AGENTS OF POLLINATION ON FRUIT SET OF *PIPER NIGRUM* L.

Abstract

The role of agents on pollination and fruit set of *Piper nigrum* L. was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvanthapuram during 2017-2019. The role of wind, gravity, rain and dew were investigated and the data was analyzed on twenty-five inflorescences of field grown black pepper plants and twenty-five inflorescences of pot grown bush pepper plants. The fruit set was superior due to the influence of wind and gravity (95.99%) in pot grown bush pepper and it was 91.98 per cent in field grown black pepper vines. The percentage of fruit set due to wind in field grown black pepper and potted bush pepper was 77.67% and 59.39 % respectively. The role of rain water in the pollination also revealed higher fruit set of 92.76% under natural conditions. Insects which visited black pepper spike were foraging nectar and common visitors were yellow crazy ant, black garden ant and pollu beetle with visit rates of 1.79, 1.41 and 0.45 respectively. The small size of pollen with high pollen availability makes it suitable to be carried away by wind. The pendulous nature of the spike, the shedding of pollen, the pollen carried by the rainwater or dew down the spike can all contributed to pollination. Integration of more factors resulted in more fruit set. Characteristics of black pepper flowers studied revealed their adaptation for wind pollination and the pendulous nature of the spike makes it efficient to carry the pollen due to gravity as well as rain and dew for pollination. The role of insects in pollination in black pepper was precluded.

Key words: Black pepper, *Piper nigrum* L. Pollination, Agents of pollination, Fruit set

1. Introduction

Black pepper (*Piper nigrum* L.) one among the distinguished spices from the family Piperaceae is rightly called the 'King of spices' in view of the legendary position it held in the international trade from centuries ago. 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). Jaramillo and Manos (2001) observed that the greatest diversity of *Piper* species occurs in the American tropics (700 spp.), followed by

Southern Asia (300 spp). In India, so far, over 100 species of *Piper* have been reported, concentrating their distribution in Western Ghats and Eastern Himalaya with about 65 species in north eastern states. According to Abril *et al.* (2006) *Piper* represents two large species components with distributions centred in the Amazonian and Andean region.

A fully developed berry in the spike contributes to the economic yield of black pepper. The poor fruit set in the spike which is a commonly noticed in black pepper plants is often due to the lack of pollination. The pollination in the plants of different species of the same genus may differ. The use of different pollination and dispersal agents in plants could be the result of habitat diversification. The selective pressure due to lower number of pollinators resulted in the evolution of self-fertilization in some plants (Levin 1972, Solbrig and Rollins 1977, Inoue *et al*., 1995). However self pollination and self fertility of the black pepper is found to be an adaptation to cultivation.

Pollination in *Piper nigrum* was attributed to various pollinating agents. Barber (1906) and Anandan (1924) carried out artificial splashing of water in black pepper, later reported that rain drops help in scattering pollen grains in different directions, either wash down the pollen grains to lower spikes or carry them to neighboring vines. 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 Venkateswran 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. Moreover, studies conducted till now could not give a conclusive report on the agents involved in the pollination of black pepper through the role of rain water, wind, insect and dew. Keeping this in view, the present study was undertaken to understand the influence of non pollinator or pollinating agent in the fruit set in black pepper.

2. Material and Methods

The experiment was carried out at the Instructional Farm located at 8.5°N latitude and 76.9ºE longitude and at an altitude of 29m above MSL, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala in field grown black pepper and bush pepper maintained in pots. The pollination study was carried out in Panniyur 1 variety between march 2018 to march 2019.

Twenty five spikes of 10-12 year old field grown black pepper plants and two year old bush pepper plants were taken as study material to understand the role of wind, insect, rain water and dew in pollination of black pepper.

Wind and gravity as pollinating agent

To understand the role of wind, inflorescences of field grown and bush pepper were selected and six treatments were imposed.

In the first treatment (FW_1) single inflorescence in the field grown black pepper plants was selected and few fully developed flowers were retained and remaining part of spike was removed and covered by nylon net to understand the role of wind (Plate 1). After flower opening, emasculation was done and covered by nylon net, so that pollination of stigma would happen only by pollens brought by wind from outside.

In the second treatment, (FW_2) three to five inflorescences were selected and covered by nylon net to understand the role of both wind and gravity. The inflorescence of lower spike was emasculated and bagged (Plate 2). The fruit set on the lower spike would be due to the pollen from the above spikes and/or those brought by the wind.

Plate 1 –Treatment (FW_1)

Plate 2 - Treatment (FW_2)

The third treatment (FC2) included twenty-five inflorescences in the field grown pepper plants. A few fully developed flowers before the start of anther emergence were marked and the remaining portion of the spike was removed and covered by polythene. The stamens were emasculated and covered by polythene to avoid wind, insects and rain. The fruits if formed can thus be due to apomixes.

The fourth treatment (PW_1) consisted of twenty-five inflorescences of bush pepper plants in pots kept under polyhouse. The inflorescences were emasculated on the selected inflorescences and single inflorescence was covered by polythene cover. The cover was removed and artificially air was passed by using electrical fan for 10 minutes at one hour interval from the stigma receptivity period.

The fifth treatment (PW_2) consisted of three to five inflorescences of bush pepper covered by polythene. The stamens were removed from the lower spike and covered by polythene. The fruit set from this treatment represented the effect of gravity on pollination.

The sixth treatment consisted of (PC_2) selected twenty-five inflorescences of pot grown bush pepper plants. The stamens were removed and covered by polythene to avoid entry wind, insects and rain.

The fruit set of all the six treatments were checked between 20 -25 days after flowering and percentage of fruit set was worked out and used as an index of successful pollination.

The characteristic of the pollen of normal black pepper grown in the field was compared with the characteristics of wind pollinated pollen. Fresh petriplates covered with vaseline was placed immediately below and between spike at 1.5m and 2 m between pepper plants kept in polyhouse (Plate 3 and 4) and at 4.5m between the plants in the field within a plant population (Plate 5). The slides were taken after anther dehiscence, stained and checked under optical microscope for the presence of pollen grains.

Plate 3 - 4.5 m between the plants in the field grown black pepper

Plate 4 - 1.5 m between plants in the in polyhouse (wind pollination)

Plate 5 - 2.0 m between the plants in polyhouse(wind pollination)

Insect as pollinating agent

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

The number of visits per inflorescence per two hours during a period of 24 hours was recorded and visitation rates were found out (Lobo *et al.,* 2016). The behaviour of insect species was monitored by field observations and the presence of pollen on their body parts was checked after the insects were anaesthetized using a stereoscopic microscope. The foraging behaviour of insects was recorded and captured using an insect net and was identified with the help of entomologists. The number of insects visiting the flower was recorded.

Rain water as pollinating agent

The role of rain water in pollination was observed in twenty-five inflorescences both from field grown and bush pepper plants. Four treatments were imposed. The first treatment (FR_1), inflorescence was covered by polythene and at the time of natural raining the inflorescence was exposed and after the rain it was covered again.

In the second treatment (FC_2), the inflorescence was kept with only a few fully developed flowers and the remaining portion was removed and the flowers were emasculated and covered by polythene to exclude wind, insects and rain as control. In the third treatment (**PR_1**) the rain water was imitated as splashing on twenty-five inflorescences of bush pepper as a whole at time of anthesis and followed by covering the whole plant to exclude wind and insects. These bush pepper plants were kept in poly house and only basin irrigation was given. The fourth treatment (PC_2) consisted of twenty-five inflorescences of pot grown bush pepper where in only few developed flowers were kept and the remaining portion of the spike was removed. These flowers were emasculated and covered by polythene to exclude wind, insects and rain and were 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. The rain water was also collected from the inflorescence and checked for the presence pollen grain.

Dew as pollinating agent

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 on the spike. The dew accumulated was collected and stained and observed for the presence of pollen.

Results and discussion

Role of wind, gravity and rain as a pollinating agent

The data on wind, gravity and rain water on pollination experiment on twenty five inflorescences of field grown black pepper and twenty five inflorescences of pot grown bush pepper plants is presented in Table 1. Among wind pollinated treatments, FW_2 were on par with PW_2 and were significantly superior compared to other treatments. FW_2 and PW_2 represented combination of gravity and wind pollination. The percentage of fruit set in treatments FW_1 and PW_1 were 77.67 and 59.39 per cent respectively which represented wind pollination alone. In this treatment whatever fruit set occurred might have been brought by the forced wind. The lower fruit set in PW_1 might be due to manual fanning within a restricted time. However, no fruit set were observed in control treatments, *i.e.*, FC_2 and PC_2 which contained single inflorescence emasculated and covered by polythene showing the absence of apomixes. Petri plates kept below the spikes of bush pepper showed the presence of pollen (Plate 6a, 6b, 6c). More over the petri plates kept at 0.75m and 1m from potted bush pepper kept at 1.5m and 2m distance in polyhouse also revealed the presence of pollen. Similarly, petri plates kept at 2.25m in between black pepper plants grown in field at a spacing of 4.5m also contained pollen grains suggesting that pollen grains are carried by wind and are moving.

Table 1. Effect of wind pollination on fruit set of *Piper nigrum* L.

S. No	Treatment	Treatment Means
1	FW1	77.674^{b}
2	FW2	91.997^{a}
3	FC2	0.000^{d}
4	PW1	59.388^{c}
5	PW2	95.999^{a}
6	PC2	0.000^{d}
	CV	**11.934**
	CD (0.05)	**5.667**

Plate 6. Presence of pollen grains in petri plates kept between the plants at different distance representing pollen carried by wind

Plate 6a – Petri plate at 4.5 m between plants in field

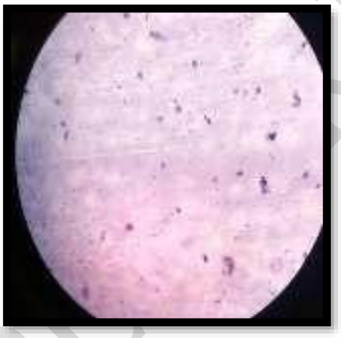

Plate 6b - 1.5 m between plants in the polyhouse

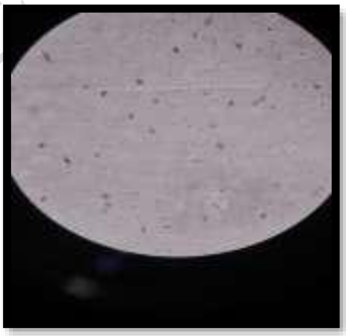

Plate 6c - 2.0 m between plants in the polyhouse

White head (1983) and Honig *et al*., (1992) had given the characteristics of wind pollinated pollen. De Waard and Zevcn (1969) stated that positive geotropism, spatial arrangement of the flowers, sequential ripening of the stigmas and nonchronological dehisence of anthers stimulate geitonogamic fertilization. Wind may be aiding the pollen dispersal by brief mechanical agitation of the spikes/branches. The present study supported the influence of both wind and gravity in pollination.

The characteristics of wind pollinated pollen and that of normal 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 (Table 2).

Table 2. Characteristics of wind pollinated pollen

Characteristics	Wind pollinated pollen	Black pepper pollen
Pollen size	Normally ranges from 10-80 µm	9-10.1 10 .14 µm
Reduced perianth (Whitehead, 1983)	To expose both anthers and stigmas to wind	Perianth absent and stigma is exposed and anthers exert out of bract.
Exserted anthers (Whitehead, 1983)	To carry the pollen by wind	Presence of exerted anthers (Ravindran, 2006)
Pendulous, catkin like inflorescence (Whitehead, 1983)	Present	Pendulous inflorescence
Protogynous flowers (Honig*et al*., 1992)	Present	Present

The data on mean percentage of fruit set due to rain water is presented in Table 4. Treatment, FR_1 were on par with PR_1 and were significantly superior compared to other treatments. FR_1, 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. PR_1, was the treatment where whole plant was covered by polythene cover and rain water was imitated as splashing on 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, FC_2 and PC_2 (Table 3). Sasikumar *et al*. (1992) evaluated three cultivars of black pepper for breeding behavior and revealed high potency of autogamy and water was found not essential for pollination. However, berry set under water-free condition was less than under open pollination. Cultivars differed in their breeding behaviour under water-free pollination.

Table 3. Effect of rain water on fruit set of *Piper nigrum* L.

S. No.	Treatment	Treatment mean
1	**FR1**	**92.760**^{a}
2	FC2	0.000^{b}
3	PR1	92.904^{a}
4	PC2	0.000^{b}
	F test	**S**
	CV	**3.097**
	CD (0.05)	**0.807**

Insect on pollination

Twenty five inflorescences in the field and twenty five inflorescences of bush pepper grown in the pot observed from the anthesis period revealed that three different floral visitors frequently visited the black pepper spike. The floral visitors and their foraging behaviour is presented in Table 4. The behavior of ant species was monitored by field observations and the body parts like mouth, abdomen and legs were checked for pollen by dissecting under stereoscopic microscope and no parts of pollen was observed in the mouth and abdomen. Hence the role of insects in pollination of black pepper was ruled out.However, collection of nectar was noticed by yellow crazy ant and black garden ant while visiting the flowers during days and night. Some of the Central American *Piper* species were pollinated mainly by stingless bees of the genus *Trigona*. Hoverflies and bees visited Piperaceae flowers for pollen. Moving along the inflorescence, flies touch the open anthers with the labell 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. Thomisidae spiders prey upon bees and flies visiting the inflorescences of *Piper crassinervium* and *P. regnelli* (Figueiredo and Sazima, 2000).

Table 4. Pollinators and their foraging behaviours in *Piper nigrum* L.

S. No	Common name	Scientific Name	Family	Visiting time	Foraging nature
1	Black garden 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	

The visitation rates of three different floral visitors visiting the black pepper spike were yellow crazy 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. Figueiredo and Sazima (2000) conducted a study in14 piperaceae species at two sites covered by semi deciduous forest in South Eastern Brazil. Of these, seven species of *Piper* were wind pollinated and three, *Piper amalago, P. crassinervium* and *P. glabratum* were exclusively insect-pollinated.

Dew in pollination

Presence of dew was observed from June to December 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. 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 De Waard and Zeven (1969) also. The contribution of dew in pollination is also confirmed though it cannot affect in large percentage.

The different type of *Piper nigrum* or different species of *Piper* climbing a single tree can result in outcrossing resulting in hybrid seedlings. Progenies of such chance seedlings grow and climbing up the nearby or same tree resulting in back crossing with the parent, clone or other seedling progenies might have resulted in variation within population. These forces acting together might have resulted in the evolution of present-day cultivars of black pepper (Ravindran *et al.*, 1990). The views of the above researchers are supported by the present experiment which showed the presence of pollen of black pepper even at 2.25 m away from the nearby black pepper plant. Thus, even black pepper plants in a black pepper population have the chance of getting pollinated by the nearby pollen from the black pepper plant. Since seedling progenies are not common in a cultivated plantation, the chances of formation of variable cultivars are thus restricted under cultivation but may be present in forest or wild or unattended black pepper gardens resulting in new cultivars.

Conclusion

Contribution of gravity, rain and wind make pollination efficient in black pepper. Even in the absence of one factor percentage of fruit set gets reduced. The combined effect of gravity and wind has resulted in higher fruit set in the experiment compared to individual factors. The role of wind and rain is equally effective in pollination. The effect of gravity, wind and rain altogether can make pollination more effective and efficient leading to higher percentage of fruit set. The movement of dew can also result in pollination provided sufficient movement of dew take place in the spike. However the role of insects in pollination of black pepper was ruled out. The flowers of pipers are small, aggregated in inflorescences called spikes. Flowers are mostly hermaphrodite with no perianth, 4 anthers, 1 stigma, and numerous pollens. These morphological features comply more with the anemophilous syndrome. The pendulous nature of spike and the shedding of pollen after stigma receptivity helps in falling of pollen from above spikes and production of numerous pollen all together help in more pollen dispersal in the nearby vicinity. The pollen carried by the rain water also increased percentage of fruitset.

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