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Floral boilogy of large cardamom (*Amomum subulatum*)

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ABSTRACT

A study on floral phenology, floral visitors, foraging nature of floral visitors, nectar production, pollination efficiency and stigma receptivity of large cadamom (*Amomum subulatum* Roxb.) of the family Zingiberaceae conducted during 2009–10. Anthesis in large cardamom took place in the early morning (around 5:30 am) and flowers offer plenty of anthers and moderate amount of nectar to floral visitors. There were four floral visitors; bumble bee (*Apis braviceps* Smith), honey bee (*Apis cerena*), fruit fly (*Bactocera* sp.) and moth (*Udaspes folus*), and among them *A. braviceps* was found to be the major pollinator due to its high pollination efficiency attributed to its big body size and foraging habit, while *A. cerena* acts as a pollen robber. Each flower receives 45.92 visits of bumble bee with the foraging time of 118.93 seconds in a day. Pollination efficiency of bumble bee was as high as 100% while honey bee could pollinate only 8.41% of flower. Bumble bee delivers pollens in the receptive cup pf stigma, in contrary honey on the non-receptive hairs surrounding stigma cup. Nectar secretion begins at 9 hr on the day anthesis and reaches to its peak at 14 hr, followed by reduction in nectar production. A temperature range of 25–28°C and air humidity of 50–60% favoured high nectar production. Increase in 0.77 unit of temperature and decrease in 2.04 unit of air humidity resulted in 1 unit increase in nectar content. The visitation frequency and foraging time of bumble bee were not correlated with the nectar production. Bumble bee and honey bee had almost equal pollen load, but pollen carrying mechanism was quite different as honey bee carries pollen in pollen basket and bumble bee on its head and thorax. Stigma became receptive 12 hr before anthesis (protogynous) and remains receptive even after 15 hr of flower senescence. The best time of pollination was between 6 and 9 hr on the day of anthesis.

Key words: Anthesis, Bumble bee, Nectar, Pollen robber, Pollination efficiency

Large cardamom (*Amomum subulatum* Roxb.), belonging to family Zingiberaceae, is the most important spice crop of Sikkim Himalayan region. It is cultivated between altitudes of 600–2000m in the tropical wet evergreen forest. It is considered to be the native of Sikkim as many wild species; *A. aromaticum*, *A. linguiforme*, *A. costatum*, *A. kingii*, *A. corynostachyum*, *A. dealbatum* and *A. plauciflorum*, and cultivars such as Ramsey, Sawney, Varlangey, Ramla, Saremna, Golsey, etc. have been grown since ages. *A. subulatum* is a shade-loving plant usually grows under tree canopy and requires an annual rainfall of 1 500–3 500 mm distributed for 7–8 months (Sharma *et al*. 2009). *A. subulatum* is a perennial herb attaining the height up to 2.0m and gives rise to shoot and short stalked spike (inflorescence) which bears flowers. Each clump produces about 15–40 spike depending on cultivars and plant age and each spike bears 35–55 flowers. Flowers are arranged acropatally and young flowers present at the centre of the spike. Yellowish flowers contain a conspicuous labellum (modified corolla), which acts as a platform for floral visitors. Solitary stamen and pistil originate from corolla tube which is attached with the ovary. Large cardamom is bisexual and allogamous due to the position of pistil with respect to stamen. The cup shaped stigma, having receptive inner wall surrounded by non-receptive hairs, placed above anther of flower (Sinu and Shivanna 2007a). In large cardamom capsule (fruit) and seeds are the productive traits. Seeds of large cardamom are used as condiment, mouth freshener and also possess medicinal properties. The flowering is initiated in mid of March and extends up to mid of May with the peak period of last week of March to April, however, flowering varies with altitude and cultivars. Flowers offer both pollen and nectar to floral visitors. Effective pollination is a major factor for sustaining yield of economically important crop species. Among many factors, pollination diversity, population density, visitation frequency, and quality and quantity of pollen that carries to stigma are major biotic factors affecting pollination and fruit set (Sinu and Shivanna 2007b) The continuous recession in yield of large cardamom, deserves more attention

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towards behaviour of floral visitors in affecting pollination and in ensuring fruit set. Limited work has been done on the floral biology and pollination biology of large cardamom; further studies are essentially required to understand the behaviour of pollinator, pollination efficiency and biological parameters associated with pollination and fruit set. The focus of our study was to document floral visitors, their foraging habit, pollen load, pollination efficiency, nectar production and stigma receptivity of large cardamom.

MATERIALS AND METHODS

The site is located at ICAR Sikkim Centre, Tadong, Gangtok (East Sikkim) Research farm is at an altitude of 4000' between the latitude of 27° 20' N and longitude of 88° 4' E. The mean monthly maximum temperature at the site ranges from 15.6 °C to 26.9 °C, the mean monthly minimum temperature from 7.6 °C to 20.3 °C, rainfall from 2 652 to 3 052 mm/annum and the average relative humidity during rainy season and winter season was 89.4 and 41.5% respectively. Investigations were carried out during March – April in 2009 and 2010 on Varlangey cultivar grown under the partial shade of *Alnus nepalensis.* For studying floral phenology (anthesis, pollen dehiscence and flower senescence), oldest flower buds (that would open in the next morning) were bagged in the evening and observed from 5 hr the next morning till the senescence of flowers. Quantitative studies and mode of foraging of floral visitors were carried out by selecting 24–30 flowers from 4–5 clumps, which were clearly visible from an observation site. The frequency of visits of floral visitors, foraging period and foraging habit were observed from 6 hr (soon after anthesis) until 17 hr (when flower ceases to attract any visitors) for 10 days (five days in March and five days in April), thus total monitoring period was about 100 hr in each year. Pollen load was measured by trapping pollen loaded visitors in polybags containing ethyl acetate. Pollens were suspended in ethyl acetate and trapped bees were let freed. The ethyl acetate containing pollen were centrifuged (RM-12C Micro Centrifuse) for three minutes at 8000 rpm, supernatant was removed and pellet (pollen) was weighed in electronic balance. The nectar content was measured by bagging 100 oldest buds (that would open in the next morning) in the evening to prevent floral visitors. Ten flowers were excised by leaving corolla/nectar tube intact, to measure nectar content in nectar tube. The nectar content was measured at hourly interval starting from 6 to 17 hr by micropipette (Ependorf 1-100ìl) and sugar content of nectar was estimated by hand refractometer (ERMA, 28-65 °Brix). The studies were replicated ten times in 2009 and 2010.

To assess pollination efficiency of floral visitors, flowers were divided into three groups, viz. bumble bee visiting flowers, honey bee visiting flowers and open-pollinated flowers. Further bee visiting flowers (bumble bee and honey bee) were divided into five subgroups on the basis of frequency of bee visit (1–5). The frequency of bee visits was monitored by allowing required number of bees (1–5) to pollinate flowers. The oldest buds (N=200) were bagged in the evening and observations on pollination efficiency were taken next day. The study was replicated ten times. Under each sub-groups 20 flowers were subjected under study and pistils were examined for the presence of pollen grain on stigma by magnifying glass (X10) and under stereo-zoom microscope with photographic attachment (Leica). Under open field condition, stigmas were examined for the presence of pollen at 16 hr. The pollination efficiency was assessed on the basis of number of pollinated stigma. In order to work out stigma receptivity emasculated flowers (N= 200) were pollinated by hand, day before anthesis, day of anthesis at three hour interval (6,9,12,15,18 hr) and next day at three hour interval (6 ,9, 12 hr), and on the basis of ovary swelling after three days of pollination stigma receptivity was worked out. Data were

subjected to analysis of variance (ANOVA) and Spearman rank correlations (r) using SPSS statistical package (11.1 version) with level of significance at P=0.05.

RESULTS AND DISCUSSION

The anthesis took place early in the morning (5–6 hr) and anther dehisces longitudinally just before anthesis, and heavy pollen mass is visible along the two dehiscing sutures in freshly opened flower. The flowers last only for a day and senescence starts around 18 hr and yellowish colour of corolla fades next day. Sinu and Shivanna (2007a) reported anthesis between 5–6 hr, flower senescence in around 14 hr and flower fading by 18 hr at an altitude of around 5000'. Investigation on floral phenology clearly implied that flower senescence and fading were varied with altitude while anthesis was unaffected. There were four floral visitors (Fig 1a–c); bumble bee (*Bombus braviceps* Smith) honey bee (*Apis cerena*), fruit fly (*Bactocera* sp.) and moth (*Udaspes folus*), and among them bumble bee and honey bee were major visitors.

It was observed that bumble bee started pollinating the flowers early in the morning and continued up to 17 hr, and its frequency of visit increased with the advancement of day and had reached to peak between 10–11hr (Fig 2). The frequency of visits of bumble bee (one way ANOVA 3, 41 =

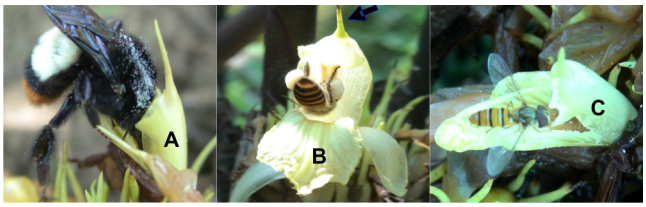

Fig 1 (*A*) Bumble bee (*Bombus braviceps*) pollinating large cardamom flower (*B*) honey bee (*Apis cerena*) collecting pollen in pollen basket, (*C*) *Bactocera* sp.

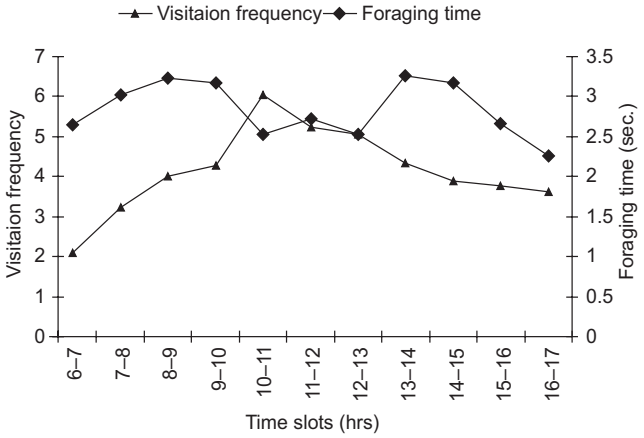

Fig 2 Visitation frequency and foraging time of bumble bee with respect to time slots

0.684) and honey bee varied significantly with time slots. The foraging time of bumble varied 2.27–3.26 sec./flower/visit. It was observed that each flower received 45.92 bees before senescence and total foraging time was 118.93 sec. Interestingly, the foraging nature of bumble bee showed sigmoid curve with periodical increase and diseases. Sinu and Shivanna (2007a) reported another species of bumble bee, *Bombus haemorrhoidalis* as floral visitor and observed that bumble bee seldom revisit the same flower on the same day, but in our studies we observed the bee revisits the flower and clump on the same day. Honey bee (*Apis cerena*) also visited flowers during morning hours but population density was relatively less. It was observed that the frequency of honey bee visit was more during morning (6–10 hr) and gradually reduced over time and no honey bee was observed after 13 hr (Fig 3). It was observed that each flower received 6.96 honey bees a day with the foraging time of 21.4 sec. The foraging time was relatively more between 6–7 hr followed by a decreasing trend (one way ANOVA, 3, 41 = 0.453). It was observed that honey bee had more foraging time in early morning than that of bumble bee, as former was engaged in collecting pollen in its pollen basket.

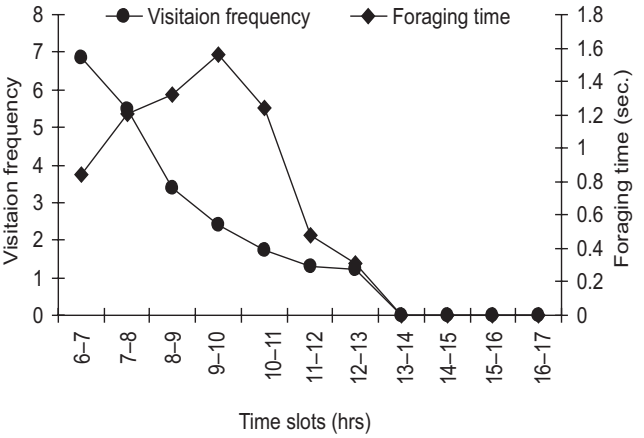

Fig 3 Visitation frequency and foraging time of honey bee with respect to time slots

The mechanism of pollination of both the bees was studied and it was observed that body size and proboscis are important factors for affecting pollination in large cardamom. Bumble bee visits flowers in search of nectar and the presence of long proboscis helps it to suck nectar from nectar tube. In search of nectar, bumble bee lands on the labellum of flower and it had to push the anther-stigma column upward to move deep into the flower and collect nectar from nectar tube through long proboscis. During this process the hairy head and thoracic portion of body come in contact with dehisced anther and pollens are adhered with. Due to the movement of bee on flower pollen laden body part come in contact with cup-shaped hairy stigma and eventually pollination is brought about. The foraging habit of *A. cerena* was completely different than that of bumble bee. Unlike bumble bee, honey bees collect pollen only and most of the pollen is collected in their pollen baskets. It was observed that honey bees don't try to collect nectar from nectar tube. The landing pattern of honey bee was just opposite than that of bumble bee as former lands directly on the dehisced anther. While collecting pollen the chance of getting stigma pollinated by honey bee is remote. Thus the foraging habit of honey bees does not make them efficient pollinators.

The investigation showed that bumble bee is the sole pollinator and honey bee as the pollen robber. The present study has shown that nectar played the most important role in effective pollination as bumble bee visits flower in search of nectar only. Social bee like *A. cerena* is effective pollinator in many plant species; however, the unique floral architecture of large cardamom marginalized it to pollen robber due to its short body size, proboscis and indifferent foraging habit. Sinu and Shivanna (2007b) reported *Apis dorsata, A. cerena* and *T. iridipennis* as pollinator in cardamom (*Elettaria cardamomum*) and observed that the first two harvest pollen as well as nectar while later harvests pollen only. Moreover, Belvadi *et al.* (1997) reported that length of proboscis is the

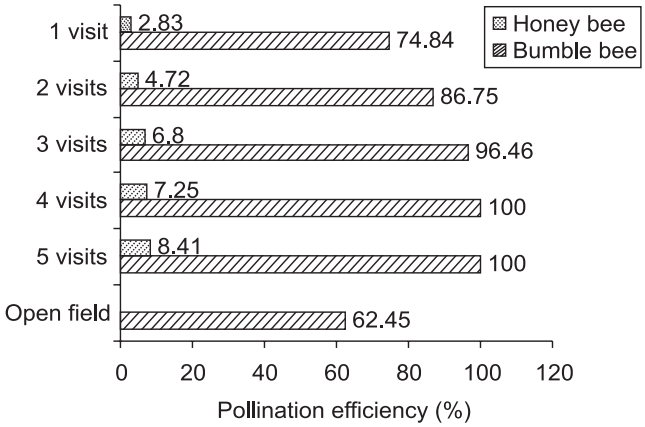

Fig 4 Pollination efficiency of bumble bee and honey bee

most important factor to harvest nectar and in affecting pollination in cardamom. Pollination efficiency of bumble bee and honey bee was determined by observing stigma of bee visited flowers. One visit of bumble bee resulted in 74.84% of pollination and increased with the frequency of visit and all flowers got pollinated with four visits, while honey bee affected only 8.41% of pollination even after five visits (Fig 4). Under open field condition the pollination was recorded to be 65.5%, the high percentage of pollination under field condition indicates visits of bumble bees to majority of flowers. The high pollination efficiency of large cardamom under field condition is attributed to adequate pollination services provided by the native bumble bee without intervention of social bees. Kuriakose *et al*. (2009) reported high pollination efficiency of wild cardamom under field condition due to the adequate pollination services of native wild bees; *Megachile* sp. and *Amegilla* sp.

Pollen load is an important indicator to brought about pollination. Pollen load of bumble bee (10.48 mg) and honey bee (9.72 mg) was not significantly different, but there was a big difference in the pollination efficiency. It is obvious from the result that though honey bee had high pollen load, it is a poor pollinator and flowers are well adapted for bumble bee pollination. Although most of these flowers had received multiple visits of honey bee; and they invariably had heavy pollen load, pollination efficiency was very low. Due to the foraging habit, *A. cerena* could able to deliver pollens on the non-receptive stigma hairs present on the margin of stigma cup, while in bumble bee pollinated flowers, pollen were trapped in the receptive inner wall of stigma cup. It may be concluded that spatial arrangement of pollens on stigma is more important aspect of pollination as the pollens trapped on non-receptive stigma hairs have very less chance to germinate. On the other hand, pollens trapped in receptive cup of stigma get better chance for germination due to availability of optimum germination medium most likely sucrose inside the receptive cup. In many of the studies, it has been reported that i*n vitro* pollen germination requires sucrose, boron and calcium and concentration thereof varies with plant species (Bhattacharya and Mandal 2009). After being successfully placed on the stigma cup the inner wall (intine) of pollen undergoes hydration and after hydration pollen wall proteins are released on to stigmatic surface which acts as a receptor for protein. Thus pollen grains are recognized and begin to germinate on the stigmatic surface in the presence of germination medium (Bhattacharya and Mandal 2009).

The nectar is accessible to those visitors having long proboscis as it is present in the nectar tube of about 3 cm long. Flowers produce hardly any nectar before 9 hr and the accumulation of nectar starts from 9 hr and gradually increases and reaches to the maximum (13.5 µl/flower) at 14 hr (Fig 5). The nectar secretion significantly varied with time (one way ANOVA, 3, 45 = 0.393). The sugar concentration of nectar varied non-significantly (35-36 °Brix) though out the secretion period. The nectar secretion rate was 2.04 µl/hr. The effect of microclimate such as temperature and relative humidity on nectar production and its sugar content was also studied (Fig 5). A conspicuous increase in nectar content was recorded between temperature range of 25–28°C and air humidity of 50–60%. The effect of temperature and air humidity on quantity and quality of nectar was assessed by

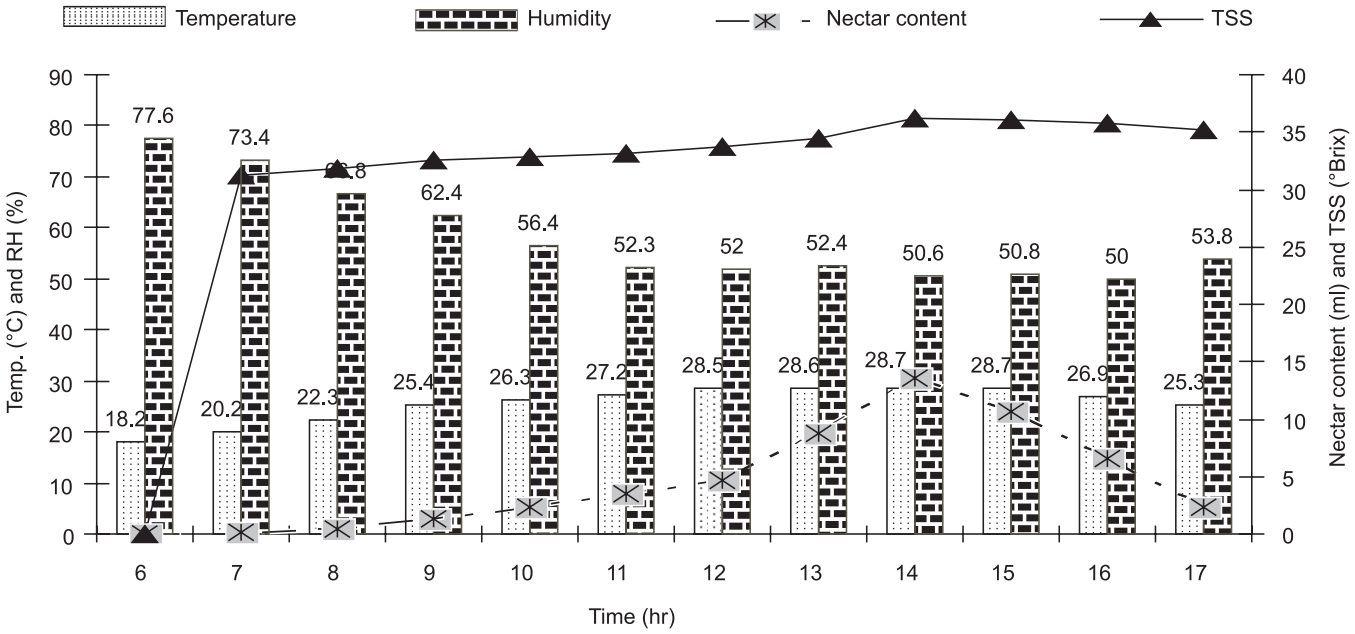

Fig 5 Influence of day temperature and air humidity on quantity and quality of nectar

correlating temperature and humidity with the amount and total soluble solids of nectar at different durations. Increase in nectar content was observed with the increase in temperature and reduction in air humidity. Increase in 0.77 unit of temperature and decrease in 2.04 unit of air humidity resulted in 1 unit increase in nectar content. Correlation studies showed that frequency of bumble bee visit (r= .281) and its foraging time (r= .046) were not influenced by the nectar secretion. On the other hand the frequency of honey bee visit (r= .819, p=.01) and its foraging time (r= .977, p=.01) were negatively correlated with nectar secretion.

Dixon *et al*. (2005) reported that flowering and pollination of avocado with regard to bee number, nectar volume and nectar sugar concentration is not the main factor limiting fruit set but the pollination efficiency. The microclimate (temperature and humidity) required for nectar production and its qualitative trait (sugar) varies with plant species, which is substantiated by the result of Mariana *et al*. (2004). They reported that the optimum temperature and relative humidity for nectar production in family Lamiaceae was between 16-25°C and 60-80%, respectively, and lower air humidity and increase in evaporation rate with temperature resulted in an elevated nectar sugar concentration. The receptivity of a stigma is a critical factor for successful completion of post pollination events. Usually, it becomes maximal soon after anthesis; however, it varies with plant species. The receptivity of stigma starts about 12 hr before anthesis and remains receptive even after 15 hr of flower senescence (Fig 6). However, the fruit set was comparatively high on the day of anthesis and reaches to its peak (95.2%) at 9 hr followed by a gradual reduction. The minimum fruit set was recorded after 15 hr of flower senescence. Data clearly indicates the best time of pollination from 6 to 9 hr day of anthesis due to the high fruit set. Wang *et al*. (2005) reported 100% stigma receptivity on the day of anthesis in *Caunokaempferia coenobialis*, and receptivity decreased with time.

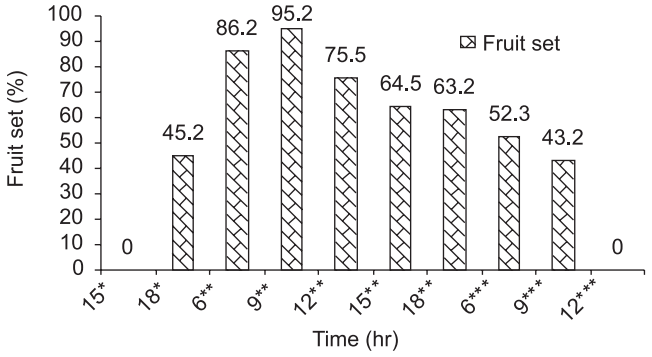

Fig 6 Stigma receptivity of large cardamom with respect to ovary swelling, * day before anthesis, ** day of anthesis, *** day after anthesis

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