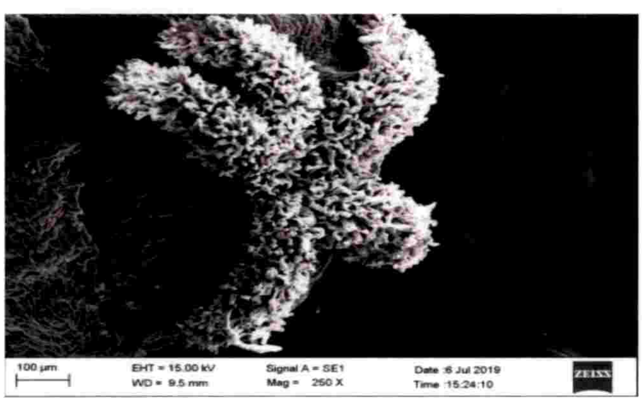
Scale bar 3µm

Plate 18. Scanning Electron Microscopic image of exine thickness of pollen grains in *Piper nigrum* L.



Scale bar -10µm

Plate 19. Scanning Electron Microscopic image of stigma of *Piper nigrum* L.



Scale bar -100µm

Table 6.Stigma receptivity through hydrogen peroxide test of *Piper nigrum* L.

No. of days	1	2	3	4	5	6	7	8	9	10	Mean number of bubbles	Percentage
First day	34	35	35	34	32	32	34	36	34	35	34	81.57
Second	37	39	37	39	40	38	39	38	37	36	38	84.44
Third day	42	45	47	46	43	45	47	44	43	48	45	93.75
Fourth day	47	48	50	47	48	46	49	50	47	48	48	94.11
Fifth day	49	52	49	51	48	53	57	48	50	53	51	100
Sixth day	28	34	29	32	29	32	30	30	34	32	31	60
Seventh day	20	25	24	24	21	25	22	21	23	25	23	74.10
Eighth day	0	0	0	0	0	0	0	0	0	0	0	0

4.1.14. Duration of pollen availability/inflorescence

Fifty inflorescences of twenty five plants were observed for 15 days from the period of full maturity of anther till all anther is dehisced and pollen is lost in each 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 (Table 7).

Table 7. Duration of pollen availability per inflorescence of *Piper nigrum* L.

No. of spike	Number of days														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total number of days
1	14	18	26	32	28	24	20	P	P	p	0	0	0	0	0	**0**
2	14	16	25	31	26	22	19	12	P	p	P	0	0	0	0	**11**
3	13	16	25	32	24	20	20	11	10	p	P	P	0	0	0	**12**
4	9	13	17	16	13	15	9	8	P	p	P	0	0	0	0	**11**
5	10	13	13	19	18	19	20	21	P	p	P	0	0	0	0	**11**
6	13	18	26	32	28	24	20	15	10	p	P	P	0	0	0	**12**
7	14	16	25	31	26	22	19	P	P	p	0	0	0	0	0	**10**
8	13	15	25	29	24	20	p	P	P	0	0	0	0	0	0	**9**
9	9	11	17	16	13	15	10	9	9	p	P	P	0	0	0	**12**
10	10	13	14	19	18	18	20	19	p	p	P	0	0	0	0	**11**
11	13	18	26	32	28	24	20	15	p	p	P	0	0	0	0	**11**
12	14	16	25	31	26	22	19	12	9	p	P	P	0	0	0	**12**
13	13	15	23	28	21	20	12	P	p	p	0	0	0	0	0	**10**
14	10	11	13	16	13	17	P	P	P	0	0	0	0	0	0	**9**
15	9	13	14	20	18	21	20	15	17	p	P	P	0	0	0	**12**
16	14	16	25	27	22	21	20	12	P	p	P	0	0	0	0	**11**
17	12	15	25	31	26	22	19	12	p	p	P	0	0	0	0	**11**
18	13	16	21	27	24	20	18	11	10	p	P	P	0	0	0	**12**
19	12	14	18	16	12	15	P	P	p	0	0	0	0	0	0	**9**
20	10	13	13	19	18	19	p	P	P	0	0	0	0	0	0	**9**
21	13	18	26	32	28	24	20	15	10	P	P	P	0	0	0	**12**
22	14	16	25	31	24	22	19	12	P	p	P	0	0	0	0	**11**
23	13	15	25	29	24	20	16	11	P	p	P	0	0	0	0	**11**
24	9	11	17	16	13	15	9	10	12	p	P	P	0	0	0	**12**
25	10	13	14	19	18	18	20	P	p	p	0	0	0	0	0	**10**
26	13	18	26	32	28	24	P	P	p	0	0	0	0	0	0	**9**
27	14	16	25	31	26	22	19	12	9	p	P	P	0	0	0	**12**
28	13	15	23	28	21	20	17	9	p	p	P	0	0	0	0	**11**
29	10	11	13	16	13	17	10	12	p	p	P	0	0	0	0	**11**
30	9	13	14	20	18	21	20	15	17	p	P	P	0	0	0	**12**

31	10	13	13	19	18	19	20	P	p	p	0	0	0	0	0	**10**
32	13	18	26	32	28	24	p	P	p	0	0	0	0	0	0	**9**
33	14	16	25	31	26	22	19	12	9	p	P	P	0	0	0	**12**
34	13	15	25	29	24	20	16	11	p	p	P	0	0	0	0	**11**
35	9	11	17	16	13	15	10	9	P	p	P	0	0	0	0	**11**
36	10	13	14	19	18	18	20	19	17	p	P	P	0	0	0	**12**
37	13	18	26	32	28	24	20	15	10	p	P	P	0	0	0	**12**
38	14	16	25	31	26	22	19	P	p	p	0	0	0	0	0	**10**
39	13	15	23	28	21	20	p	P	p	0	0	0	0	0	0	**9**
40	10	11	13	16	13	17	12	11	8	p	P	P	0	0	0	**12**
41	9	13	14	20	18	21	20	15	p	p	P	0	0	0	0	**11**
42	14	16	25	27	22	21	20	12	P	p	P	0	0	0	0	**11**
43	12	15	25	31	26	22	19	12	9	p	P	P	0	0	0	**12**
44	13	16	21	27	24	20	18	P	p	p	0	0	0	0	0	**10**
45	12	14	18	16	12	15	P	P	p	0	0	0	0	0	0	**9**
46	10	13	13	19	18	19	20	21	17	p	P	P	0	0	0	**12**
47	13	18	26	32	28	24	20	15	p	p	P	0	0	0	0	**11**
48	14	18	26	32	28	24	20	15	p	p	P	0	0	0	0	**11**
49	14	16	25	31	26	22	19	12	9	p	P	P	0	0	0	**12**
50	13	16	25	32	24	20	p	p	p	0	0	0	0	0	0	**9**
Mean																**10.84**

4.1.15. Duration of pollen availability per plant population.

Five plants were observed for a period till the pollen was available in a plant for one year from March 2018to March 2019. The maximum pollen availability was in the month of June- July. The details of individual observation is shown in appendix XII.

Table 8. Duration of pollen availability per plant population of *Piper nigrum* L.

Month	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**Mean**
March 2018	0	0	0	0	0	0

April 2018	8	9	8	8	8	8.25
May 2018	14	14	13	14	14	14
June 2018	22	20	22	22	21	22
July 2018	25	26	25	27	26	27
Aug 2018	21	20	21	21	20	20.75
Sep 2018	17	18	18	17	16	17.5
Oct 2018	16	15	16	18	16	16.25
Nov 2018	15	14	15	15	14	14.75
Dec 2018	11	12	11	12	11	11.5
Jan 2019	8	8	9	8	8	7.75
Feb 2019	7	8	7	8	8	7.5
March 2019	8	7	8	7	7	8.5

4.1.16. Duration of stigma receptivity

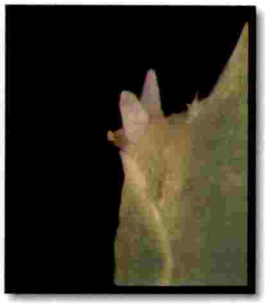
The stigma receptivity was observed for 8 days. The duration of stigma receptivity was for seven days as revealed from hydrogen peroxide test (Table 9). The different developmental stages of stigma observed through stereomicroscope can be described as follows (Plate 20).

Table 16. Different development stages of stigma receptivity of *Piper nigrum* L.

Days	Stages of stigma receptivity
First day	First emergence of stigma
Second	Elongation of stigma
Third day	Elongation complete and spreading of stigma
Fourth day	Elongation complete and spreading of stigma
Fifth day	Wide spreading of stigma
Sixth day	Wide spreading of stigma
Seventh day	Tip of stigma starts blackening
Eighth day	Complete stigma becomes black

Plate 20. Stereomicroscopic image of different stages of stigma receptivity of *Piper nigrum* L.

1 day



2 day

3 day

4 day

5 day

6 day

7 day

Plate 21. Bubbles released on treatment with hydrogen per oxide

4.1.17. 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 (Appendix 13).

4.1.18. Pollen fertility

Pollen fertility studies by Acetocarmine test revealed that 91 % of the pollen were fertile. Steromicroscopic image of pollen fertility by Acetocarmine shown in the Plate 22.

Table 10. Pollen fertility test by Acetocarmine in *Piper nigrum* L.

Anther	Total number of pollen	Number of stained pollen	Fertility percentage
Replication 1	78	72	92.3
Replication 2	69	61	88.4
Replication 3	85	79	92.9
Replication 4	59	52	88.1
Replication 5	62	57	91.5
Replication 6	70	65	92.8
Mean			**91**

4.1.19. Pollen viability

The pollen viability percentage by TTC stain and IKI stain was 91.03 % and 92.4% respectively. Stereomicroscopic image of pollen fertility by TTC and IKI shown in the Plate 23 and Plate 24.

Table 11. Pollen viability test by TTC in *Piper nigrum* L.

Anther	Total number of pollen	Number of stained pollen	Fertility percentage
Replication 1	82	75	91.4
Replication 2	67	62	92.5

Plate 22.Pollen fertility test by Acetocarmine in *Piper nigrum*L.

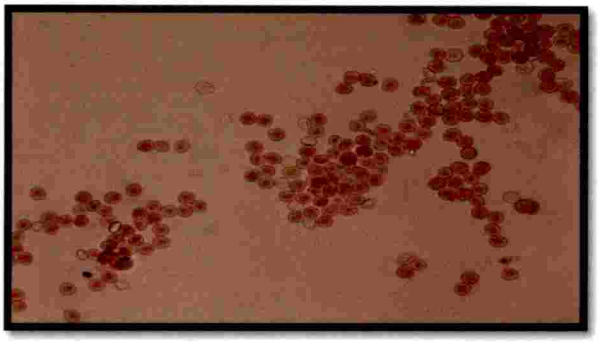


Plate 23.Pollen viability test by TTC in *Piper nigrum*L.

Plate 24.Pollen viability test by IKI in*Piper nigrum*L.

Replication 3	65	60	92.3
Replication 4	72	65	90.2
Replication 5	51	46	90.1
Replication 6	78	70	89.7
Mean			**91.03**

Table 12. Pollen viability test by IKI in *Piper nigrum* L.

Anther	**Total no of pollen**	**No of stained pollen**	**Fertility percentage**
Replication 1	76	71	93.4
Replication 2	74	69	93.2
Replication 3	56	51	91.0
Replication 4	67	62	92.5
Replication 5	61	56	91.8
Replication 6	52	48	92.3
Mean			**92.4**

4.1.19. Pollen viability

a. *In vitro* germination

In vitro germination in Brewbakers- Kwack medium at 5, 10, 20 and 30 % sucrose showed highest pollen germination at 5% sucrose. Steromicroscopic image of Pollen gerimination is shown in the Plate 25.

Table 13. Pollen viability through *in vitro* germination (Brewbakers-Kwack medium)

Sl No	**Sucrose solution /Brewbakers–Kwackmedium**	**Total number of pollen grains**	**No of pollen grains germinated**	**Pollen viability (percentage)**

Plate 25. Pollen germination at different concentration of sucrose (Brewbakers-Kwack medium)

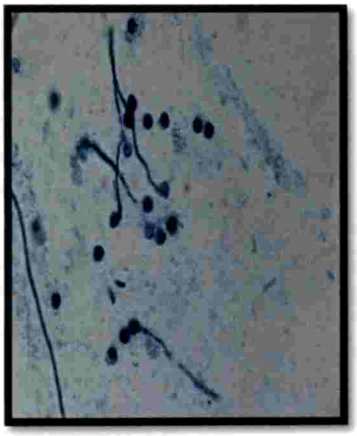


Plate 25a. 5% sucrose

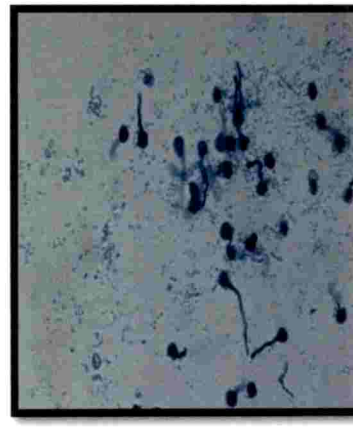


Plate 25b. 10% sucrose

Plate 25c. 20% sucrose

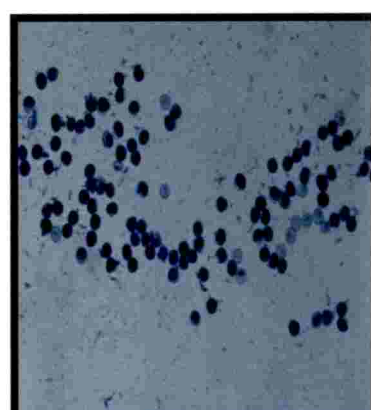


Plate 25d. 30% sucrose

Plate 26. Pollen viability test through *In vivo* germination

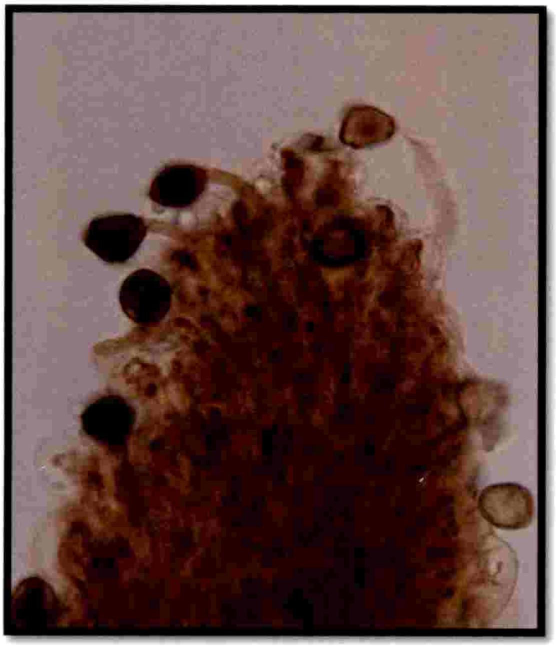


Plate 26a. Stereomicroscopic image *In vivo* germination of pollen by DAB method.

Plate 26b. Pollen viability through *In vivo* by aniline blue fluorescence method

	(percentage)			
1	5	64	58	90.62
2	10	58	49	84.48
3	20	76	63	82.89
4	30	51	42	82.35
Mean				**85.08**

b. *In vivo* germination

Ten flowers from five inflorescences of black pepper plants grown in field selected and pollinated artificially were observed for *in vivo* germination of pollen. *In vivo* germination done through DAB test and aniline blue fluorescence method for 6 hrs and 24 hours were viewed under fluorescent microscope and the images were taken and shown in Plate 26a and Plate 26b.

14.1.20. 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 Plate 27 and Plate 28.

14.1.21. Flower emergence duration

The time period taken from bud initiation of individual flower to full emergence of flower is 19-20 days (Appendix XIV).

14.1.22. Flowering frequency

The Flowering frequency in black pepper was maximum in the month of July and minimum in January and February.

Table 14. Flowering frequency of *Piper nigrum* L.

Plate 27. Anatomical observation of floral morphology (longitudinal section)

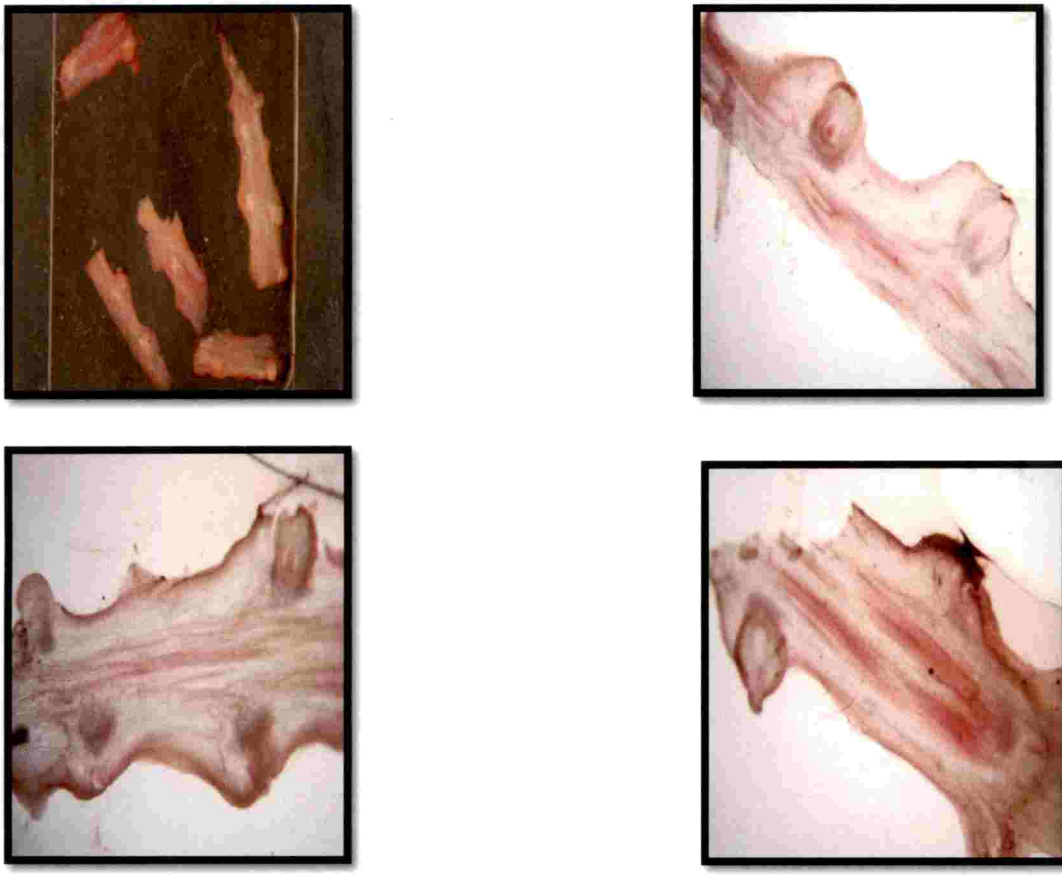


Plate 28. Anatomical observation of floral morphology (cross section)

Months	**No. of plants observed**	**No. of plants which were in flowering**	**Percentage (%)**
April	25	13	**52**
May	25	18	**72**
June	25	24	**96**
July	25	25	**100**
Aug	25	20	**80**
Sep	25	16	**64**
Oct	25	10	**40**
Nov	25	7	**28**
Dec	25	2	**8**
Jan	25	1	**4**
Feb	25	1	**4**
March	25	9	**36**

4.1.23. Flowering intensity

The average number of flowers opened per inflorescence each day represented as flowering intensity was maximum on the fifth day of anthesis.

Table 15. Flowering intensity of *Piper nigrum* L.

No of spikes	**Number of days**														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	1	6	0	9	12	0	10	5	0	12	15	0	7	0	0
2	0	0	7	0	11	13	0	9	0	6	10	8	0	9	0
3	0	6	9	0	9	12	8	0	9	0	12	0	5	10	0
4	4	0	0	11	14	0	13	0	10	0	9	0	5	0	0
5	0	0	0	0	9	0	6	8	10	12	15	17	8	0	0

6	5	0	9	12	0	16	0	15	0	6	14	5	0	5	0
7	0	9	12	0	16	10	0	7	0	0	8	9	0	9	0
8	0	5	10	0	15	0	14	0	0	10	0	9	0	8	0
9	4	6	0	0	14	10	5	0	3	0	0	7	6	0	0
10	5	0	8	12	0	16	9	0	0	6	7	0	0	5	0
11	0	7	9	0	14	15	0	6	0	3	5	5	0	0	0
12	7	0	12	0	13	0	5	0	6	7	0	3	0	2	0
13	0	8	14	0	10	0	7	9	0	5	0	5	0	0	0
14	7	9	0	16	0	10	0	7	9	0	0	0	7	5	0
15	5	0	9	13	0	5	10	0	3	0	3	5	0	0	0
16	0	7	6	0	6	10	0	0	4	5	0	0	2	0	0
17	0	9	12	0	6	15	0	2	0	9	0	7	0	0	0
18	7	10	0	8	15	0	7	0	7	0	7	5	0	0	0
19	0	6	9	0	0	16	10	6	0	0	7	9	0	0	0
20	0	0	8	14	0	9	0	0	11	7	0	14	8	0	0
21	0	0	0	0	6	8	0	13	16	0	9	11	0	8	0
22	7	9	0	15	0	0	9	12	0	0	8	10	0	0	0
23	0	0	8	0	12	0	0	14	0	0	6	4	8	3	0
24	9	0	0	14	8	6	0	0	14	0	7	0	8	0	0
25	0	0	7	5	0	0	7	5	0	4	8	0	4	0	0
Mean	**2.44**	**3.88**	**5.96**	**5.16**	**7.6**	**6.84**	**4.8**	**4.72**	**4.08**	**3.68**	**6**	**5.32**	**2.72**	**2.56**	**0**

4.1.24. Anthesis period in an inflorescence

The anthesis period in inflorescence varied from 9days (6 cm) in small inflorescence and 12 days (9 cm) in long inflorescence.

Table 16. Anthesis period in an inflorescence of *Piper nigrum* L.

No of spikes	**No of days**														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Total days**
1	1	6	0	9	12	0	10	5	0	12	15	0	7	0	0	**10**
2	0	0	7	0	11	13	0	9	0	6	10	8	0	9	0	**11**

3	0	6	9	0	9	12	8	0	9	0	12	0	5	10	0	**12**
4	4	0	0	11	14	0	13	0	10	0	9	0	5	0	0	**11**
5	0	0	0	0	9	0	6	8	10	12	15	17	8	0	0	**11**
6	5	0	9	12	0	16	0	15	0	6	14	5	0	5	0	**12**
7	0	9	12	0	16	10	0	7	0	0	8	9	0	9	0	**10**
8	0	5	10	0	15	0	14	0	0	10	0	9	0	8	0	**9**
9	4	6	0	0	14	10	5	0	3	0	0	7	6	0	0	**12**
10	5	0	8	12	0	16	9	0	0	6	7	0	0	5	0	**11**
11	0	7	9	0	14	15	0	6	0	3	5	5	0	0	0	**11**
12	7	0	12	0	13	0	5	0	6	7	0	3	0	2	0	**12**
13	0	8	14	0	10	0	7	9	0	5	0	5	0	0	0	**10**
14	7	9	0	16	0	10	0	7	9	0	0	0	7	5	0	**9**
15	5	0	9	13	0	5	10	0	3	0	3	5	0	0	0	**12**
16	0	7	6	0	6	10	0	0	4	5	0	0	2	0	0	**11**
17	0	9	12	0	6	15	0	2	0	9	0	7	0	0	0	**11**
18	7	10	0	8	15	0	7	0	7	0	7	5	0	0	0	**12**
19	0	6	9	0	0	16	10	6	0	0	7	9	0	0	0	**9**
20	0	0	8	14	0	9	0	0	11	7	0	14	8	0	0	**9**
21	0	0	0	0	6	8	0	13	16	0	9	11	0	8	0	**12**
22	7	9	0	15	0	0	9	12	0	0	8	10	0	0	0	**11**
23	0	0	8	0	12	0	0	14	0	0	6	4	8	3	0	**11**
24	9	0	0	14	8	6	0	0	14	0	7	0	8	0	0	**12**
25	0	0	7	5	0	0	7	5	0	4	8	0	4	0	0	**10**
Mean																**10.84**

4.1.25. 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.

Table 17. Anthesis period in the plant of *Piper nigrum* L.

Month	**1**	**2**	**3**	**4**	**5**	**Mean**
March, 2018	0	0	0	0	0	0
April, 2018	9	9	8	8	9	8.6

May, 2018	14	15	14	15	15	14.6
June, 2018	23	24	23	24	24	23.6
July,2018	28	29	28	29	28	28.4
Aug, 2018	24	25	24	25	25	24.6
Sep, 2018	19	20	20	19	20	19.6
Oct, 2018	18	18	17	19	18	18
Nov, 2018	16	15	15	16	15	15.4
Dec, 2018	12	12	11	12	11	11.6
Jan, 2019	9	7	8	7	8	7.8
Feb, 2019	7	8	7	8	8	7.6
March, 2019	8	9	8	9	8	8.4

4.1.26. Duration of spiking in the plant

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

4.1.27. 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.

Table 18. Duration of spiking in the plant population of *Piper nigrum* L.

Month	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Mean
March, 2018	0	0	0	0	0	0
April, 2018	26	25	26	26	26	25.8
May, 2018	28	27	28	28	27	27.6
June, 2018	29	30	30	29	30	29.6
July,2018	30	29	30	29	29	29.4

Aug, 2018	26	25	26	25	26	25.6
Sep, 2018	24	23	25	24	23	23.8
Oct, 2018	23	21	23	22	21	22
Nov, 2018	19	18	19	19	18	18.6
Dec, 2018	16	17	17	16	17	16.6
Jan, 2019	15	15	14	15	15	14.8
Feb, 2019	12	12	11	12	11	11.6
March, 2019	13	13	14	14	14	13.6

4.1.28. 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.

Table 19. Fruit maturation period from fertilization of *Piper nigrum* L.

Number of spikes	Number of days
1	155
2	165
3	159
4	171
5	165
6	165
7	175
8	159
9	165
10	172

11	170
12	150
13	171
14	175
15	168
16	175
17	155
18	167
19	174
20	170
21	155
22	170
23	165
24	170
25	165
Mean	**166.44**

4.2. Experiment on agents of pollination

4.2.1. Wind pollination

The data on wind pollination experiment on twenty-five inflorescences of field grown black pepper plants and twenty five inflorescences of pot grown bush pepper plants is presented in Appendix XVIII. The mean percentage fruit set of different treatments is presented in Table 20. The different treatments were significantly superior. Treatment, PW2 (Plate 29d) were on par with FW2 (Plate 29b) and were significantly superior compared to other treatments. The percentage of fruit set of treatment FW1 (Plate 29a) and PW1 (Plate 29c) was77.67 and 59.39 respectively. No fruit set were observed in control treatments, FC2 and PC2. Presence of pollen grains grains between the plants at different distances (wind pollination. The pollen on petriplate kept at 4.5 m from the base of *Piper nigrum* L.

Plate 29.Effect of wind pollination on fruit set of *Piper nigrum* L.

Plate 29a- Fruit set of FW1

Plate 29b - Fruit set of FW2

Plate 29c. Fruit set of treatment PW1

Plate 29d. Fruit set of treatment PW2

Plate 30. Presence of pollen grains between the plants in different distance (wind pollination)

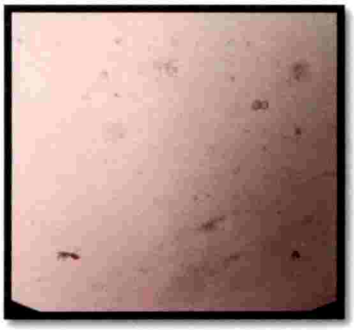


Plate 30a. Pollen on petriplate kept at 4.5 m from the base of *Piper nigrum* L.

30b. Pollen on petriplate kept at 1.5 m from the base of *Piper nigrum* L.

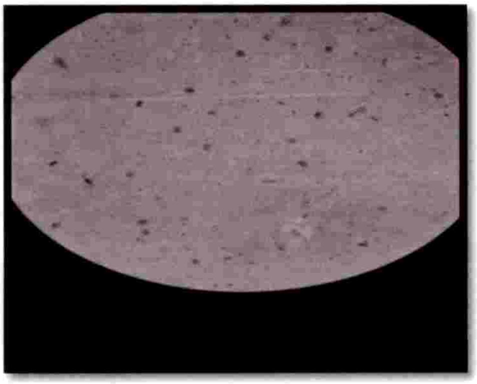


Plate30c. Pollen on petriplate kept at 2 m from the base of *Piper nigrum* L.

(Plate 30a). The pollen on petriplate kept at 1.5 m from the base of *Piper nigrum* L. (Plate 30b). The pollen on petriplate kept at 2.0 m from the base of *Piper nigrum* L. (Plate 30c).

Table 20. Effect of wind pollination on fruit set of *Piper nigrum* L.

Sl No.	Treatments	Treatment mean
1	FW1(Single inflorescence emasculated and covered with nylon cover)	77.67[b]
2	FW2 (Three-five Inflorescences with lower spike emasculated and covered with nylon cover)	92.00[a]
3	FC2 (Single inflorescence emasculated and covered with polythene cover)	0.000[d]
4	PW1(Single inflorescence emasculated and covered with polythene cover and manual fanning)	59.39[c]
5	PW2 (Three-five Inflorescences with lower spike emasculated and covered by polythene cover)	96.00[a]
6	PC2 (Single inflorescence emasculated and covered by polythene cover)	0.000[d]
	F test	**S**
	CV	**11.93**
	CD (0.05)	**5.667**

4.2.2. Insect pollination

Twenty-five inflorescences in the field and twenty-five in the pot grown were observed from the anthesis period revealed the visitation of insects. Hence the insects making the visit were collected and identified and visitation rates of insects were determined. Three different floral visitors visiting the black pepper spike noticed were fire ant (Plate 32b), black garden ant (Plate 32a) and pollu beetle visiting day and night is presented in Table 21. The behaviour of ant species was monitored by field observations and the presence of pollen on their body parts like mouth, abdomen and legs were checked by dissecting under stereoscopic microscope. No pollen was obtained from the mouth, abdomen and legs. Hence the role of insects in pollination could not be found out. However, collection of nectar was noticed by fire ant and black garden while visiting the flowers during days and night (Plate 33b and Plate 33a).

Table 21. Pollinators and their foraging behaviors in *Piper nigrum* L.

Sl No.	Common name	Scientific name	Family	Visiting time	Foraging nature
1	Black ant	*(Componotus compressus)*	Formicidae	Day and night	Nectar
2	Yellow crazy ant	(*Anoplolepis gracilipes*)	Formicidae	Day and night	Nectar
3	Pollu beetle	*Lanka rama krishnai*	Chrysomelidae	Day and night	

Table 22. Visitation rates of pollinators and insect in *Piper nigrum* L.

Time	*Oecophylla smaragdina* (visits per inflorescence)	(visits per inflorescence)	Pollu beetle (*Lanka rama krishnai*)
6-8am	6	5	2
8-10am	5	5	4
10am-12pm	3	4	2
12-2pm	5	3	2
2-4pm	3	3	1

Plate 31. Stereomicroscopic image of anthers in *Piper nigrum* L.

Plate 32. Presence of black garden ant and fire ant on the inflorescence

Plate 32a- Black ant

Componotus compressus

32b- Yellow crazy ant

Anoplolepis gracilipes

4-6pm	6	3	0
6-8pm	4	4	0
8-10 pm	3	2	0
10 pm – 12 am	4	2	0
12 am	2	1	0
4 am	2	2	0
Visitation rate	**1.79**	**1.41**	**0.45**

The visitation rates of three different floral visitors visiting the black pepper spike was Black ant (1.79), Yellow crazy ant (1.41) and Pollu beetle (0.45).

4.2.3. Rain water

The data on rain water experiment on twenty-five inflorescences of field grown black pepper plants and twenty five inflorescences of pot grown bush pepper plants is presented in Appendix XIX. The mean percentage of fruit set differed significantly among treatments and is presented Table 22. Treatment, FR1 (Plate 34a) were on par with PR1 (Plate 34b) and were significantly superior compared to other treatments. No fruit set were observed in control treatments, FC2 and PC2. The presence of pollen grains in rain water (Plate 34c).

Table 23. Effect of rain water on fruit set of *Piper nigrum* L.

Sl No.	Treatments	Treatment mean
1	FR1(Single inflorescence covered by polythene cover and at time of natural raining the inflorescence will be exposed and after the rain it was covered with polythene)	92.76^{a}
2	FC2 (Single inflorescence emasculated and covered by polythene cover)	0.00^{b}
3	PR1 (The whole plant is covered by polythene cover and rain water was imitated as splashing	92.90^{a}

Plate 33. Sucking of nectar from the flowers by black garden ant and fire ant

Plate 33a- Black ant

Componotus compressus

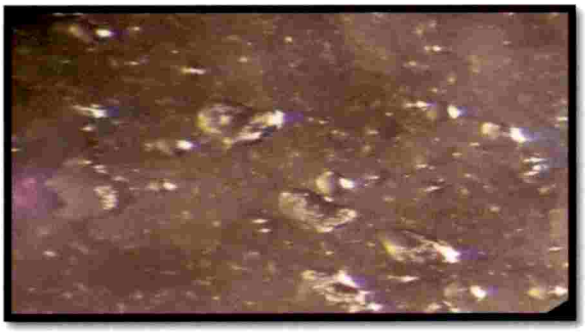
Plate 33b- Yellow crazy ant

Anoplolepis gracilipes

Plate 34. Effect of rain water on fruit set of *Piper nigrum* L.

Plate 34a. Fruit set of treatment FR1

Plate 34b. Fruit set of treatment PR1



34c.Presence of pollen grains in the rain water collected

	on ten bush pepper plants at time of anthesis and then once again covered by polythene cover)	
4	PC2 (Single inflorescence emasculated and covered by polythene cover)	0.00 [b]
	F test	**S**
	CV	**3.097**
	CD (0.05)	**0.807**

4.2.4. Dew

Presence of dew were observed from June to December and the dew collected from the inflorescence showed the presence of pollen grains (Plate 35a and 35b) which suggests the role of dew also in the pollination of pepper.

4.3. Experiments on breeding system of *Piper nigrum* L.

The data on breeding system experiment on fifty pot grown bush pepper plants is presented in Appendix XX. The mean percentage fruit set differed significantly among treatments and is presented Table 23. Treatment, B5 (Plate 36e) were on par with B6 (Plate 36f), B4 (Plate 36), B3 (Plate 36c), B2 (Plate 36b) and B1 (Plate 36a)and were significantly superior compared to other treatments. The percentage of fruit set of treatment B7 (83.97) Plate 36g, B8 (82.85) Plate 36h, and B10 (89.15) Plate 36i. No fruit set were observed in treatment, B9.

Table 24. Effect of breeding system on fruit set of *Piper nigrum* L.

Sl No	Treatment	Treatment mean
1	**B1**(Single inflorescence was selected, marked and kept for open pollination)	92.84 [a]
2	**B2** (Three flowers from top, middle and base were selected and covered with cloth bag, Autogamy)	93.32 [a]
3	**B3**(Single flower was selected and covered with cloth bag,	92.00 [a]

	Autogamy)	
4	**B4**(Four- five mature inflorescences were covered by cloth bag and emasculation of lower spike, Geitonogamy)	93.32^{a}
5	**B5**(Single inflorescence will be emasculated and hand self pollinated with pollen collected from the same plant and covered by cloth bag, Geitonogamy)	95.20^{a}
6	**B6** (Four – five mature inflorescences were covered by a cloth bag and emasculation of lower spike during anthesis period and sprayed with water imitating rain to carry pollen, Geitonogamy)	92.65^{a}
7	**B7**(Single inflorescence were emasculated and the buds upon opening were hand cross-pollinated with pollen collected from other plant using brush and covered with polythene cover, Xenogamy)	83.97^{b}
8	**B8**(Three-four inflorescences were emasculated and another pot containing inflorescence were kept near and both the inflorescences were covered together with polythene cover, Xenogamy)	82.85^{b}
9	**B9**(Single inflorescence were emasculated and covered with polythene cover, Apomixis)	0.00^{c}
10	**B10**(Single inflorescence were covered with polythene cover without emasculation, Control)	89.15^{b}
	F Test	**S**
	C V	14.61
	CD (0.05)	6.604

Plate 35. Presence of dew

Plate 35a. Presence of dew on the spike

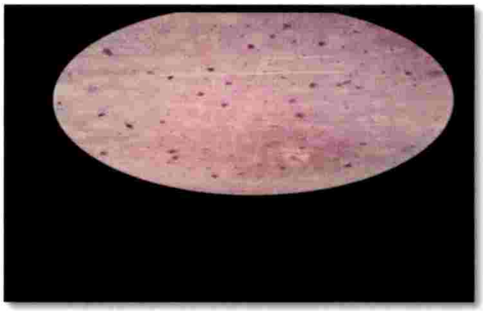


Plate 35b . Presence of pollens in dew collected

plate 36. Experiment on breeding system

Plate 36a. Fruit set of treatment B1)

Plate 36b. Fruit set of treatmenyB2

Plate 36c- Fruit set of treatment B3

Plate 36d- Fruit set of treatment B4

Plate 36e – Fruit set of treatment B5

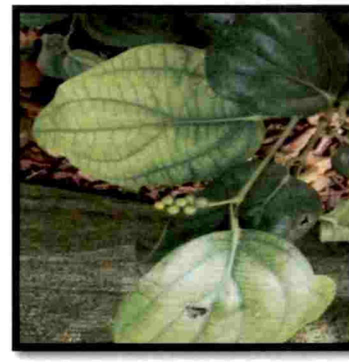


Plate 36f– Fruit set of treatment B6

Plate 36g. Fruit set of treatment B7

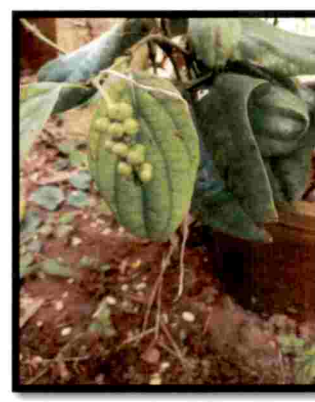


Plate 36h. Fruit set of treatment B8

Plate 36i. Fruit set of treatment B10

Discussion

5. DISCUSSION

The study entitled "Pollination biology of black pepper (*Piper nigrum* L)." was undertaken in the Department of Plantation Crops and Spices from March 2018 to March 2019. Floral morphology, floral phenology, agents of pollination and breeding system carried out are discussed here based on the results obtained.

5.1. FLORAL MORPHOLOGY AND FLORAL PHENOLOGY OF BLACK PEPPER

The flower colour of black pepper observed were Light green, 149 C (52%) and were Dark green, 140 A (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 bract colour varied from Yellow (3B) to Light green (140A) as per the Royal Horticultural Society colour charts. The presence of fresh floral fruity odour of black pepper flowers were 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. arboretum, 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). Presence of nectar was noticed in the black pepper flower between the bract and stigma and the amount of nectar varied from 23 to 25 μl as observed from thirty-five stigma of twenty inflorescences. The mean of nectar content from thirty-five stigma of fifty inflorescences were 24.08μl (Table 3). The mean nectar content of one flower was 0.69 μl. Nectar was not discernible in the flowers, as observed for other *Piper* species according to Fleming (1985).

The maximum flower opening in black pepper was observed at 6-7 pm followed by 4-5pm and 7-8 pm (Figure 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 6-6.30 pm. The length and breadth of thirty flower was taken on third day, fourth day and fifth day to understand the size of black pepper flower. On third day and fourth day the mean length of flower was 1.51 mm and mean breadth of flower was 1.32 mm. The flower size was maximum (1.53 x 1.34 mm) on the fifth day of stigma receptivity. The number of flowers per spike varied from 49-98 flowers and it depended on the length of the spike (Figure 2). The number of anthers in each flower was four and was uniform in all fifty flowers and Stereomicroscopic and Scanning Electron Microscopic image are shown plate 6. Ravindran (2006) reported that stamens are two in number and anthers are dithecous.

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 is presented in appendix 3. 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 (Figure 3). According to Nybe *et al.* (2007) anther dehiscence took place between 14.30-15.30 h (2.30-3.30 pm). 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 (Table 9). Stereomicroscopic observation of anther dehiscence opening revealed longitudinal splitting of anther to release the pollen (Plate 7).

The number of pollen grains per inflorescence was calculated by haemocytometer (plate 8) and it varied from 5,01,500 - 7,00,000 pollen grains

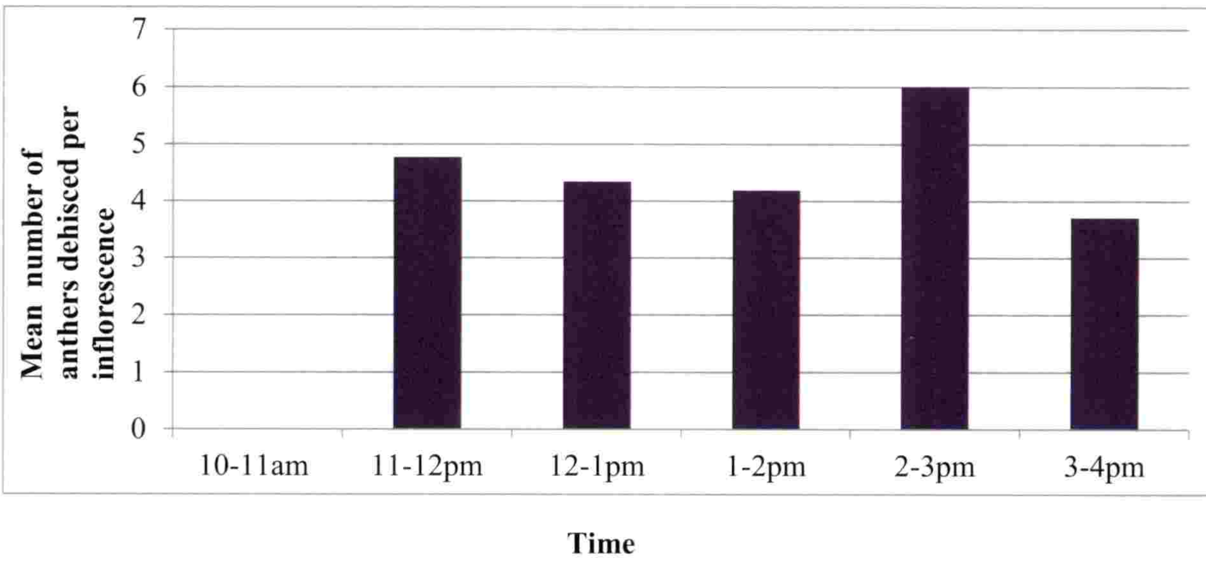


Figure 3. Anther dehiscence time in *Piper nigrum* L.

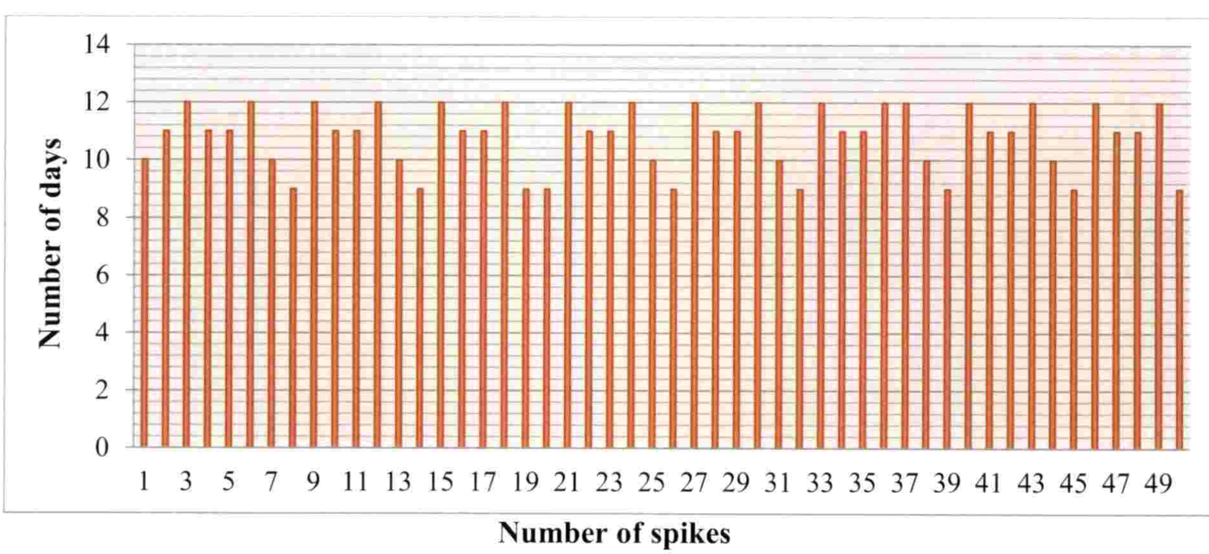


Figure 4. Duration of pollen availability per inflorescence of *Piper nigrum* L.

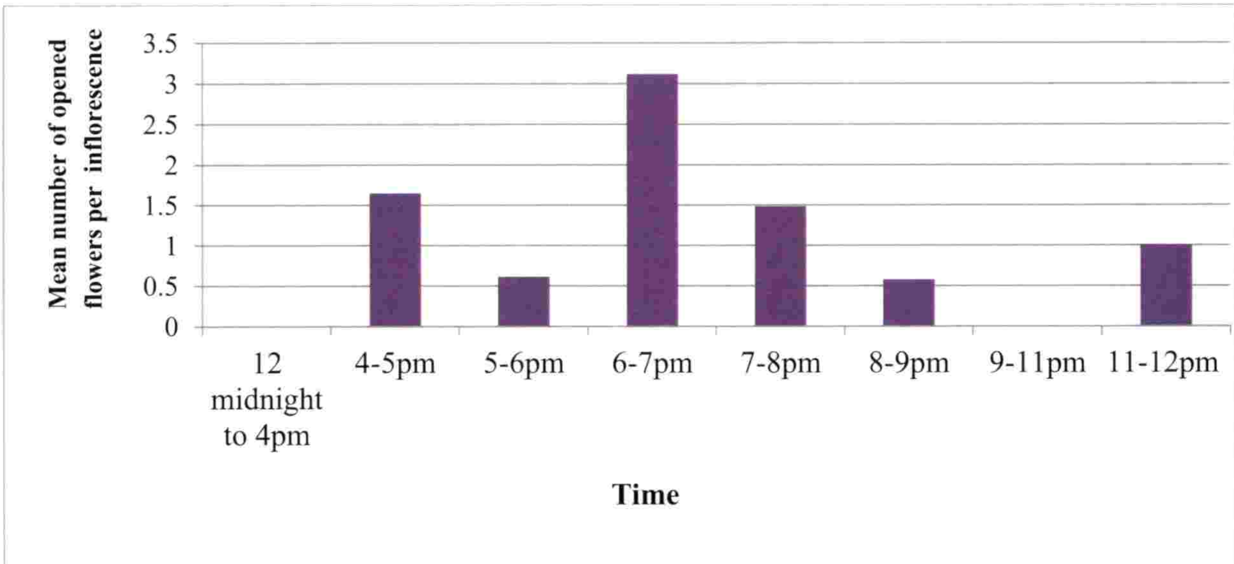


Figure 1. Flower opening time of *Piper nigrum* L.

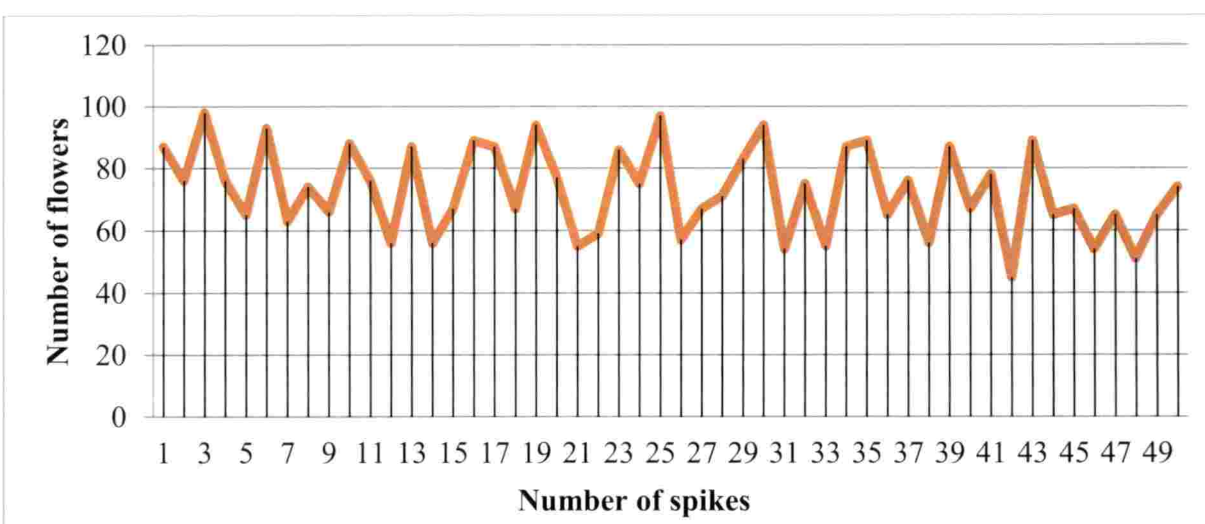


Figure 2. **Number of flowers per spike in *Piper nigrum* L.**

(Table 10) and it depended on the number of flowers in a spike. The number of pollen grains per flower varied from 8,300 - 10,000 (plate 9). According to Nybe *et al.* (2007) the pollen production varied from 7000-13000 pollen grains per flower.

The pollen morphology studied using Scanning Electron Microscope revealed monosulcate pollen grains with the mean polar diameter of 10.414 μm and the mean equatorial diameter of 6.309 μm having an exine thickness of 924.8 nm as shown in the table 11. Martin and Gregory (1962) reported the mean diameter of pollen grain of black pepper as approximately 10 μm. The pollen grains measured along the equatorial axis varied from 9.5–13.0 μm, the mean being 11.0 μm and along the polar axis was 7.0–10.5 μm having a mean of 8.84 μm (Rahiman,1981). According to Divya *et al.* (2015) the pollen grains of black pepper was monosulcate. According to *Nybe et al.* (2007), the mean diameter of pollen grain was 9.86 μm.

Twenty-five stigma of fifty inflorescences taken from twenty-five black pepper plants grown in the field revealed the presence of four lobes in each stigma. Stigma was wet type and papillate (Table 12). Scanning Electron Microscopic image of stigma is shown in plate 11. Ravindran (2006) observed the stigma of black pepper as 3-5 lobed and papillate.

Stigma receptivity observed for 8 days from the emergence of stigma tested using hydrogen peroxide (Table 13) revealed stigma receptivity for 7 days. Maximum bubbles were counted on fifth day of emergence of stigma. This can be substantiated by the stereomicroscopic image of stigma through hydrogen peroxide (Plate 12). 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. 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). 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.

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 (Figure 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.

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 (Figure 5).

Pollen being present and remained fresh in a flower after 14 - 15 days of stigma emergence. This was taken as the longevity of the flower (Figure 6). Pollen fertility studies by Acetocarmine test revealed that 91 % of the pollen were fertile. The pollen viability percentage by TTC stain and IKI stain was 91.03 % and 92.4% respectively. *In vitro* germination in Brewbakers-Kwack medium at 5, 10, 20 and 30 % sucrose showed highest pollen germination at 5% sucrose. Ten flowers from five inflorescences of black pepper plants grown in field selected and pollinated artificially were observed for *in vivo* germination of pollen. *In vivo* germination done through DAB test and aniline blue fluorescence method for 6 h and 24 h were viewed under fluorescent microscope and the images were shown in Plate 17 and Plate18. The cross and longitudinal section of the immature spikes

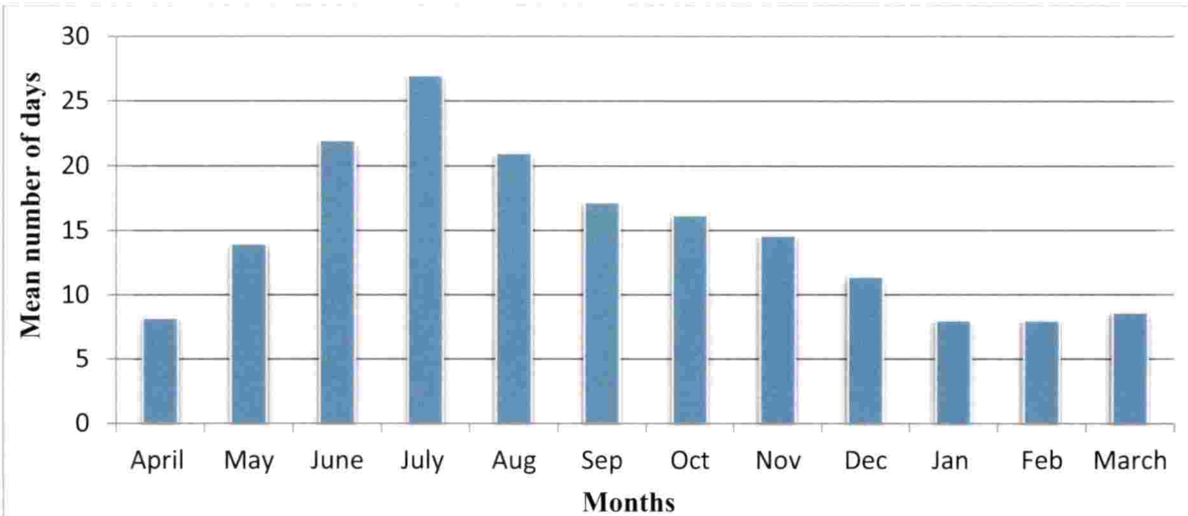


Figure 5. Duration of pollen availability per plant of *Piper nigrum* L.

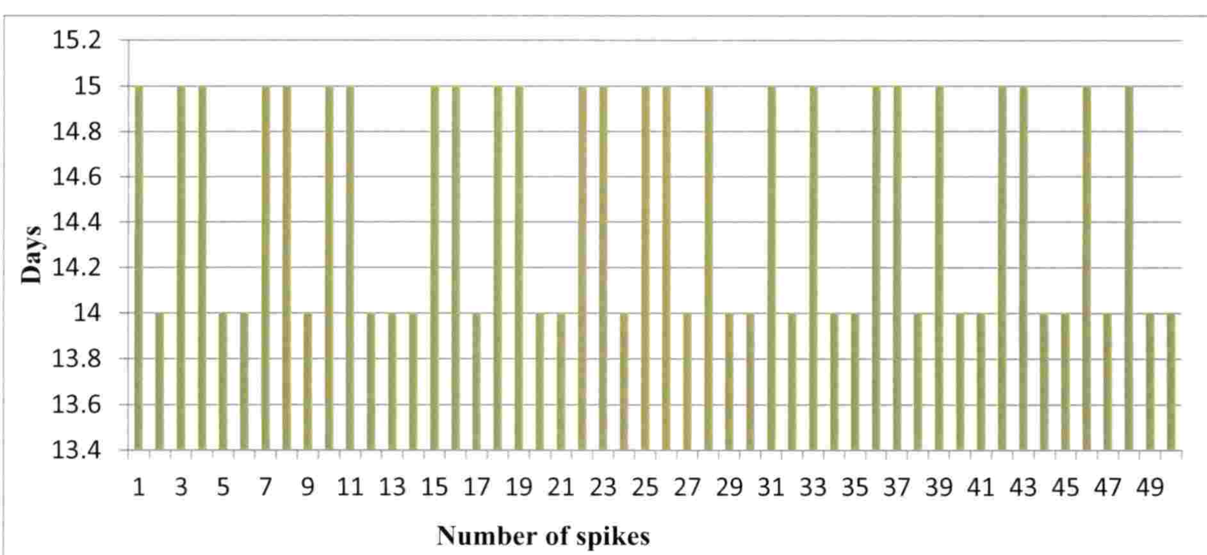


Figure 6. Flower longevity of *Piper nigrum* L.

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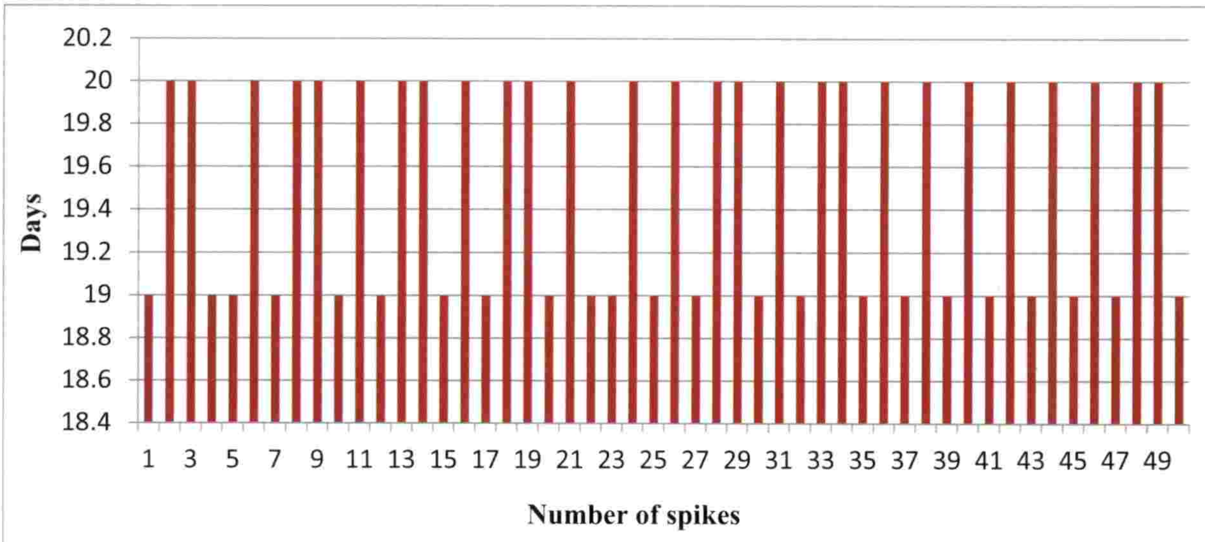
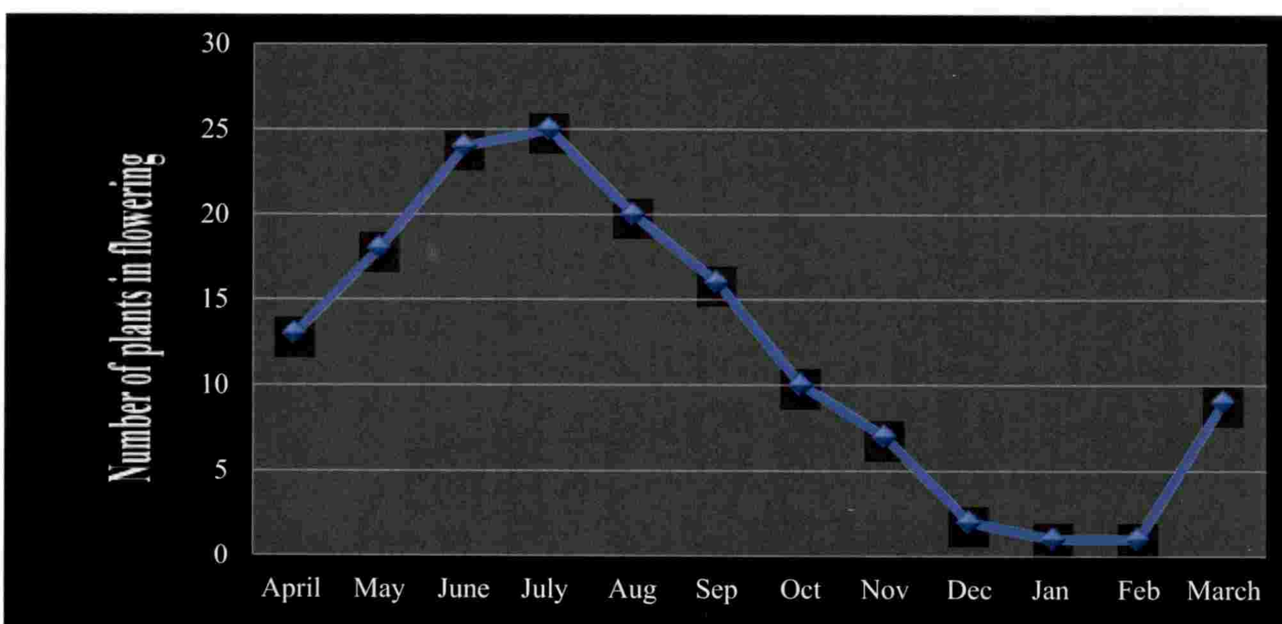


Figure 7. Flower emergence duration in *Piper nigrum* L.



Months

Figure 8. Flowering frequency in *Piper nigrum* L.

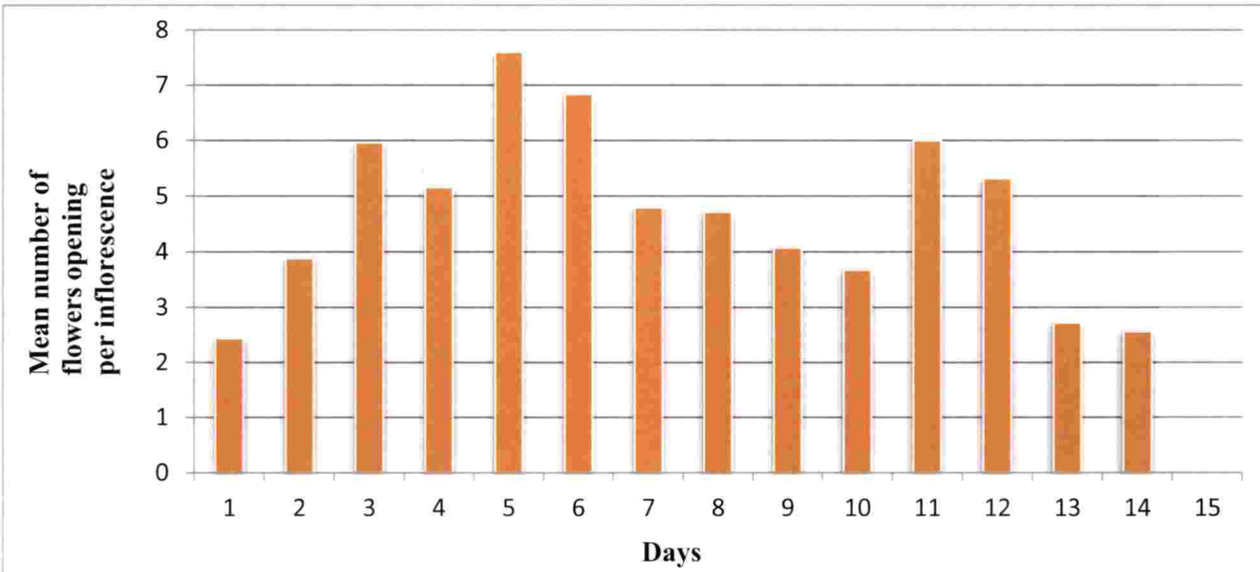
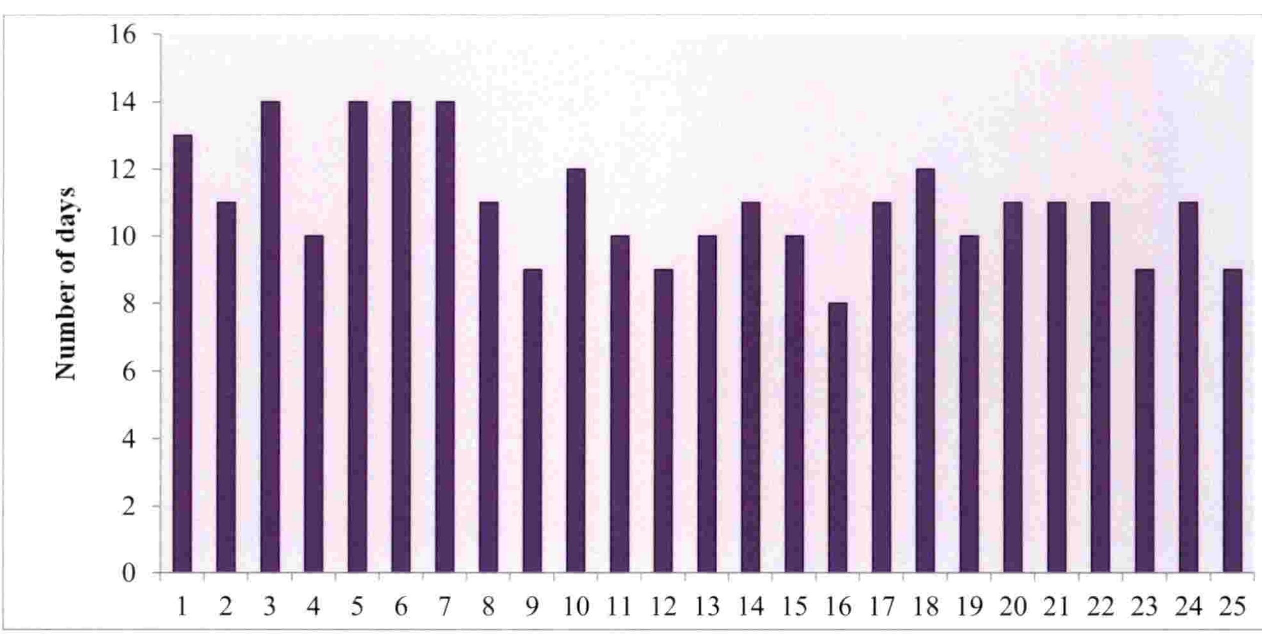
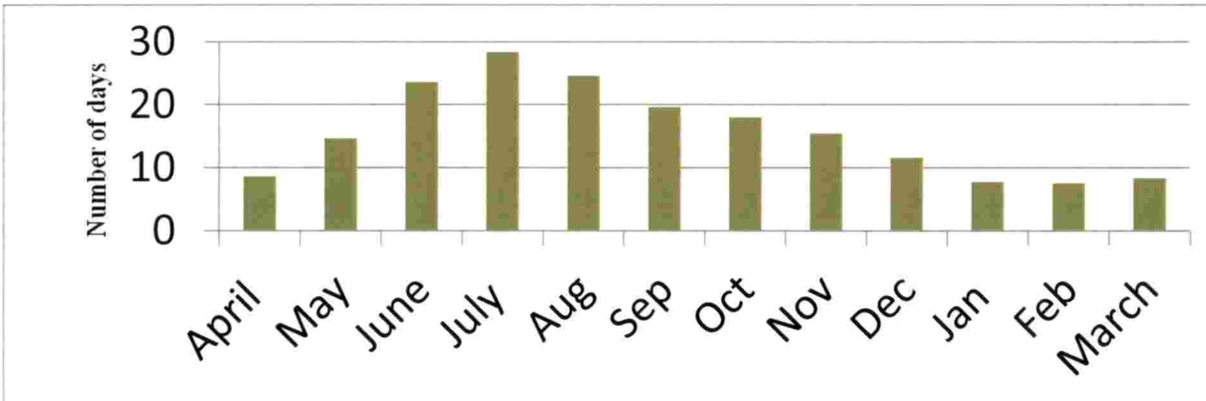


Figure 9. Flowering intensity in *Piper nigrum* L.



Number of Spikes

Figure 10. Anthesis period in an inflorescence of *Piper nigrum* L.



Number of Months

Figure 11. Anthesis period in a plant of *Piper nigrum* L.

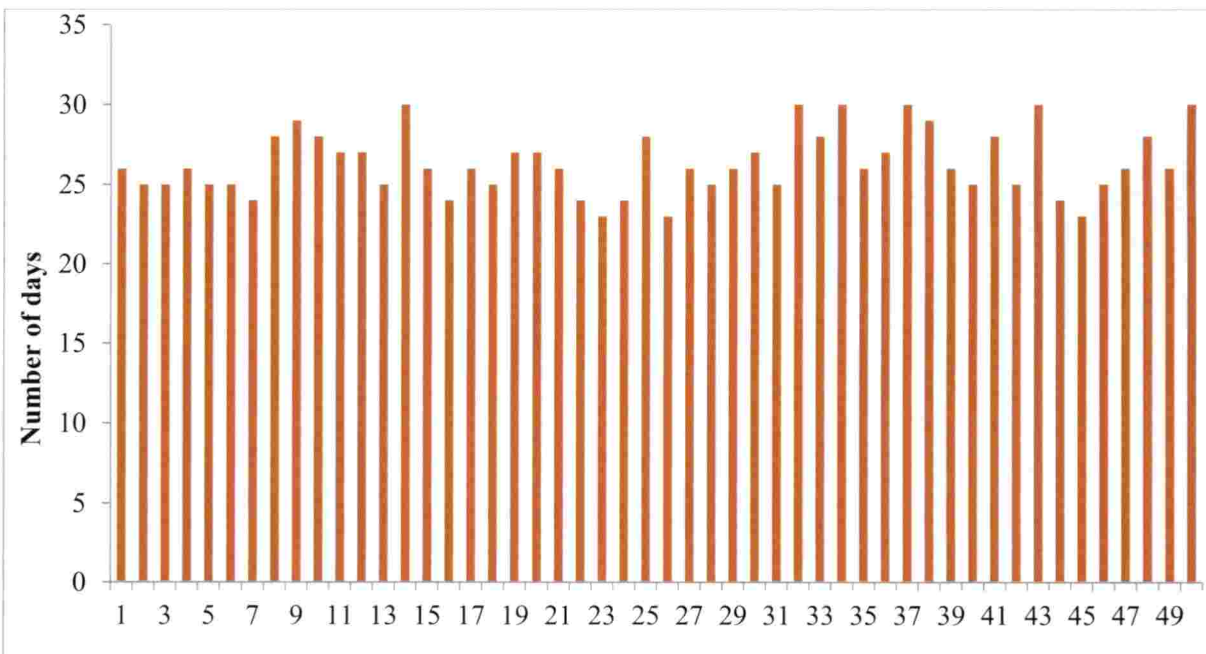
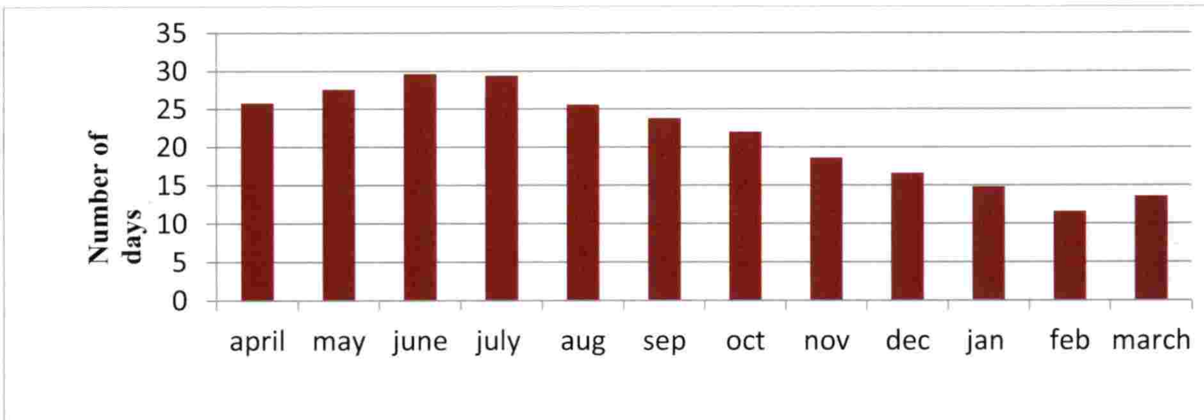


Figure 12. Duration of spiking in the plant of *Piper nigrum* L.



Month

Figure 13. Duration of spiking in a plant population of *Piper nigrum* L.

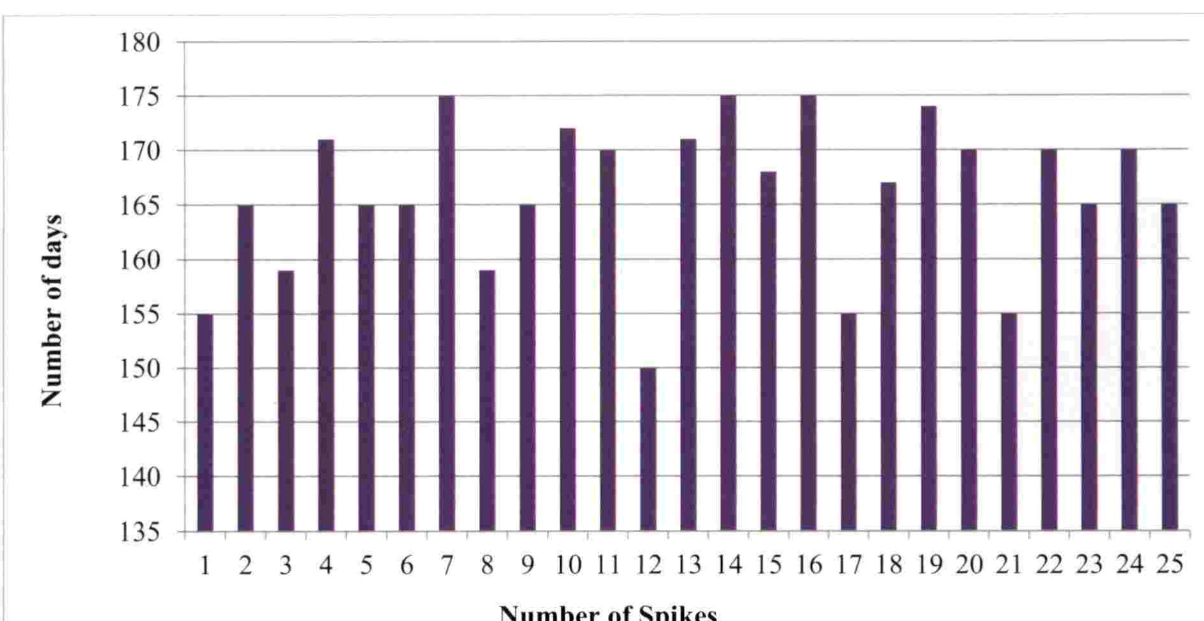


Figure 14. Fruit maturation period from fertilization of *Piper nigrum* L.

revealed the rudimentary structures of ovary and stamens. Through these tests it was possible to observe the germination of pollen tube.

The time period taken from bud initiation of individual flower to full emergence of flower is 19-20 days and is represented in the Figure 7.

The flowering frequency in black pepper was maximum in the month of July and minimum in January and February is shown in the Figure 8. The flowering intensity was maximum on the fifth day of anthesis and it is shown in the Figure 9. The anthesis period in inflorescence varied from 9days (6 cm) in small inflorescence and 12 days (9 cm) in long inflorescence and it is represented in the Figure 10. According to Nybe *et al.* (2007) the period of anthesis varied from 6-10 days. The anthesis period in a plant was maximum in the month of July and minimum in the month of February and January and is shown in the Figure 11. The emergence of spike varied from 23-30 days as shown in the Figure 12. According to Ravindran (2006) it took three to four weeks for the full spike emergence. The mean duration of spiking in a plant population was maximum in the month of June – July and minimum in the month of February and is represented in the Figure 13. 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 (Figure 14). Pepper fruit takes 6–8 months for full maturity from flowering, depending upon variety and the average being 7 months (Ravindran, 2006).

5.2 AGENT/AGENTS OF POLLINATION IN *PIPER NIGRUM* L.

The role of different agents of pollination like rainwater, wind, insect and dew were investigated. The mean percentage fruit set under wind pollination is presented in Table 28. The different treatments were significantly superior. Treatment, PW2 were on par with FW2 and were significantly superior compared to other treatments. These are the treatments where three to five inflorescences with lower spike emasculated and covered with nylon cover in field as well as on pot. So the fertilization in lower spike might have occurred due to the pollen fallen from the above spikes or from the pollen carried by wind from outside. The percentage of fruit set of treatment FW1, where single inflorescence was emasculated and covered with nylon cover was 77.67%. Here whatever fruit set occurred might be due to the pollen brought by the wind. PW1, where single inflorescence was selected, emasculated and covered with polythene cover and manual fanning done had resulted in a fruit set of 59.39 %. In this treatment whatever fruit set occurred might have been brought by the forced wind from outside the inflorescence. However no fruit set were observed in control treatments, FC2 and PC2 which contained single inflorescence emasculated and covered by polythene cover. Petri plates kept below the spikes showed the presence of pollen suggesting that pollen grains are carried by wind and are moving. Petri plates were kept between plants also collected pollen grains of black pepper.

Table 33. Characteristics of wind pollinated pollen

Characteristics	Wind pollinated pollen	Black pepper pollen
Pollen size	Normally ranges from 10-80 µm	9-10 µm
Reduced perianth (Whitehead, 1983)	To expose both anthers and stigmas to wind	Perianth absent and anthers and stigma are exposed
Exserted anthers (Whitehead, 1983	To carry the pollen by wind	Presence of exserted anthers (Ravindran, 2006)
Pendulous, catkin like inflorescence (Whitehead, 1983)	Usually present	Pendulous inflorescence
Protogynous flowers (Honig *et al.*, 1992)	Present	Present

The characteristics of wind pollinated pollen and that of black pepper shows very much similarity. Hence from the results of the experiment as well as from the characteristics of pollen of black pepper we can assume that the pollen of black pepper is suited for wind pollination.

Twenty-five inflorescences in the field and twenty-five in the pot grown black pepper observed from the anthesis period revealed the visitation of insects. Hence the insects making the visit were collected, identified and visitation rates of insects were determined. Three different floral visitors visiting the black pepper spike noticed were black ant , yellow crazy ant and pollu beetle visiting day and night. The behaviour of ant species were monitored by field observations However no pollen was obtained from the mouth, abdomen and legs of the insects collected. Hence the role of insects in pollination could not be found out. However, collection of nectar was noticed by fire ant and black garden while visiting the flowers during days and night. The visitation rates of three different floral visitors visiting the black pepper spike was fire ant (1.79), black garden ant (1.41) and pollu beetle (0.45). The presence of insects had been reported by Martin and Gregory (1962) and Semple (1974) suggestive of their role in pollination. Semple (1974) found that in Costa Rica several species of *Trigona* bees are the most common visitors on *Piper* spp. for 14 Piperaceae species at two sites covered by semideciduous forest in South Eastern Brazil. In a study conducted in Central American species of Piper, seven species were wind pollinated and three, Piper *amalago, P. crassinervium* and *P. glabratum* were exclusively insect-pollinated (Figueiredo and Sazima,2000).

The role of rain water in pollination was undertaken with four treatments. The mean percentage of fruit set differed significantly among treatments and is presented Table 31. Treatment, FR1 were on par with PR1 and were significantly superior compared to other treatments.FR1, where single inflorescence was covered by polythene cover and at time of natural raining the inflorescence was exposed and after the rain it was covered with polythene produced 92.76 % of fruit set. PR1, was the treatment where whole plant was covered by polythene cover and rain water was imitated as splashing on ten bush pepper plants at time of anthesis and then once again covered by polythene cover. Here the fruit set was 92.9 %. No fruit set were observed in control treatments, FC2 and PC2, where single inflorescence was emasculated and covered by polythene cover.

Presence of dew were observed from June to December and the dew collected from the inflorescence showed the presence of pollen grains which suggests the role of dew also in the pollination of pepper. Martin and Gregory (1962) reported that accumulation of dew may cause the disintegration of the pollen lumps. Drops collected from the spikes were reported to contain considerable quantities of pollen by Waard and Zeven (1969) also.

5.3 BREEDING SYSTEM OF *PIPER NIGRUM* L.

The different types of breeding system investigated in black pepper were open pollination, autogamy, geitonogamy, xenogamy and apomixis. The mean percentage fruit set differed significantly among treatments and is presented Figure 15. Treatment, B5 which was single inflorescence emasculated and hand self-pollinated with pollen collected from the same plant and covered by cloth bag (Geitonogamy) were on par with B4 which was four to five mature inflorescences were covered by cloth bag and emasculation of lower spike (Geitonogamy), B3, where single flower was selected and covered with cloth bag (Autogamy), B2 where three flowers from top, middle and base were selected and covered with cloth bag after removing all other flowers (Autogamy), B6 in which four to five mature inflorescences were covered by a cloth bag and doing emasculation of lower spike during anthesis period and sprayed with water imitating rain to carry pollen (Geitonogamy) and B1 where single inflorescence was selected, marked and kept for open pollination and were significantly superior compared to other treatments. The percentage of fruit set of treatment B7 (83.97), B8 (82.85), and B10 (89.15). No fruit set were observed in treatment, B9 where single inflorescence was emasculated and covered with polythene cover (Apomixis). It can be inferred that in black pepper open pollination, autogamy and geitonogamy equivalently contributed to fruit set while the fruit set due to xenogamy was less compared to the open pollination, autogamy and geitonogamy breeding system mentioned breeding system. However, apomixes was not noticed. Gentry (1955) suggested agamospermy in *Piper nigrum*. The breeding systems of Piperaceae are known for only very few species, the paleotropical *Piper nigrum* and the neotropical *P. Arieianum* which are self-compatible (Martin and Gregory, 1962; Sasikumar *et al.*, 1992), and the paleotropical *P. methysticum* which is self-incompatible (Prakash *et al.*, 1994). Apomixis in black pepper was first reported by Gentry (1955) on male sterile cv *Uthirankotta*. Fruiting in female plants of *P. longum*and *P. chaba* growing in the germplasm conservatory of IISR has been reported. In the Western Ghat forests, small populations of either male or female plants are met with quite frequently and in such female populations good fruiting was noticed. All evidences tend to point to the presence of apomixis in the South Indian species of *Piper* (Ravindran, 2006*)* Apomixis seems to disappear or at least gets suppressed to a great extent with the gain of bisexuality (Ravindran, 2006) However Chen (2013) reported that occurrence of apomixes in *P. nigrum*, was not proved and hence apomixes do not contribute to fruit set in pepper

In a study conducted by Sasikumar *et al.* (1992) highest fruit setting was observed under open pollination in the three most popular cultivars, Karimunda, Panniyur1 and Aimpiriyan. The extent of autogamy in three cultivars were 86 per cent in *Karimunda,* 92 per cent in *Panniyur*1 and 95 per cent in *Aimpiriyan* and the percentage of self compatibility was 84 per cent, 91 per cent and 86.5 per cent respectively. These results indicated that selfing is the predominant form of fertilization in cultivated bisexual pepper. Thus, though protogyny occurred, it appeared ineffective to prevent selfing as the pendent spike was abundantly assured of pollen from upper flowers.

A basic study of floral structure and phenology was essential to understand the pollination systems of *Piper nigrum* L. For successful pollination, pollen production and availability of matured pollen is an essential requirement for fruit set and seed set. The peak emergence of spike and the availability of pollen were maximum during June - July corresponding to rainy periods. Characteristics of black pepper flowers studied revealed their adaptation for wind pollination and can take place even without rain water. The role of insects in pollination of black pepper could not be confirmed. Predominant form of breeding system in black pepper was geitonogamy and autogamy. However, apomixis could not be proved from the experiment. Thus, the study on pollination biology of black pepper revealed the floral biology, phenology and the role of rain, wind and dew in pollination and supported geitonogamy, autogamy, open pollination and xenogamy.

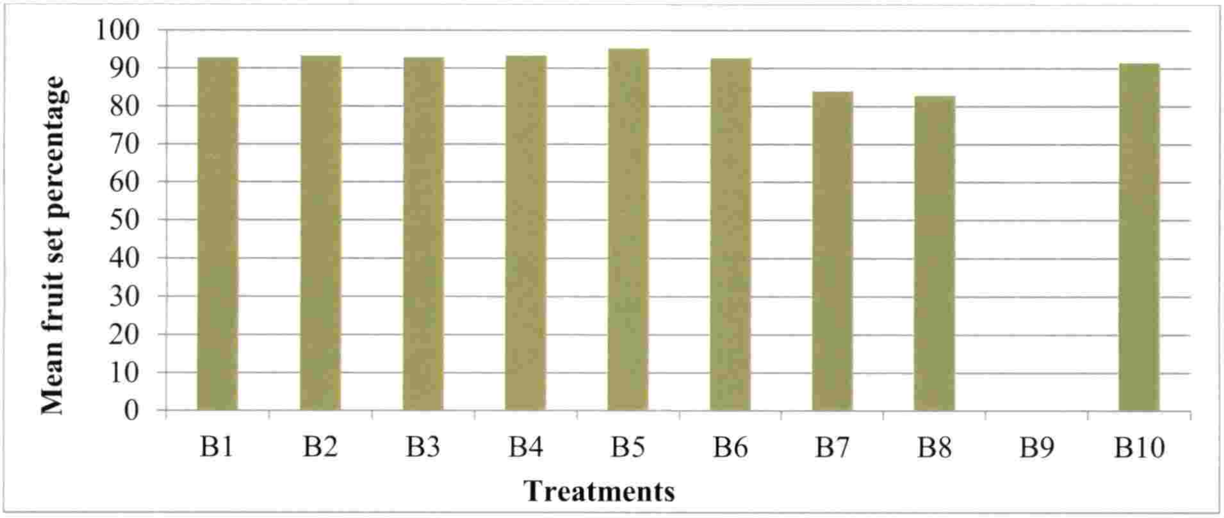


Figure 15. Effect of breeding system on fruit set of *Piper nigrum* L.

Summary

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6. SUMMARY

The salient findings obtained from the study on "Pollination biology of black pepper (*Piper nigrum* L.)" is summarized in this chapter.

The experiments on floral morphology and floral phenology were carried out in twenty five field grown black pepper plants of variety Panniyur 1 maintained by Instructional Farm, Vellayani. The experiments on the role of wind, insect, rain water and dew on pollination and different types of breeding system were carried out in both field and fifty bush pepper plants of Panniyur 1 maintained in pots at Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The plants were observed from March 2018 to March 2019 for studying the floral morphology, floral phenology, agents of pollination and breeding system. 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. 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. Presence of nectar was noticed in the flower between the bract and stigma and the amount of nectar content of one flower was 0.69 μl. 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. The size of the flower was maximum on fifth day of stigma opening and the length and breadth of flower was 1.53 x 1.34 mm. The average number of flowers per spike was carried out from fifty inflorescences of twenty-five black pepper plants grown in the field and it varied from 49-98 flowers depending on the length of the spike. The number of anthers in each flower was four and was uniform in all fifty flowers. 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 and it revealed that anther dehiscence occurred from 11 am and continued till 4 pm and was maximum at 2-3 pm. Anther dehiscence mode showed longitudinal dehiscence. The number of pollen grains per inflorescence was calculated by haemocytometer and it varied from 5,01,500 - 7,00,000 pollen grains and depending on the number of flowers in a spike. The number of pollen grains per flower varied from 8,300 - 10,000. The pollen morphology studied using Scanning Electron Microscope revealed monosulcate pollen grains with the mean polar diameter of 10.414 μm and a mean equatorial diameter of 6.309 μm having an exine thickness of 924.8 nm. Stigma of Panniyu-1 was wet type, papillate and had four lobes. Two flowers each were taken from five inflorescences of black pepper plants grown in the field for studying the stigma receptivity from the emergence of stigma up to 8 days. Stereomicroscopic image of opening of stigma revealed the different stages of stigma receptivity. The stigma receptivity by hydrogen peroxide test revealed stigma receptivity for 7 days. Maximum bubbles were counted on fifth day of emergence of stigma. 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. Five plants were observed for a period till the pollen was available in a plant for one year from March 2018 to March 2019. The maximum pollen availability was in the month of June - July. Pollen being present and remain fresh in a flower after 14- 15 days of stigma emergence and was considered as the longevity of the flower. Pollen fertility studies by Acetocarmine test revealed that 91 % of the pollen were fertile. The pollen viability percentage by TTC stain and IKI stain was 91.03 % and 92.4% respectively. *In vitro* germination in Brewbakers- Kwack medium at 5, 10, 20 and 30 % sucrose showed highest pollen germination at 5% sucrose. Ten flowers from five inflorescences of black pepper plants grown in field selected and pollinated artificially were observed for *in vivo* germination of pollen. *In vivo* germination done through DAB test and aniline blue fluorescence method for 6 hrs and 24 hours viewed under fluorescent microscope revealed *in vitro* pollen tube growth. The cross and longitudinal section of the immature spikes revealed the rudimentary structures of ovary and stamens under Stereomicroscope. The time period taken from bud initiation of individual flower to full emergence of flower was 19-20 days. Flowering frequency in black pepper was maximum in the month of July and minimum in January and February. The average number of flowers opened per inflorescence each day represented as flowering intensity was maximum on the fifth day of anthesis. The anthesis period in inflorescence varied from 9 days (6 cm) in small inflorescence and 12 days (9 cm) in long inflorescence. The anthesis period in a plant was maximum in the month of July and minimum in the month of February and January.

The emergence of spike from the first day of appearing to the full emergence varied from 23-30 days. The mean duration of spiking was 26.84 days in a plant. 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. 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 to find out the fruit maturity period. The fruit was drupe and the period taken from fertilization to maturity varied from 150-175 days.

The data on wind pollination experiment on twenty five inflorescences of field grown black pepper plants and twenty five inflorescences of pot grown bush pepper plants. The different treatments were significantly superior. Treatment, PW2 were on par with FW2 and were significantly superior compared to other treatments. The percentage of fruit set of treatment FW1 and PW1 was 77.67 and 59.39 respectively. No fruit set were observed in control treatments, FC2 and PC2.

The insects making the visit of black pepper inflorescences were collected, identified and visitation rates of insects were determined. Three different floral visitors visiting the black pepper spike noticed were fire ant, black garden ant and pollu beetle visiting day and night. The behaviour of ant species were monitored by field observations and the presence of pollen on their body parts like mouth, abdomen and legs were checked by dissecting under stereoscopic microscope. No pollen was obtained from the mouth, abdomen and legs. Hence the role of insects in pollination could not be found out. However collection of nectar was noticed by fire ant and black garden while visiting the flowers during days and night. The visitation rates of three different floral visitors visiting the black pepper spike was fire ant (1.79), black garden ant (1.41) and pollu beetle (0.45).

The treatments of pollination by rain water revealed treatment, FR1 on par with PR1 and were significantly superior compared to other treatments. FR1, where single inflorescence was covered by polythene cover and at time of natural raining the inflorescence was exposed and after the rain it was covered with polythene produced 92.76 % of fruit set. PR1, was the treatment where whole plant was covered by polythene cover and rain water was imitated as splashing on ten bush pepper plants at time of anthesis and then once again covered by polythene cover. Here the fruit set was 92.9 %. No fruit set were observed in control treatments, FC2 and PC2, where single inflorescence was emasculated and covered by polythene cover.

Presence of dew were observed from June to December in the inflorescences of black pepper and the dew collected from the inflorescence showed the presence of pollen grains which suggested the role of dew also in the pollination of pepper.

The different types of breeding system investigated in black pepper were open pollination, autogamy, geitonogamy, xenogamy and apomixes. Treatment, B5 were on par with B4, B3, B2, B1 and were significantly superior compared to other treatments. The percentage of fruit set of treatment B7 (83.97), B8 (82.85), and B10 (89.15). No fruit set were observed in treatment, B9. Thus, in black pepper open pollination, autogamy and geitonogamy equivalently contributed to fruit set while the fruit set due to xenogamy was less compared to the open pollination, autogamy and geitonogamy breeding system. However, apomixis was not noticed.

Thus, the study on pollination biology of black pepper revealed the floral biology, phenology agents of pollination and the breeding system in black pepper. The floral morphology and breeding system could explain the role of rain, wind and dew in pollination which was supported by geitonogamy, autogamy, open pollination and xenogamy.

A basic study of floral structure and phenology was essential to understand the pollination systems of *Piper nigrum* L. For successful pollination, pollen production and availability of matured pollen is an essential requirement for fruit set and seed set. The peak emergence of spike and the availability of pollen were maximum during June - July corresponding to rainy periods. Characteristics of black pepper flowers studied revealed their adaptation for wind pollination and can take place even without rain water. The role of insects in pollination of black pepper could not be confirmed. Predominant form of breeding system in black pepper was geitonogamy and autogamy. However, apomixis could not be proved from the experiment. Thus, the study on pollination biology of black pepper revealed the floral biology, phenology and the role of rain, wind and dew in pollination and supported geitonogamy, autogamy, open pollination and xenogamy.

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Abstract

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POLLINATION BIOLOGY OF BLACK PEPPER

(*Piper nigrum* L.)

by

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ABSTRACT

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ABSTRACT

The present study entitled "Pollination biology of black pepper (*Piper nigrum* L.) was carried out at College of Agriculture, Vellayani with specific objectives to study the floral morphology, floral phenology, mode of pollination and breeding system in black pepper.

The experiments on floral morphology and floral phenology were carried out in twenty five field grown black pepper plants of variety Panniyur 1. The experiments on the role of wind, insect, rain water and dew on pollination and different types of breeding system were carried out in both field and fifty bush pepper plants of Panniyur 1 maintained in pots. The plants were observed from March 2018 to March 2019 for studying the floral morphology, floral phenology, agents of pollination and breeding system.

The floral morphology revealed light green (149 B) colour in 52 % and dark green colour (140 A) in 48 % of the observed flowers as per the Royal Horticulture Society Colour Charts. The odour of the flower was slightly minty during the first five minutes and then increased to strong minty after 30 minutes and became light minty six hours after keeping in closed containers. Presence of nectar was noticed on the black pepper flowers. Anthesis started from 4-5 pm and was maximum between 6-7 pm and extended upto 12 midnight. The flower size was maximum (1.53 x 1.34 mm) on fifth day of stigma receptivity. The number of flowers in a spike varied from 48 to 98 flowers depending on the length of spike. The number of anthers in each flower was four and anther dehiscence was longitudinal. The anther dehiscence occurred from 11 am and continued till 4 pm and was maximum at 2-3 pm. The number of pollen grains was counted using haemocytometer and it varied from 5,00,000 – 7,00,000 pollen grains per inflorescence. The pollen morphology studied using Scanning Electron Microscope (SEM) revealed monosulcate pollen grains with mean polar diameter of 10.414 μm and mean equatorial diameter of 7.803 μm having

an exine thickness of 924.8 nm. The stigma was 4 lobed, wet type and papillate. The duration of stigma receptivity was for 7 days with peak stigma receptivity was on fifth day of anthesis. Pollen was available in an inflorescence from 9 to 12 days from the first day of anthesis depending on length of spike and was available throughout the year in a plant. The longevity of flowers varied from 14 to15 days. Pollen fertility studies by Acetocarmine test revealed that 91% of the pollen were fertile. The pollen viability by 2,3,5-triphenyl tetrazolium chloride (TTC) stain and iodine potassium iodide (IKI) stain was 91.03% and 92.4% respectively. *In vitro* germination in Brewbakers medium showed highest pollen germination at 5% sucrose. *In vivo* germination was done through diaminobenzidine (DAB) test and aniline blue fluorescence method for 6 h and 24 h respectively and imaged through fluorescent microscopy. The cross and longitudinal section of the immature spikes revealed the emergence of ovary and stamens. Flower emergence duration ranged from 19 to 20 days. Flowering frequency in black pepper was maximum in the month of July and the flowering intensity was maximum in the fifth day of anthesis. The anthesis period in an inflorescence varied from 9 to 12 days depending on length of spike and was noticed throughout the year in a plant. Duration of spiking was 26.84 days while spiking extended for 259.5 days in a plant population. The fruit is a drupe and the mean period taken from fertilization to maturity was 150-175 days.

The percentage of fruit set with rain water in field grown pepper was 92.76% and that for pot grown bush pepper was 92.90%. The percentage of fruit set by experiments on wind pollination resulted in 77.67% with wind alone and 92% with wind and geitonogamy in field grown black pepper plants and 59.39% with simulated wind and 96% with wind and geitonogamy in pot grown bush pepper plants. The floral biology of the black pepper revealed numerous pollen with very small grain size and pendulous spike supporting the characteristics of wind pollinated plant. Presence of dew was observed from June to December and the dew collected from the inflorescence showed the presence of pollen grains which suggests the role of dew also in the pollination of pepper. Three different floral visitors of the black pepper spike were and black ant, yellow crazy and pollu beetle visting day and night. However the role of insects in pollination could not be confirmed, but collection of nectar was noticed by black ant and yellow crazy ant while visiting the flowers. Studies on breeding system revealed high fruit set in geitonogamy followed by autogamy, open pollination and xenogamy. However no fruit set was noticed due to apomixis. The study on pollination biology of black pepper revealed the floral biology, phenology and the role of rain followed by wind and dew in pollination and supported geitonogamy, autogamy, open pollination and xenogamy.

Appendices

APPENDIX I

Weather parameters from 1th March 2018 to 30th March, 2019

Standard weeks	Temperature(°C)		Relative humidity(%)		Rainfall (mm)	Rainy days
	Max	Min	Max	Min		
22	31.8	25	93.17	79.17	68	6
23	30.6	24.68	96.43	85.57	126.6	6
24	31.17	25.06	92	80.57	63.5	5
25	31	24.57	92.4	83.7	57	3
26	31.46	24.4	89.7	80.7	25.2	4
27	31.56	24.69	86.6	75.4	10.2	1
28	29.6	23	93.9	85.4	69.3	6
29	30.4	23.5	91.1	79.1	56.3	4
30	31.4	23.6	89.3	73.3	13.1	2
31	29.5	23.9	90.4	80.9	136.2	3
32	30.3	23.3	91	85.1	107.3	4
33	29.1	22.6	94.9	89.9	205.2	6
34	31	24	89	77	2.8	1
35	32	24.5	89.1	71.9	0	0
36	32.2	24.1	87.1	72	0	0
37	33	24.1	85.1	70.9	0	0

APPENDIX II

Flower colour of *Piper nigrum* L.

No. of flowers	Colour of the flower	RHS colour chart code
1	Light green	149 C
2	Dark green	140 A
3	Dark green	140 A
4	Light green	149 C
5	Light green	149 C
6	Dark green	140 A
7	Light green	149 C
8	Light green	149 C
9	Dark green	140 A
10	Dark green	140 A
11	Light green	149 C
12	Dark green	140 A
13	Light green	149 C
14	Dark green	140 A
15	Light green	149 C
16	Dark green	140 A
17	Dark green	140 A
18	Light green	149 C
19	Light green	145 C
20	Light green	145 C
21	Light green	145 C
22	Dark green	140 A
23	Dark green	140 A
24	Light green	145 C
25	Light green	145 C

26	Dark green	140 A
27	Light green	145 C
28	Dark green	140 A
29	Light green	149 C
30	Dark green	140 A
31	Dark green	140 A
32	Light green	149 C
33	Light green	149 C
34	Light green	149 C
35	Light green	149 C
36	Light green	149 C
37	Light green	149 C
38	Dark green	140 A
39	Dark green	140 A
40	Dark green	140 A
41	Light green	149 C
42	Light green	149 C
43	Light green	149 C
44	Dark green	140 A
45	Dark green	140 A
46	Light green	149 C
47	Light green	149 C
48	Light green	149 C
49	Light green	149 C
50	Light green	149 C
51	Dark green	140 A
52	Dark green	140 A
53	Dark green	140 A
54	Light green	149 C
55	Dark green	140 A

56	Dark green	140 A
57	Light green	149 C
58	Dark green	140 A
59	Light green	149 C
60	Dark green	140 A
61	Dark green	140 A
62	Light green	149 C
63	Dark green	140 A
64	Dark green	140 A
65	Dark green	140 A
66	Dark green	140 A
67	Light green	149 C
68	Light green	149 C
69	Light green	149 C
70	Light green	149 C
71	Light green	149 C
72	Dark green	140 A
73	Dark green	140 A
74	Dark green	140 A
75	Dark green	140 A
76	Light green	149 C
77	Light green	149 C
78	Light green	149 C
79	Light green	149 C
80	Dark green	140 A
81	Dark green	140 A
82	Dark green	140 A
83	Dark green	140 A
84	Dark green	140 A
85	Dark green	140 A

86	Dark green	140 A
87	Light green	149 C
88	Light green	149 C
89	Light green	149 C
90	Light green	149 C
91	Light green	149 C
92	Light green	149 C
93	Light green	149 C
94	Dark green	140 A
95	Dark green	140 A
96	Light green	149 C
97	Light green	149 C
98	Light green	149 C
99	Dark green	140 A
100	Dark green	140 A

APPENDIX III.

Flower odour of *Piper nigrum* L.

Panel members	After 5 minutes	After 30 minutes	After 6 hours
1	Slightly minty	Strong minty	Light minty
2	Slightly minty	Strong minty	Light minty
3	Slightly minty	Strong minty	Light minty
4	Slightly minty	Strong minty	Light minty
5	Slightly minty	Strong minty	Light minty
6	Slightly minty	Strong minty	Light minty
7	Slightly minty	Strong minty	Light minty
8	Slightly minty	Strong minty	Light minty
9	Slightly minty	Strong minty	Light minty
10	Slightly minty	Strong minty	Light minty

APPENDIX IV

Presence of nectar in *Piper nigrum* L.

Twenty five inflorescences with thirty five stigma each	Amount of nectar measured in µl
1	25
2	24
3	25
4	23
5	25
6	23
7	23
8	24
9	25
10	24
11	25
12	23
13	25
14	23
15	23
16	24
17	24
18	25
19	23
20	23
21	25
22	25
23	24
24	25
25	24
Mean	**24.08**

APPENDIX V

Flower opening time in *Piper nigrum* L.

First day of flower opening in the spike

No. of spike	Time									
	12 midnight to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11pm -12am	No. of flowers opening per day
1	0	2	0	3	2	1	0	0	1	9
2	0	1	1	2	1	0	0	0	2	7
3	0	1	1	3	1	1	0	0	0	7
4	0	2	0	2	2	1	0	0	2	9
5	0	2	1	3	1	0	0	0	1	8
6	0	1	0	2	1	1	0	0	0	5
7	0	2	1	3	2	0	0	0	1	9
8	0	1	1	3	2	1	0	0	1	9
9	0	2	0	2	2	0	0	0	1	7
10	0	2	1	3	1	1	0	0	2	10
11	0	1	2	2	1	1	0	0	1	8

12	0	2	1	3	2	0	0	0	2	10
13	0	1	0	2	2	1	0	0	1	7
14	0	2	1	2	1	1	0	0	1	8
15	0	2	1	2	1	0	0	0	1	7
16	0	1	0	2	2	1	0	0	1	7
17	0	2	1	3	1	1	0	0	2	10
18	0	1	1	2	1	0	0	0	0	5
19	0	1	1	3	2	1	0	0	1	9
20	0	2	1	2	1	0	0	0	2	8
21	0	2	0	3	2	1	0	0	1	9
22	0	1	1	3	1	1	0	0	0	7
23	0	1	1	2	2	0	0	0	1	7
24	0	1	1	3	1	1	0	0	0	7
25	0	2	1	2	1	0	0	0	2	8
26	0	2	0	2	2	0	0	0	1	7
27	0	1	1	3	1	1	0	0	0	7
28	0	2	1	2	1	0	0	0	2	8
29	0	2	0	3	2	1	0	0	1	9
30	0	1	1	2	2	1	0	0	1	8

31	0	2	0	3	2	0	0	0	1	8
32	0	1	1	3	1	1	0	0	0	7
33	0	1	1	2	2	0	0	0	1	7
34	0	2	0	2	1	1	0	0	1	7
35	0	2	1	3	1	1	0	0	2	10
36	0	2	0	2	2	0	0	0	1	7
37	0	1	1	2	2	0	0	0	1	7
38	0	2	1	3	1	1	0	0	2	10
39	0	2	0	2	2	0	0	0	1	7
40	0	1	1	2	1	1	0	0	0	6
41	0	1	1	3	2	0	0	0	1	8
42	0	2	1	3	1	0	0	0	2	9
43	0	2	0	2	2	1	0	0	1	8
44	0	1	1	3	2	1	0	0	1	9
45	0	2	1	2	1	0	0	0	2	8
46	0	2	0	3	2	1	0	0	1	9
47	0	2	0	2	2	1	0	0	1	8
48	0	1	1	2	1	0	0	0	1	6
49	0	1	1	3	2	1	0	0	1	9

50	0	2	1	3	1	1	0	0	2	10
Mean	0	1.56	0.7	2.46	1.5	0.58	0	0	1.1	

Second dayof flower opening in the spike

No. of spike	12 midnight to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers opening per day
1	0	1	1	3	2	1	0	0	1	9
2	0	2	0	2	1	0	0	0	2	7
3	0	1	1	2	1	1	0	0	0	6
4	0	2	0	2	2	1	0	0	2	9
5	0	1	1	3	1	0	0	0	1	7
6	0	1	0	2	1	1	0	0	0	5
7	0	2	1	3	2	0	0	0	1	9
8	0	1	0	3	2	1	0	0	1	8
9	0	2	0	2	2	0	0	0	1	7
10	0	1	1	3	1	1	0	0	1	8
11	0	2	0	2	1	1	0	0	1	7
12	0	1	1	3	2	0	0	0	2	9

13	0	1	0	2	2	1	0	0	1	7
14	0	2	1	2	1	1	0	0	1	8
15	0	1	0	2	1	0	0	0	1	5
16	0	1	0	2	2	1	0	0	1	7
17	0	2	1	3	1	1	0	0	2	10
18	0	1	0	2	1	0	0	0	0	4
19	0	1	1	3	2	1	0	0	1	9
20	0	2	1	2	1	0	0	0	2	8
21	0	2	0	3	2	1	0	0	1	9
22	0	1	1	3	1	1	0	0	0	7
23	0	2	0	2	2	0	0	0	1	7
24	0	1	1	3	1	1	0	0	0	7
25	0	2	0	2	1	0	0	0	2	7
26	0	2	0	2	2	0	0	0	1	7
27	0	1	1	3	1	1	0	0	0	7
28	0	2	1	2	1	0	0	0	2	8
29	0	2	0	3	2	1	0	0	1	9
30	0	1	1	2	2	1	0	0	1	8
31	0	2	0	3	2	0	0	0	1	8

32	0	1	1	3	1	1	0	0	0	7
33	0	1	1	2	2	0	0	0	1	7
34	0	2	0	2	1	1	0	0	2	8
35	0	1	1	3	1	1	0	0	1	8
36	0	2	0	2	2	0	0	0	1	7
37	0	1	1	3	2	0	0	0	2	9
38	0	2	1	3	1	1	0	0	1	9
39	0	2	0	2	2	0	0	0	1	7
40	0	1	1	2	1	1	0	0	0	6
41	0	1	1	2	2	0	0	0	1	7
42	0	2	1	3	1	0	0	0	0	7
43	0	2	0	2	2	1	0	0	1	8
44	0	1	1	3	2	1	0	0	1	9
45	0	2	1	3	1	0	0	0	2	9
46	0	1	0	2	1	1	0	0	1	6
47	0	2	0	2	2	1	0	0	1	8
48	0	1	1	2	1	0	0	0	0	5
49	0	1	1	3	2	1	0	0	1	9
50	0	2	1	2	1	1	0	0	2	9

Mean	0	1.48	0.56	2.44	1.48	0.58	0	0	1.02	

Third dayof flower opening in the spike

No of spike	12 midnight to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers opening per day
1	0	1	1	4	2	1	0	0	1	10
2	0	2	0	3	1	0	0	0	2	8
3	0	1	1	4	1	1	0	0	0	8
4	0	2	0	3	2	1	0	0	2	10
5	0	1	1	3	1	0	0	0	1	7
6	0	1	0	4	1	1	0	0	0	7
7	0	2	1	3	2	0	0	0	1	9
8	0	1	0	3	2	1	0	0	1	8
9	0	2	0	4	2	0	0	0	1	9
10	0	1	1	4	1	1	0	0	1	9
11	0	2	0	3	1	1	0	0	1	8
12	0	1	1	4	2	0	0	0	2	10
13	0	1	0	3	2	1	0	0	1	8

14	0	2	1	3	1	1	0	0	1	9
15	0	1	0	4	1	0	0	0	1	7
16	0	1	0	4	2	1	0	0	1	9
17	0	2	1	3	1	1	0	0	2	10
18	0	1	0	3	1	0	0	0	0	5
19	0	1	1	4	2	1	0	0	1	10
20	0	2	1	3	1	0	0	0	2	9
21	0	2	0	4	2	1	0	0	1	10
22	0	1	1	3	1	1	0	0	0	7
23	0	2	0	3	2	0	0	0	1	8
24	0	1	1	4	1	1	0	0	0	8
25	0	2	0	3	1	0	0	0	2	8
26	0	2	0	4	2	0	0	0	1	9
27	0	1	1	4	1	1	0	0	0	8
28	0	2	1	3	1	0	0	0	2	9
29	0	2	0	4	2	1	0	0	1	10
30	0	1	1	4	2	1	0	0	1	10
31	0	2	0	3	2	0	0	0	1	8
32	0	1	1	3	1	1	0	0	0	7

33	0	1	1	4	2	0	0	0	1	9
34	0	2	0	3	1	1	0	0	2	9
35	0	1	1	4	1	1	0	0	1	9
36	0	2	0	3	2	0	0	0	1	8
37	0	1	1	3	2	0	0	0	0	7
38	0	2	1	4	1	1	0	0	1	10
39	0	1	0	3	2	0	0	0	1	7
40	0	1	1	4	1	1	0	0	0	8
41	0	1	1	4	2	0	0	0	1	9
42	0	2	1	3	1	0	0	0	0	7
43	0	2	0	4	2	1	0	0	1	10
44	0	2	1	3	2	1	0	0	0	9
45	0	1	1	4	1	0	0	0	2	9
46	0	1	0	3	1	1	0	0	1	7
47	0	2	0	4	2	1	0	0	1	10
48	0	1	1	3	1	0	0	0	0	6
49	0	1	1	3	2	1	0	0	1	9
50	0	2	1	4	1	1	0	0	2	11
Mean	0	1.46	0.56	3.48	1.48	0.58	0	0	0.96	

Fourth dayof flower opening in the spike

No of spike	12 midnight to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers opening per day
1	0	2	1	4	2	1	0	0	1	11
2	0	2	0	6	1	0	0	0	2	11
3	0	2	1	6	1	1	0	0	0	11
4	0	1	0	4	2	1	0	0	2	10
5	0	2	1	6	1	0	0	0	1	11
6	0	2	0	4	1	1	0	0	0	8
7	0	2	1	6	2	0	0	0	1	12
8	0	1	0	6	2	1	0	0	1	11
9	0	2	0	4	2	0	0	0	1	9
10	0	1	1	4	1	1	0	0	1	9
11	0	2	0	6	1	1	0	0	1	11
12	0	2	1	6	2	0	0	0	2	13
13	0	1	0	4	2	1	0	0	1	9
14	0	2	1	6	1	1	0	0	1	12
15	0	1	0	4	1	0	0	0	1	7

16	0	2	0	4	2	1	0	0	1	10
17	0	2	1	6	1	1	0	0	2	13
18	0	1	0	6	1	0	0	0	0	8
19	0	2	1	4	2	1	0	0	1	11
20	0	2	1	6	1	0	0	0	2	12
21	0	2	0	4	2	1	0	0	1	10
22	0	1	1	6	1	1	0	0	0	10
23	0	2	0	4	2	0	0	0	1	9
24	0	1	1	6	1	1	0	0	0	10
25	0	2	0	4	1	0	0	0	2	9
26	0	2	0	4	2	0	0	0	1	9
27	0	1	1	6	1	1	0	0	0	10
28	0	2	1	4	1	0	0	0	2	10
29	0	2	0	6	2	1	0	0	1	12
30	0	1	1	4	2	1	0	0	1	10
31	0	2	0	6	2	0	0	0	1	11
32	0	1	1	4	1	1	0	0	0	8
33	0	2	1	4	2	0	0	0	1	10
34	0	2	0	6	1	1	0	0	2	12

35	0	1	1	4	1	1	0	0	1	9
36	0	2	0	6	2	0	0	0	1	11
37	0	2	1	6	2	0	0	0	0	11
38	0	1	1	4	1	1	0	0	1	9
39	0	1	0	6	2	0	0	0	1	10
40	0	1	1	4	1	1	0	0	0	8
41	0	1	1	4	2	0	0	0	1	9
42	0	2	1	6	1	0	0	0	0	10
43	0	2	0	4	2	1	0	0	1	10
44	0	2	1	4	2	1	0	0	0	10
45	0	1	1	6	1	0	0	0	2	11
46	0	1	0	6	1	1	0	0	1	10
47	0	2	0	4	2	1	0	0	1	10
48	0	1	1	6	1	0	0	0	0	9
49	0	2	1	4	2	1	0	0	1	11
50	0	2	1	4	1	1	0	0	2	11
	0	1.62	0.56	4.96	1.48	0.58	0	0	0.96	

Fifth dayof flower opening in the spike

No of spike	12 midnight to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers opening per day
1	0	3	1	7	2	1	0	0	1	11
2	0	2	0	5	1	0	0	0	2	11
3	0	2	1	7	1	1	0	0	1	11
4	0	3	0	6	2	1	0	0	2	10
5	0	2	1	7	1	0	0	0	1	11
6	0	3	0	6	1	1	0	0	2	8
7	0	2	1	7	2	0	0	0	1	12
8	0	3	0	7	2	1	0	0	2	11
9	0	3	0	5	2	0	0	0	1	9
10	0	2	1	6	1	1	0	0	1	9
11	0	3	0	5	1	1	0	0	1	11
12	0	3	1	6	2	0	0	0	2	13
13	0	2	0	7	2	1	0	0	1	9
14	0	2	1	5	1	1	0	0	2	12
15	0	3	0	7	1	0	0	0	1	7
16	0	2	0	7	2	1	0	0	1	10

17	0	2	1	6	1	1	0	0	2	13
18	0	3	0	6	1	0	0	0	1	11
19	0	2	1	7	2	1	0	0	1	14
20	0	2	1	6	1	0	0	0	2	12
21	0	3	0	5	2	1	0	0	1	12
22	0	3	1	6	1	1	0	0	1	13
23	0	2	0	7	2	0	0	0	1	12
24	0	3	1	6	1	1	0	0	0	12
25	0	2	0	5	1	0	0	0	2	10
26	0	2	0	5	2	0	0	0	1	10
27	0	3	1	6	1	1	0	0	0	12
28	0	2	1	5	1	0	0	0	2	11
29	0	2	0	6	2	1	0	0	1	12
30	0	3	1	5	2	1	0	0	1	13
31	0	3	0	7	2	0	0	0	2	14
32	0	2	1	5	1	1	0	0	0	10
33	0	3	1	7	2	0	0	0	1	14
34	0	2	0	6	1	1	0	0	2	12
35	0	3	1	5	1	1	0	0	1	12

36	0	3	0	7	2	0	0	0	1	13
37	0	2	1	6	2	0	0	0	1	12
38	0	3	1	7	1	1	0	0	2	15
39	0	2	0	6	2	0	0	0	1	11
40	0	3	1	5	1	1	0	0	1	12
41	0	2	1	5	2	0	0	0	1	11
42	0	2	1	5	1	0	0	0	1	10
43	0	3	0	7	2	1	0	0	0	13
44	0	3	1	5	2	1	0	0	1	13
45	0	2	1	7	1	0	0	0	2	13
46	0	3	0	6	1	1	0	0	1	12
47	0	2	0	5	2	1	0	0	0	10
48	0	3	1	6	1	0	0	0	0	11
49	0	2	1	5	2	1	0	0	1	12
50	0	2	1	7	1	1	0	0	2	14
	0	2.48	0.56	6	1.48	0.58	0	0	1.18	

Sixth day of flower opening in the spike

No of spike	12	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers

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	midnight to 4pm									opening per day
1	0	2	0	3	2	1	0	0	1	9
2	0	1	1	2	1	0	0	0	2	7
3	0	1	1	3	1	1	0	0	0	7
4	0	2	0	2	2	1	0	0	2	9
5	0	2	1	3	1	0	0	0	1	8
6	0	1	0	2	1	1	0	0	0	5
7	0	2	1	3	2	0	0	0	1	9
8	0	1	1	3	2	1	0	0	1	9
9	0	2	0	2	2	0	0	0	1	7
10	0	2	1	3	1	1	0	0	2	10
11	0	1	2	2	1	1	0	0	1	8
12	0	2	1	3	2	0	0	0	2	10
13	0	1	0	2	2	1	0	0	1	7
14	0	2	1	2	1	1	0	0	1	8
15	0	2	1	2	1	0	0	0	1	7
16	0	1	0	2	2	1	0	0	1	7
17	0	2	1	3	1	1	0	0	2	10

18	0	1	1	2	1	0	0	0	0	5
19	0	1	1	3	2	1	0	0	1	9
20	0	2	1	2	1	0	0	0	2	8
21	0	2	0	3	2	1	0	0	1	9
22	0	1	1	3	1	1	0	0	0	7
23	0	1	1	2	2	0	0	0	1	7
24	0	1	1	3	1	1	0	0	0	7
25	0	2	1	2	1	0	0	0	2	8
26	0	2	0	2	2	0	0	0	1	7
27	0	1	1	3	1	1	0	0	0	7
28	0	2	1	2	1	0	0	0	2	8
29	0	2	0	3	2	1	0	0	1	9
30	0	1	1	2	2	1	0	0	1	8
31	0	2	0	3	2	0	0	0	1	8
32	0	1	1	3	1	1	0	0	0	7
33	0	1	1	2	2	0	0	0	1	7
34	0	2	0	2	1	1	0	0	1	7
35	0	2	1	3	1	1	0	0	2	10
36	0	2	0	2	2	0	0	0	1	7

37	0	1	1	2	2	0	0	0	1	7
38	0	2	1	3	1	1	0	0	2	10
39	0	2	0	2	2	0	0	0	1	7
40	0	1	1	2	1	1	0	0	0	6
41	0	1	1	3	2	0	0	0	1	8
42	0	2	1	3	1	0	0	0	2	9
43	0	2	0	2	2	1	0	0	1	8
44	0	1	1	3	2	1	0	0	1	9
45	0	2	1	2	1	0	0	0	2	8
46	0	2	0	3	2	1	0	0	1	9
47	0	2	0	2	2	1	0	0	1	8
48	0	1	1	2	1	0	0	0	0	5
49	0	1	1	3	2	1	0	0	1	9
50	0	2	1	3	1	1	0	0	2	10
	0	1.56	0.7	2.48	1.5	0.58	0	0	1.08	

Seventh dayof flower opening in the spike

No of spike	12 midnight	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers opening per

	to 4pm									day
1	0	2	0	1	1	1	0	0	1	6
2	0	1	1	2	1	0	0	0	0	5
3	0	1	1	1	1	1	0	0	0	5
4	0	2	0	2	2	1	0	0	0	7
5	0	2	1	1	1	0	0	0	1	6
6	0	1	0	2	1	1	0	0	0	5
7	0	2	1	1	2	0	0	0	1	7
8	0	1	1	2	2	1	0	0	1	8
9	0	2	0	2	2	0	0	0	1	7
10	0	2	1	1	1	1	0	0	0	6
11	0	1	2	2	1	1	0	0	1	8
12	0	2	1	2	2	0	0	0	0	7
13	0	1	0	1	2	1	0	0	1	6
14	0	2	1	2	1	1	0	0	1	8
15	0	2	1	2	1	0	0	0	1	7
16	0	1	0	1	2	1	0	0	1	6
17	0	2	1	2	1	1	0	0	0	7
18	0	1	1	1	1	0	0	0	0	4

19	0	1	1	2	2	1	0	0	1	8
20	0	2	1	2	1	0	0	0	0	6
21	0	2	0	1	2	1	0	0	1	7
22	0	1	1	2	1	1	0	0	0	6
23	0	1	1	2	2	0	0	0	1	7
24	0	1	1	1	1	1	0	0	0	5
25	0	2	1	2	1	0	0	0	2	8
26	0	2	0	2	2	0	0	0	1	7
27	0	1	1	1	1	1	0	0	0	5
28	0	2	1	2	1	0	0	0	2	8
29	0	2	0	3	2	1	0	0	1	9
30	0	1	1	1	2	1	0	0	1	7
31	0	2	0	1	2	0	0	0	1	6
32	0	1	1	2	1	1	0	0	0	6
33	0	1	1	1	2	0	0	0	1	6
34	0	2	0	2	1	1	0	0	1	7
35	0	2	1	2	1	1	0	0	2	9
36	0	2	0	1	2	0	0	0	1	6
37	0	1	1	2	2	0	0	0	1	7

38	0	2	1	1	1	1	0	0	2	8
39	0	2	0	2	2	0	0	0	1	7
40	0	1	1	2	1	1	0	0	0	6
41	0	1	1	1	2	0	0	0	1	6
42	0	2	1	1	1	0	0	0	2	7
43	0	2	0	2	2	1	0	0	1	8
44	0	1	1	1	2	1	0	0	1	7
45	0	2	1	2	1	0	0	0	2	8
46	0	2	0	1	2	1	0	0	1	7
47	0	2	0	2	2	1	0	0	1	8
48	0	1	1	2	1	0	0	0	0	5
49	0	1	1	1	2	1	0	0	1	7
50	0	2	1	1	1	1	0	0	2	8
Mean	0	1.56	0.7	1.58	1.48	0.58	0	0	0.84	

Eight dayof flower opening in the spike

No of spike	12 midnight to 4pm	4-5pm	5-6pm	6-7pm	7-8pm	8-9pm	9-10pm	10-11pm	11-12pm	No of flowers opening per day

175

1	0	1	1	2	2	1	0	0	1	**8**
2	0	2	0	1	1	0	0	0	2	**6**
3	0	1	1	2	1	1	0	0	0	**6**
4	0	2	0	2	2	1	0	0	2	**9**
5	0	1	1	1	1	0	0	0	1	**5**
6	0	1	0	2	1	1	0	0	0	**5**
7	0	2	1	1	2	0	0	0	1	**7**
8	0	1	0	2	2	1	0	0	1	**7**
9	0	2	0	1	2	0	0	0	1	**6**
10	0	1	1	1	1	1	0	0	1	**6**
11	0	2	0	2	1	1	0	0	1	**7**
12	0	1	1	2	2	0	0	0	2	**8**
13	0	1	0	1	2	1	0	0	1	**6**
14	0	2	1	2	1	1	0	0	1	**8**
15	0	1	0	1	1	0	0	0	1	**4**
16	0	1	0	2	2	1	0	0	1	**7**
17	0	2	1	1	1	1	0	0	2	**8**
18	0	1	0	2	1	0	0	0	0	**4**
19	0	1	1	2	2	1	0	0	1	**8**

20	0	2	1	1	1	0	0	0	2	**7**
21	0	2	0	1	2	1	0	0	1	**7**
22	0	1	1	2	1	1	0	0	0	**6**
23	0	2	0	2	2	0	0	0	1	**7**
24	0	1	1	1	1	1	0	0	0	**5**
25	0	2	0	1	1	0	0	0	2	**6**
26	0	2	0	2	2	0	0	0	1	**7**
27	0	1	1	1	1	1	0	0	0	**5**
28	0	2	1	2	1	0	0	0	2	**8**
29	0	2	0	1	2	1	0	0	1	**7**
30	0	1	1	2	2	1	0	0	1	**8**
31	0	2	0	2	2	0	0	0	1	**7**
32	0	1	1	1	1	1	0	0	0	**5**
33	0	1	1	2	2	0	0	0	1	**7**
34	0	2	0	1	1	1	0	0	2	**7**
35	0	1	1	2	1	1	0	0	1	**7**
36	0	2	0	2	2	0	0	0	1	**7**
37	0	1	1	1	2	0	0	0	2	**7**
38	0	2	1	1	1	1	0	0	1	**7**

39	0	2	0	2	2	0	0	0	1	**7**
40	0	1	1	1	1	1	0	0	0	**5**
41	0	1	1	2	2	0	0	0	1	**7**
42	0	2	1	2	1	0	0	0	0	**6**
43	0	2	0	1	2	1	0	0	1	**7**
44	0	1	1	2	2	1	0	0	0	**7**
45	0	2	1	2	1	0	0	0	0	**6**
46	0	1	0	1	1	1	0	0	1	**5**
47	0	2	0	2	2	1	0	0	1	**8**
48	0	1	1	1	1	0	0	0	0	**4**
49	0	1	1	2	2	1	0	0	1	**8**
50	0	2	1	2	1	1	0	0	0	**7**
Mean	0	1.48	0.56	1.56	1.48	0.58	0	0	0.92	

Appendix VI.

Flower size of *Piper nigrum* L.

No of flowers	Third day		Fourth day		Fifth day	
	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)
1	1.51	1.32	1.51	1.32	1.52	1.33
2	1.51	1.32	1.51	1.32	1.52	1.33
3	1.51	1.32	1.51	1.32	1.52	1.33
4	1.51	1.32	1.51	1.32	1.52	1.33
5	1.51	1.32	1.51	1.32	1.52	1.33
6	1.51	1.32	1.51	1.32	1.52	1.33
7	1.51	1.32	1.51	1.32	1.52	1.33
8	1.51	1.32	1.51	1.32	1.52	1.33
9	1.51	1.32	1.51	1.32	1.52	1.33
10	1.51	1.32	1.51	1.32	1.52	1.33
11	1.51	1.32	1.51	1.32	1.52	1.33
12	1.51	1.32	1.51	1.32	1.52	1.33
13	1.51	1.32	1.51	1.32	1.52	1.33
14	1.51	1.32	1.51	1.32	1.52	1.33
15	1.51	1.32	1.51	1.32	1.52	1.33
16	1.51	1.32	1.51	1.32	1.52	1.33
17	1.51	1.32	1.51	1.32	1.52	1.33
18	1.51	1.32	1.51	1.32	1.52	1.33
19	1.51	1.32	1.51	1.32	1.52	1.33
20	1.51	1.32	1.51	1.32	1.52	1.33
21	1.51	1.32	1.51	1.32	1.52	1.33
22	1.51	1.32	1.51	1.32	1.52	1.33
23	1.51	1.32	1.51	1.32	1.52	1.33

24	1.51	1.32	1.51	1.32	1.52	1.33
25	1.51	1.32	1.51	1.32	1.52	1.33
26	1.51	1.32	1.51	1.32	1.52	1.33
27	1.51	1.32	1.51	1.32	1.52	1.33
28	1.51	1.32	1.51	1.32	1.52	1.33
29	1.51	1.32	1.51	1.32	1.52	1.33
30	1.51	1.32	1.51	1.32	1.52	1.33
Mean	**1.51mm**	**1.32mm**	**1.51mm**	**1.32mm**	**1.52mm**	**1.33mm**

APPENDIX VII

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

No. of spike	No. of flowers
1	87
2	76
3	98
4	76
5	65
6	93
7	63
8	74
9	66
10	88
11	76
12	56
13	87
14	56
15	67
16	89
17	87
18	67
19	94
20	77
21	55
22	59
23	86
24	75
25	97
26	57

27	67
28	71
29	83
30	94
31	54
32	75
33	55
34	87
35	89
36	65
37	76
38	56
39	87
40	67
41	78
42	45
43	89
44	65
45	67
46	54
47	65
48	51
49	65
50	74
Mean	**73**

APPENDIX VIII

Number of anthers per flower in *Piper nigrum* L.

Number of flowers	Number of anthers in each flowers
1	4
2	4
3	4
4	4
5	4
6	4
7	4
8	4
9	4
10	4
11	4
12	4
13	4
14	4
15	4
16	4
17	4
18	4
19	4
20	4
21	4
22	4
23	4
24	4
25	4
26	4

27	4
28	4
29	4
30	4
31	4
32	4
33	4
34	4
35	4
36	4
37	4
38	4
39	4
40	4
41	4
42	4
43	4
44	4
45	4
46	4
47	4
48	4
49	4
50	4

APPENDIX IX

Anther dehiscence time of *Piper nigrum* L.

First day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	7	7	7	8	7
2	0	6	6	5	7	5
3	0	7	6	6	8	7
4	0	4	3	3	5	3
5	0	4	2	3	5	3
6	0	8	7	8	9	8
7	0	6	6	5	7	6
8	0	7	4	7	6	4
9	0	5	4	5	6	4
10	0	7	5	5	8	7
11	0	5	4	5	6	4
12	0	6	5	5	7	6
13	0	8	7	6	8	5
14	0	4	5	4	6	4
15	0	7	6	6	8	4
16	0	6	5	4	7	6
17	0	7	6	5	8	4
18	0	5	5	4	6	5
19	0	4	5	5	6	4
20	0	5	6	5	7	4
21	0	7	5	6	8	3
22	0	3	4	3	6	3
23	0	3	4	3	5	3
24	0	5	5	5	6	4

25	0	6	5	4	7	4
26	0	6	4	4	8	5
27	0	7	5	4	6	3
28	0	6	7	6	8	4
29	0	4	5	4	6	4
30	0	5	6	5	7	5
31	0	6	4	5	7	4
32	0	4	5	6	7	4
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	6	5	4	7	4
36	0	6	5	4	7	6
37	0	7	6	5	8	4
38	0	6	5	5	7	6
39	0	8	7	6	8	5
40	0	4	5	4	6	4
41	0	7	6	6	8	4
42	0	6	5	4	7	6
43	0	7	6	5	8	4
44	0	7	7	7	8	7
45	0	6	6	5	7	5
46	0	7	6	6	8	7
47	0	4	3	3	5	3
48	0	4	2	3	5	3
49	0	7	6	5	8	4
50	0	5	4	5	6	3
Mean	0	5.7	5.12	4.86	6.86	4.56

Second day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	5	6	6	7	3
2	0	6	5	5	7	5
3	0	5	6	6	8	7
4	0	4	3	3	5	4
5	0	3	2	3	4	3
6	0	7	6	8	9	6
7	0	6	5	5	7	5
8	0	6	4	6	8	4
9	0	4	4	5	6	4
10	0	6	5	5	8	7
11	0	4	4	5	6	3
12	0	7	5	5	6	4
13	0	8	6	6	8	5
14	0	4	4	4	6	3
15	0	5	5	6	8	4
16	0	6	5	4	7	3
17	0	6	5	5	8	5
18	0	4	5	4	6	4
19	0	4	5	5	6	3
20	0	5	5	5	7	4
21	0	7	5	6	8	4
22	0	3	4	3	5	3
23	0	6	4	3	5	3
24	0	5	5	5	6	4
25	0	5	5	4	7	3
26	0	6	4	4	7	5

27	0	7	5	5	6	3
28	0	7	7	6	8	4
29	0	5	5	4	6	4
30	0	5	4	5	7	5
31	0	6	4	5	7	4
32	0	4	5	6	7	4
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	6	5	4	7	4
36	0	6	4	4	7	6
37	0	5	6	5	8	4
38	0	5	5	4	7	3
39	0	8	6	6	8	5
40	0	5	5	4	6	4
41	0	7	6	5	8	4
42	0	6	5	4	7	5
43	0	7	6	5	8	4
44	0	7	5	7	8	7
45	0	6	4	5	7	5
46	0	7	6	4	8	7
47	0	4	4	3	5	3
48	0	3	2	3	5	3
49	0	5	6	5	8	4
50	0	5	4	5	7	3
Mean	0	5.42	4.8	4.76	6.82	4.2

Third day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	5	5	6	8	3
2	0	4	5	5	6	5
3	0	5	6	6	7	3
4	0	4	3	3	5	4
5	0	3	2	3	4	3
6	0	5	6	6	8	6
7	0	4	5	5	6	3
8	0	5	4	3	7	4
9	0	3	4	4	6	3
10	0	6	5	5	7	4
11	0	5	4	5	6	3
12	0	6	5	5	7	4
13	0	4	6	4	6	5
14	0	4	5	4	6	3
15	0	5	4	6	8	4
16	0	6	5	4	7	3
17	0	6	3	4	6	5
18	0	4	5	5	6	4
19	0	4	5	5	6	3
20	0	5	5	4	6	4
21	0	4	5	6	7	4
22	0	3	4	3	6	3
23	0	5	4	3	7	3
24	0	3	3	5	6	4
25	0	5	4	4	6	3

26	0	5	4	4	7	5
27	0	7	5	5	6	3
28	0	5	7	7	8	4
29	0	5	5	4	6	4
30	0	5	4	5	7	5
31	0	4	4	5	6	4
32	0	4	5	5	7	3
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	6	5	4	6	4
36	0	6	4	4	8	6
37	0	5	6	5	8	4
38	0	5	5	4	7	3
39	0	8	6	6	8	5
40	0	5	5	4	6	4
41	0	7	6	5	8	4
42	0	6	5	4	7	5
43	0	5	6	5	8	4
44	0	7	5	7	8	7
45	0	6	4	5	7	5
46	0	6	6	4	7	4
47	0	4	4	3	5	3
48	0	3	2	3	5	3
49	0	5	6	5	8	4
50	0	5	4	5	7	3
Mean	0	4.9	4.68	4.58	6.62	3.92

Fourth day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	7	7	7	8	7
2	0	6	6	5	7	5
3	0	7	6	6	8	7
4	0	4	3	3	5	3
5	0	4	2	3	5	3
6	0	8	7	8	9	8
7	0	6	6	5	7	6
8	0	7	4	7	6	4
9	0	5	4	5	6	4
10	0	7	5	5	8	7
11	0	5	4	5	6	4
12	0	6	5	5	7	6
13	0	8	7	6	8	5
14	0	4	5	4	6	4
15	0	7	6	6	8	4
16	0	6	5	4	7	6
17	0	7	6	5	8	4
18	0	5	5	4	6	5
19	0	4	5	5	6	4
20	0	5	6	5	7	4
21	0	7	5	6	8	3
22	0	3	4	3	6	3
23	0	3	4	3	5	3
24	0	5	5	5	6	4
25	0	6	5	4	7	4
26	0	6	4	4	8	5

27	0	7	5	4	6	3
28	0	6	7	6	8	4
29	0	4	5	4	6	4
30	0	5	6	5	7	5
31	0	6	4	5	7	4
32	0	4	5	6	7	4
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	6	5	4	7	4
36	0	6	5	4	7	6
37	0	7	6	5	8	4
38	0	6	5	5	7	6
39	0	8	7	6	8	5
40	0	4	5	4	6	4
41	0	7	6	6	8	4
42	0	6	5	4	7	6
43	0	7	6	5	8	4
44	0	7	7	7	8	7
45	0	6	6	5	7	5
46	0	7	6	6	8	7
47	0	4	3	3	5	3
48	0	4	2	3	5	3
49	0	7	6	5	8	4
50	0	5	4	5	6	3
Mean	0	5.7	5.12	4.86	6.86	4.56

Fifth day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	5	6	6	7	3
2	0	6	5	5	7	5
3	0	5	6	6	8	7
4	0		3	3	5	4
5	0	3	2	3	4	3
6	0	5	6	6	7	4
7	0	5	5	5	7	5
8	0	6	4	6	8	4
9	0	5	4	5	6	4
10	0	6	5	5	8	7
11	0	4	4	5	6	3
12	0	7	5	5	6	4
13	0	7	6	6	8	5
14	0	4	4	4	6	3
15	0	5	5	6	8	4
16	0	6	5	4	7	3
17	0	6	5	5	7	5
18	0	4	5	4	6	4
19	0	4	5	5	6	3
20	0	5	5	5	7	4
21	0	7	5	6	8	4
22	0	3	4	3	5	3
23	0	6	4	3	5	3
24	0	5	5	5	6	4
25	0	5	5	4	7	3
26	0	6	4	4	7	5

27	0	7	5	5	6	3
28	0	7	7	6	8	4
29	0	5	5	4	6	4
30	0	5	4	5	7	5
31	0	4	4	5	6	4
32	0	4	5	6	7	3
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	5	5	4	7	4
36	0	6	4	4	7	3
37	0	5	6	5	8	4
38	0	5	5	4	7	3
39	0	7	6	6	8	5
40	0	5	5	4	6	4
41	0	7	6	5	8	4
42	0	6	5	4	7	5
43	0	7	6	5	8	4
44	0	6	5	7	8	7
45	0	6	4	5	7	5
46	0	5	6	4	8	7
47	0	4	4	3	5	3
48	0	3	2	3	5	3
49	0	5	6	5	8	4
50	0	5	4	5	7	3
Mean	0	5.24	4.8	4.72	6.74	4.08

Sixth day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	5	5	6	8	3
2	0	4	5	5	6	5
3	0	5	6	6	7	3
4	0	4	3	3	5	4
5	0	3	2	3	4	3
6	0	5	6	6	8	6
7	0	4	5	5	6	3
8	0	5	4	3	7	4
9	0	3	4	4	6	3
10	0	6	5	5	7	4
11	0	5	4	5	6	3
12	0	6	5	5	7	4
13	0	4	6	4	6	5
14	0	4	5	4	6	3
15	0	5	4	6	8	4
16	0	6	5	4	7	3
17	0	6	3	4	6	5
18	0	4	5	5	6	4
19	0	4	5	5	6	3
20	0	5	5	4	6	4
21	0	4	5	6	7	4
22	0	3	4	3	6	3
23	0	5	4	3	7	3
24	0	3	3	5	6	4
25	0	5	4	4	6	3
26	0	5	4	4	7	5

27	0	7	5	5	6	3
28	0	5	7	7	8	4
29	0	5	5	4	6	4
30	0	5	4	5	7	5
31	0	4	4	5	6	4
32	0	4	5	5	7	3
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	6	5	4	6	4
36	0	6	4	4	8	6
37	0	5	6	5	8	4
38	0	5	5	4	7	3
39	0	8	6	6	8	5
40	0	5	5	4	6	4
41	0	7	6	5	8	4
42	0	6	5	4	7	5
43	0	5	6	5	8	4
44	0	7	5	7	8	7
45	0	6	4	5	7	5
46	0	6	6	4	7	4
47	0	4	4	3	5	3
48	0	3	2	3	5	3
49	0	5	6	5	8	4
50	0	5	4	5	7	3
Mean	0	4.9	4.68	4.58	6.62	3.92

Seventh day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	7	7	7	8	7
2	0	6	6	5	7	5
3	0	7	6	6	8	7
4	0	4	3	3	5	3
5	0	4	2	3	5	3
6	0	8	7	8	9	8
7	0	6	6	5	7	6
8	0	7	4	7	6	4
9	0	5	4	5	6	4
10	0	7	5	5	8	7
11	0	5	4	5	6	4
12	0	6	5	5	7	6
13	0	8	7	6	8	5
14	0	4	5	4	6	4
15	0	7	6	6	8	4
16	0	6	5	4	7	6
17	0	7	6	5	8	4
18	0	5	5	4	6	5
19	0	4	5	5	6	4
20	0	5	6	5	7	4
21	0	7	5	6	8	3
22	0	3	4	3	6	3
23	0	3	4	3	5	3
24	0	5	5	5	6	4
25	0	6	5	4	7	4
26	0	6	4	4	8	5

27	0	7	5	4	6	3
28	0	6	7	6	8	4
29	0	4	5	4	6	4
30	0	5	6	5	7	5
31	0	6	4	5	7	4
32	0	4	5	6	7	4
33	0	3	4	3	5	3
34	0	5	5	5	6	4
35	0	6	5	4	7	4
36	0	6	5	4	7	6
37	0	7	6	5	8	4
38	0	6	5	5	7	6
39	0	8	7	6	8	5
40	0	4	5	4	6	4
41	0	7	6	6	8	4
42	0	6	5	4	7	6
43	0	7	6	5	8	4
44	0	7	7	7	8	7
45	0	6	6	5	7	5
46	0	7	6	6	8	7
47	0	4	3	3	5	3
48	0	4	2	3	5	3
49	0	7	6	5	8	4
50	0	5	4	5	6	3
Mean	0	5.7	5.12	4.86	6.86	4.52

Eight day of anther dehiscence in a spike

No of spike	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	6	7	7	8	6
2	0	0	0	0	0	0
3	0	7	5	5	8	5
4	0	0	0	0	0	0
5	0	5	3	4	6	3
6	0	5	4	5	6	2
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	5	4	5	6	3
11	0	0	0	0	0	0
12	0	6	7	6	8	5
13	0	7	5	6	8	5
14	0	0	0	0	0	0
15	0	6	6	5	7	4
16	0	0	0	0	0	0
17	0	0	0	0	0	0
18	0	4	5	4	7	3
19	0	0	0	0	0	0
20	0	5	6	5	7	4
21	0	6	5	4	7	5
22	0	0	0	0	0	0
23	0	6	4	5	8	4
24	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	3	4	3	5	3

27	0	0	0	0	0	0
28	0	5	5	5	6	4
29	0	5	4	4	6	3
30	0	0	0	0	0	0
31	0	4	5	4	6	3
32	0	0	0	0	0	0
33	0	5	5	4	6	3
34	0	4	3	4	5	3
35	0	0	0	0	0	0
36	0	4	3	5	4	3
37	0	0	0	0	0	0
38	0	0	0	0	0	0
39	0	0	0	0	0	0
40	0	5	6	6	7	3
41	0	0	0	0	0	0
42	0	6	7	5	8	3
43	0	0	0	0	0	0
44	0	5	5	4	7	4
45	0	0	0	0	0	0
46	0	5	6	4	7	4
47	0	5	4	5	6	3
48	0	5	4	5	7	4
49	0	0	0	0	0	0
50	0	0	0	0	0	0
Mean	0	2.58	2.44	2.38	3.32	1.84

Ninth day of anther dehiscence in a spike

No of spike	Time					
	10-11am	11am-12pm	12-1pm	1-2pm	2-3pm	3-4 pm
1	0	7	6	6	7	6
2	0	0	0	0	0	0
3	0	5	4	5	6	4
4	0	0	0	0	0	0
5	0	6	5	5	7	4
6	0	5	4	3	6	4
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	5	4	3	6	3
11	0	0	0	0	0	0
12	0	6	5	4	7	4
13	0	5	4	4	6	2
14	0	0	0	0	0	0
15	0	3	4	3	5	3
16	0	0	0	0	0	0
17	0	0	0	0	0	0
18	0	5	5	4	7	4
19	0	0	0	0	0	0
20	0	6	5	5	7	4
21	0	7	6	4	8	3
22	0	0	0	0	0	0
23	0	7	5	6	8	4
24	0	0	0	0	0	0
25	0	0	0	0	0	0

26	0	6	5	4	7	3
27	0	0	0	0	0	0
28	0	7	5	5	8	3
29	0	4	3	3	5	3
30	0	0	0	0	0	0
31	0	4	4	3	6	4
32	0	0	0	0	0	0
33	0	5	5	4	6	4
34	0	6	5	6	7	3
35	0	0	0	0	0	0
36	0	6	4	3	7	3
37	0	0	0	0	0	0
38	0	0	0	0	0	0
39	0	0	0	0	0	0
40	0	4	5	4	6	3
41	0	0	0	0	0	0
42	0	6	5	4	7	4
43	0	0	0	0	0	0
44	0	0	0	0	0	0
45	0	6	5	5	7	3
46	0	5	4	4	6	3
47	0	6	5	4	8	4
48	0	7	5	4	8	4
49	0	0	0	0	0	0
50	0	0	0	0	0	0
Mean	0	2.78	2.34	2.11	3.36	1.78

APPENDIX X.

Anther dehiscence mode of *Piper nigrum*

Number of flowers	Anther dehiscence mode
1	Longitudinal
2	Longitudinal
3	Longitudinal
4	Longitudinal
5	Longitudinal
6	Longitudinal
7	Longitudinal
8	Longitudinal
9	Longitudinal
10	Longitudinal
11	Longitudinal
12	Longitudinal
13	Longitudinal
14	Longitudinal
15	Longitudinal
16	Longitudinal
17	Longitudinal
18	Longitudinal
19	Longitudinal
20	Longitudinal
21	Longitudinal
22	Longitudinal
23	Longitudinal
24	Longitudinal
25	Longitudinal
26	Longitudinal

27	Longitudinal
28	Longitudinal
29	Longitudinal
30	Longitudinal
31	Longitudinal
32	Longitudinal
33	Longitudinal
34	Longitudinal
35	Longitudinal
36	Longitudinal
37	Longitudinal
38	Longitudinal
39	Longitudinal
40	Longitudinal
41	Longitudinal
42	Longitudinal
43	Longitudinal
44	Longitudinal
45	Longitudinal
46	Longitudinal
47	Longitudinal
48	Longitudinal
49	Longitudinal
50	Longitudinal

APPENDIX XI

Stigma type in *Piper nigrum* L.

Sl. No.	**No. of lobes in stigma**	**Stigma type**
1	Four lobes	Wet and papillate
2	Four lobes	Wet and papillate
3	Four lobes	Wet and papillate
4	Four lobes	Wet and papillate
5	Four lobes	Wet and papillate
6	Four lobes	Wet and papillate
7	Four lobes	Wet and papillate
8	Four lobes	Wet and papillate
9	Four lobes	Wet and papillate
10	Four lobes	Wet and papillate
11	Four lobes	Wet and papillate
12	Four lobes	Wet and papillate
13	Four lobes	Wet and papillate
14	Four lobes	Wet and papillate
15	Four lobes	Wet and papillate
16	Four lobes	Wet and papillate
17	Four lobes	Wet and papillate
18	Four lobes	Wet and papillate
19	Four lobes	Wet and papillate
20	Four lobes	Wet and papillate
21	Four lobes	Wet and papillate
22	Four lobes	Wet and papillate
23	Four lobes	Wet and papillate
24	Four lobes	Wet and papillate
25	Four lobes	Wet and papillate

APPENDIX XII

Duration of pollen availability/plant of *Piper nigrum* L.

No of days	Month														
	April					May					June				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
2	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
3	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
4	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1
5	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1
6	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
7	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
8	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
9	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
10	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
11	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
12	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
13	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
14	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
19	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1
20	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1
21	1	1	1	0	1	0	0	0	0	0	1	1	1	1	1
22	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1
23	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1
24	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1

25	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	July					August					September				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0
2	0	1	1	1	1	0	1	0	1	0	1	1	0	1	0
3	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
21	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
22	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
23	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
24	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
25	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
26	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0
27	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0
28	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
29	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	October					November					December				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	1	0	0	1	1	1	1	0	1	0
2	0	0	0	0	1	1	0	1	1	1	1	1	0	1	0
3	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0
4	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
5	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

13	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
14	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
15	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
16	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
17	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
18	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
19	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
20	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
21	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
22	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
23	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	January					February					March				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
11	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
12	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0
13	1	1	1	1	1	0	1	0	1	1	0	0	0	0	0
14	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
16	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
17	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
18	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0
19	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1
20	0	0	0	0	0	1	1	1	0	1	0	1	1	0	1
21	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
22	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
23	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
24	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
25	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
26	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
27	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
28	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

APPENDIX XIII

Flower longevity of *Piper nigrum* L.

No. of flowers	No. of days
1	15
2	14
3	15
4	15
5	14
6	14
7	15
8	15
9	14
10	15
11	15
12	14
13	14
14	14
15	15
16	15
17	14
18	15
19	15
20	14
21	14
22	15
23	15
24	14
25	15
26	15

27	14
28	15
29	14
30	14
31	15
32	14
33	15
34	14
35	14
36	15
37	15
38	14
39	15
40	14
41	14
42	15
43	15
44	14
45	14
46	15
47	14
48	15
49	14
50	14

APPENDIX XIV

Flower emergence duration in *Piper nigrum* L.

No. of flowers	Number of days
1	19
2	20
3	20
4	19
5	19
6	20
7	19
8	20
9	19
10	20
11	19
12	20
13	20
14	19
15	20
16	20
17	19
18	20
19	20
20	19
21	20
22	20
23	19
24	19
25	20

26	19
27	20
28	19
29	20
30	19
31	20
32	20
33	19
34	19
35	20
36	19
37	20
38	19
39	20
40	19
41	20
42	19
43	20
44	20
45	19
46	20
47	19
48	20
49	20
50	19

APPENDIX XV

Anthesis period in plant of *Piper nigrum* L.

No	Month														
of	April					May					June				
days	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
2	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
3	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
4	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1
5	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1
6	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
7	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
8	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
9	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
10	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
11	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
12	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
13	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
14	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
19	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1
20	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1
21	1	1	1	0	1	0	0	0	0	0	1	1	1	1	1
22	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1
23	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1
24	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1

25	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	July					August					September				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0
2	0	1	1	1	1	0	1	0	1	0	1	1	0	1	0
3	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
21	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
22	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
23	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
24	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
25	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
26	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0
27	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0
28	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
29	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	October					November					December				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	1	0	0	1	1	1	1	0	1	0
2	0	0	0	0	1	1	0	1	1	1	1	1	0	1	0
3	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0
4	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
5	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

13	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
14	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
15	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
16	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
17	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
18	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
19	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
20	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
21	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
22	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
23	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	January					February					March				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
11	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
12	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0
13	1	1	1	1	1	0	1	0	1	1	0	0	0	0	0
14	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
16	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
17	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
18	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0
19	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1
20	0	0	0	0	0	1	1	1	0	1	0	1	1	0	1
21	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
22	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
23	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
24	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
25	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
26	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
27	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
28	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX XVI

Duration of spiking in the plant of *Piper nigrum* L.

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

27	26
28	25
29	26
30	27
31	25
32	30
33	28
34	30
35	26
36	27
37	30
38	29
39	26
40	25
41	28
42	25
43	30
44	24
45	23
46	25
47	26
48	28
49	26
50	30
Mean	**26.36**

APPENDIX XVII

Duration of spiking in plant population of *Piper nigrum* L.

No of days	Month														
	April					May					June				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
2	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
3	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
4	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
5	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1
29	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1
30	1	1	1	1	1	0	0	0	0	0	0	1	1	0	1

No of days	Month														
	July					August					September				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0
2	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0
3	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
4	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
26	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
27	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
28	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0
29	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
30	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	October					November					December				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0
2	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0
3	0	1	0	1	1	1	0	1	1	1	0	0	0	0	0
4	1	1	0	1	1	1	0	1	1	1	0	0	0	0	0
5	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
6	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
7	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0
8	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
20	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1
21	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1
22	1	0	1	1	1	0	1	0	0	0	1	1	1	1	1
23	1	0	1	0	1	0	0	0	0	0	1	1	1	1	1
24	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1
25	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1
26	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
27	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No of days	Month														
	January					February					March				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

8	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
9	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
10	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
11	0	1	0	0	0	0	0	0	0	0	1	1	1	1	1
12	1	1	0	1	0	0	0	0	0	0	1	1	1	1	1
13	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1
14	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1
15	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
20	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
21	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1
22	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1
23	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0
24	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
25	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
26	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0
27	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX VIII.

Wind pollination in *Piper nigrum* L.

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
FW1R1	**7**	**6**	**85.71**
FW1R2	7	6	85.71
FW1R3	7	5	71.42
FW1R4	7	6	85.71
FW1R5	7	5	71.42
FW1R6	7	5	71.42
FW1R7	7	7	100
FW1R8	7	4	57.1
FW1R9	7	6	85.71
FW1R10	7	3	42.8
FW1R11	7	7	100
FW1R12	7	6	85.71
FW1R13	7	7	100
FW1R14	7	4	42.8
FW1R15	7	7	100
FW1R16	7	5	71.2
FW1R17	7	4	42.8
FW1R18	7	6	85.71
FW1R19	7	7	100
FW1R20	7	7	100
FW1R21	7	6	85.71
FW1R22	7	5	71.2
FW1R23	7	4	42.8
FW1R24	7	5	71.2
FW1R25	7	6	85.71

FW2 Treatment

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
FW2R1	7	7	100
FW2R2	7	7	100
FW2R3	7	6	85.71
FW2R4	7	7	100
FW2R5	7	6	85.71
FW2R6	7	7	100
FW2R7	7	6	85.71
FW2R8	7	6	85.71
FW2R9	7	6	85.71
FW2R10	7	7	100
FW2R11	7	7	100
FW2R12	7	6	85.71
FW2R13	7	6	85.71
FW2R14	7	5	71.4
FW2R15	7	7	100
FW2R16	7	6	85.71
FW2R17	7	6	85.71
FW2R18	7	7	100
FW2R19	7	6	85.71
FW2R20	7	7	100
FW2R21	7	7	100
FW2R22	7	6	85.71
FW2R23	7	6	85.71
FW2R24	7	7	100
FW2R25	7	7	100

PW1 Treatment

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
PW1R1	**7**	**5**	**71.4**
PW1R2	7	4	57.1
PW1R3	7	5	71.4
PW1R4	7	4	57.1
PW1R5	7	5	71.4
PW1R6	7	3	42.8
PW1R7	7	5	71.4
PW1R8	7	4	57.1
PW1R9	7	3	42.8
PW1R10	7	4	57.1
PW1R11	7	5	71.4
PW1R12	7	5	57.1
PW1R13	7	3	42.8
PW1R14	7	5	57.1
PW1R15	**7**	**5**	**71.4**
PW1R16	7	3	42.8
PW1R17	7	5	71.4
PW1R18	7	3	42.8
PW1R19	7	4	57.1
PW1R20	7	4	57.1
PW1R21	7	5	71.4
PW1R22	7	3	42.8
PW1R23	7	5	71.4
PW1R24	7	5	71.4
PW1R25	7	4	57.1

PW2 Treatment

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
PW2R1	7	7	100
PW2R2	7	7	100
PW2R3	7	7	100
PW2R4	7	6	85.71
PW2R5	7	7	100
PW2R6	7	6	85.71
PW2R7	7	7	100
PW2R8	7	7	100
PW2R9	7	7	100
PW2R10	7	7	100
PW2R11	7	6	85.71
PW2R12	7	7	100
PW2R13	7	6	85.71
PW2R14	7	7	100
PW2R15	**7**	**7**	**100**
PW2R16	7	6	85.71
PW2R17	7	7	100
PW2R18	7	7	100
PW2R19	7	7	100
PW2R20	7	6	85.71
PW2R21	7	7	100
PW2R22	7	7	100
PW2R23	7	6	85.71
PW2R24	7	7	100
PW2R25	7	7	100

APPENDIX XIX.

Water pollination in *Piper nigrum* L.

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
FRI	75	71	94.6
FR2	82	76	92.6
FR3	65	59	90.7
FR4	66	61	92.4
FR5	70	66	94.2
FR6	54	49	90.7
FR7	89	85	95.5
FR8	76	72	94.7
FR9	85	81	95.2
FR10	66	60	90.9
FR11	67	61	91
FR12	77	72	93.5
FR13	57	52	91.2
FR14	83	78	93.9
FR15	**78**	**72**	92.3
FR16	71	65	91.5
FR17	57	53	92.9
FR18	68	63	92.6
FR19	65	59	90.7
FR20	88	84	95.4
FR21	75	69	92
FR22	58	53	91.3
FR23	55	51	92.7
FR24	61	57	93.4
FR25	73	68	93.1

PR1 Treatment

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
PR1R1	56	52	92.8
PR1R2	65	60	92.3
PR1R3	61	58	95.0
PR1R4	68	63	92.6
PR1R5	65	60	92.3
PR1R6	72	66	91.6
PR1R7	59	55	93.2
PR1R8	81	76	93.8
PR1R9	78	70	89.7
PR1R10	75	71	94.6
PR1R11	90	85	94.4
PR1R12	82	78	95.1
PR1R13	45	42	93.3
PR1R14	56	51	91.0
PR1R15	56	52	92.8
PR1R16	61	56	91.8
PR1R17	64	60	93.7
PR1R18	59	51	86.4
PR1R19	82	79	96.3
PR1R20	74	70	94.5
PR1R21	63	59	93.6
PR1R22	79	71	89.8
PR1R23	75	71	94.6
PR1R24	63	59	93.6
PR1R25	65	60	92.3

APPENDIX XX

Breeding system of *Piper nigrum* L.

Open pollination

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
OPR1	75	70	93.3
OPR2	66	60	92
OPR3	63	55	91
OPR4	72	65	92
OPR5	54	50	92.5
OPR6	87	73	83.9
OPR7	74	66	92.9
OPR8	74	71	90
OPR9	58	52	93
OPR10	60	56	93.3
OPR11	59	54	93
OPR12	68	60	93
OPR13	70	65	94
OPR14	71	68	95.7
OPR15	68	63	97
OPR16	78	73	93.5
OPR17	65	60	93.7
OPR18	58	55	94.8
OPR19	87	84	92
OPR20	84	80	92
OPR21	74	72	94
OPR22	89	85	96
OPR23	56	55	92
OPR24	79	74	93.6

OPR25	67	62	93

Autogamy (B2)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
AB2R1	3	3	100
AB2R2	3	3	100
AB2R3	3	3	100
AB2R4	3	2	66.6
AB2R5	3	3	100
AB2R6	3	3	100
AB2R7	3	2	66.6
AB2R8	3	3	100
AB2R9	3	2	66.6
AB2R10	3	3	100
AB2R11	3	3	100
AB2R12	3	3	100
AB2R13	3	3	100
AB2R14	3	3	100
AB2R15	3	3	100
AB2R16	3	2	66.6
AB2R17	3	3	100
AB2R18	3	3	100
AB2R19	3	3	100
AB2R20	3	3	100
AB2R21	3	3	100
AB2R22	3	2	66.6
AB2R23	3	3	100
AB2R24	3	3	100
AB2R25	3	3	100

Autogamy (B3)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
AB3R1	1	1	100
AB3R2	1	1	100
AB3R3	1	1	100
AB3R4	1	1	100
AB3R5	1	1	100
AB3R6	1	1	100
AB3R7	1	0	0
AB3R8	1	1	100
AB3R9	1	1	100
AB3R10	1	0	0
AB3R11	1	1	100
AB3R12	1	1	100
AB3R13	1	1	100
AB3R14	1	1	100
AB3R15	1	1	100
AB3R16	1	1	100
AB3R17	1	0	0
AB3R18	1	1	100
AB3R19	1	1	100
AB3R20	1	1	100
AB3R21	1	1	100
AB3R22	1	1	100
AB3R23	1	1	100
AB3R24	1	1	100
AB3R25	1	1	100

Geitonogamy (B4)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
B4R1	6	6	100
B4R2	6	5	83.3
B4R3	6	6	100
B4R4	6	5	83.3
B4R5	6	6	100
B4R6	6	5	83.3
B4R7	6	6	100
B4R8	6	5	83.3
B4R9	6	6	100
B4R10	6	5	83.3
B4R11	6	6	100
B4R12	6	6	100
B4R13	6	5	83.3
B4R14	6	6	100
B4R15	6	6	100
B4R16	6	5	83.3
B4R17	6	6	100
B4R18	6	5	83.3
B4R19	6	6	100
B4R20	6	6	100
B4R21	6	5	83.3
B4R22	6	6	100
B4R23	6	6	100
B4R24	6	5	83.3
B4R25	6	6	100

Geitonogamy(B5)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
GB5R1	10	9	90
GB2	10	10	100
GB3	10	9	90
GB4	10	10	100
GB5	10	9	90
GB6	10	10	100
GB7	10	9	90
GB8	10	10	100
GB9	10	9	90
GB10	10	10	100
GB11	10	10	100
GB12	10	9	90
GB13	10	10	100
GB14	10	10	100
GB15	**10**	**9**	90
GB16	10	10	100
GB17	10	9	90
GB18	10	10	100
GB19	10	9	90
GB20	10	9	90
GB21	10	10	100
GB22	10	10	100
GB23	10	9	90
GB24	10	9	90
GB25	10	10	100

Getinogamy (B6)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
B6R1	6	6	100
B6R2	6	5	83.3
B6R3	6	6	100
B6R4	6	6	100
B6R5	6	6	100
B6R6	6	5	83.3
B6R7	6	6	100
B6R8	6	5	83.3
B6R9	6	5	83.3
B6R10	6	6	100
B6R11	6	5	83.3
B6R12	6	6	100
B6R13	6	6	100
B6R14	6	5	83.3
B6R15	6	6	100
B6R16	6	5	83.3
B6R17	6	6	100
B6R18	6	6	100
B6R19	6	5	83.3
B6R20	6	6	100
B6R21	6	5	83.3
B6R22	6	6	100
B6R23	6	5	83.3
B6R24	6	6	100
B6R25	6	5	83.3

Xenogamy (B7)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
B7R1	6	5	83.3
B7R2	6	5	83.3
B7R3	6	4	66.6
B7R4	6	5	83.3
B7R5	6	4	66.6
B7R6	6	6	100
B7R7	6	4	66.6
B7R8	6	5	83.3
B7R9	6	6	100
B7R10	6	5	83.3
B7R11	6	4	66.6
B7R12	6	5	83.3
B7R13	6	6	100
B7R14	6	5	83.3
B7R15	6	6	100
B7R16	6	5	83.3
B7R17	6	6	100
B7R18	6	6	100
B7R19	6	4	66.6
B7R20	6	5	83.3
B7R21	6	6	100
B7R22	6	5	83.3
B7R23	6	4	66.6
B7R24	6	6	100
B7R25	6	4	66.6

Xenogamy (B8)

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
B8R1	7	6	85.71
B8R2	7	5	71.42
B8R3	7	6	85.71
B8R4	7	7	100
B8R5	7	5	71.42
B8R6	7	5	71.42
B8R7	7	5	71.42
B8R8	7	7	100
B8R9	7	6	85.71
B8R10	7	5	71.42
B8R11	7	6	85.71
B8R12	7	7	100
B8R13	7	5	71.42
B8R14	7	6	85.71
B8R15	7	7	100
B8R16	**7**	**5**	71.42
B8R17	7	6	85.71
B8R18	7	6	85.71
B8R19	7	7	100
B8R20	7	5	71.42
B8R21	7	6	85.71
B8R22	7	6	85.71
B8R23	7	5	71.42
B8R24	7	6	85.71
B8R25	7	5	71.42

Control B10

Number of spikes	Number of flowers	Number of fruit set	Fruit set percentage
B10R1	56	50	89.2
B10R2	65	58	88.5
B10R3	61	54	88.4
B10R4	69	61	89.2
B10R5	65	58	88.9
B10R6	72	64	89.8
B10R7	59	53	89.3
B10R8	84	75	89.7
B10R9	78	70	89.3
B10R10	75	67	89.4
B10R11	85	76	89.2
B10R12	82	73	88.9
B10R13	45	40	89.3
B10R14	56	50	89.4
B10R15	66	59	87.5
B10R16	61	53	89.1
B10R17	64	56	89.4
B10R18	57	51	88.6
B10R19	79	70	90.5
B10R20	74	67	88.5
B10R21	61	54	89.6
B10R22	77	69	89.5
B10R23	78	68	89
B10R24	63	55	89.2