ICIMOD Working Paper 2017/8

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The Indigenous Honeybee, *Apis cerana* – A Pollen Robber or Pollinator of Large Cardamom?

The Mountain Institute-India

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Cover photo: An *Apis cerana* bee pollinating large cardamom flower.

Corresponding author: Uma Partap, uma.partap@icimod.org

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The Indigenous Honeybee, *Apis cerana* – A Pollen Robber or Pollinator of Large Cardamom?

Studies on the Pollinators and Pollination of Large Cardamom with a Particular Focus on the Role of *Apis cerana* in Sikkim

Contributing Authors:

Uma Partap[1], Durga P Sharma[2], Ghanashyam Sharma[2], Kailash S Gaira[3], Golam Rasul[1], Eklabya Sharma[1]

[1] International Centre for Integrated Mountain Development (ICIMOD), PO Box 3226, Kathmandu, Nepal

[2] The Mountain Institute – India, Gangtok, Sikkim-737101, India

[3] GB Pant Institute of Himalayan Environment and Development, Sikkim Unit, India

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Christopher Butler (Editor)
Dharma R Maharjan (Layout and design)
Asha Kaji Thaku (Editorial assistant)

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Executive Summary

Large cardamom (*Amomum subulatum* Roxb.) is the main cash crop and an important livelihood option for farming communities in the eastern Himalayas – especially Sikkim, parts of West Bengal, eastern Nepal, and southern Bhutan. The crop is self-fertile but requires cross pollination for production of capsules. Scientific information on the eco-biology of large cardamom pollination is scarce, but studies have shown bumblebees to be the main pollinators. Previous studies have indicated contradictory results on the role of indigenous honeybees, particularly *Apis cerana*, in pollination of large cardamom. Some studies reported that *Apis cerana* was a 'pollen robber' that collects all the pollen from a flower without actually pollinating it, resulting in severe crop decline. However, beekeeping is common in large cardamom farming areas of Sikkim and Nepal and the bee is a common visitor to the flowers. Farmers in these areas have never observed a decline in large cardamom yield due to the presence of *Apis cerana*.

This publication describes the results of a detailed investigation into the role of *Apis cerana* in large cardamom pollination, designed to establish whether the bee is a pollinator or pollen robber and determine its impact on crop yield and quality. The research was conducted on farmers' fields located at different sites in Sikkim, India: Lingee Payong (1,100 masl) and Hee Martam (1,500 masl) in West Sikkim, and Jaubari (2,000 masl) in South Sikkim.

The methodology consisted of on-farm experimental research and data collection through regular field observations, and a review of the literature on large cardamom pollination. Three experimental procedures were used at each site: (i) observations of the foraging behaviour of *Apis cerana* bees on cardamom flowers, and the impact of pollination by *Apis cerana* and other pollinators on capsule yield and quality (capsule weight and number of seeds per capsule), in a plot with a medium-sized healthy colony of *Apis cerana* placed at the centre; (ii) observations of the foraging behaviour of bumblebees and other pollinators, and of capsule yield and quality, in a plot about 250 m away from the plot containing the *Apis cerana* colony; and (iii) observations of capsule yield and quality in plants caged in a nylon net (3 x 3 x 3 m) to exclude all pollinators as a control.

The results of the study showed that *Apis cerana* is an effective pollinator of large cardamom. Although the *Apis cerana* bees are relatively small, they landed on the anther-stigma column of the cardamom flowers multiple times from different directions while collecting pollen which ensured that they also touched the stigma and thus pollinated the flowers. Other favourable foraging attributes included foraging throughout the day, visiting all flowers on a panicle and then moving on to another, and multiple visits to flowers. The favourable attributes translated into a 45% increase in yield compared to natural pollination in fields with a supplementary *Apis cerana* colony. The fruit set, seed set, and fruit and seed weight (capsule quality) were all significantly higher in plots with an *Apis cerana* colony than in plots without a colony or with all pollinators excluded. The results suggest that especially in areas where bumblebee populations and other natural pollinators are scarce, *Apis cerana* can be used to pollinate large cardamom to ensure a reasonable harvest and better quality capsules.

Keywords: Large cardamom, *Apis cerana*, bumblebees, pollinators, Sikkim Himalaya

A young large cardamom farm in West Sikkim

Introduction

Pollination has long been recognized as an important ecosystem service that is necessary for maintaining agricultural productivity as well as sustaining natural ecosystems (Kevan, 1991; Buchmann & Nabhan 1996; Kevan & Phillips, 2001; Millennium Ecosystem Assessment (MA), 2005; Eardley et al., 2006). Thus, it is vital to ensuring human wellbeing and food security. Nearly ninety per cent of all plants and more than three-quarters of the leading global food crops rely on animal pollinators (insects, birds, mammals, and others) for yield and quality (McGregor, 1976; Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES), 2016). Studies conducted in different parts of the world have shown that pollinators make a huge economic contribution to agriculture (Winston & Scott, 1984; Matheson & Schrader, 1987; Pimentel et al., 1997; Carreck & Williams, 1998; Morse & Calderone, 2001; Ruijter, 2002); estimated by Gallai et al. (2009) to be Euro 153 billion (USD 161 billion) annually. Partap et al. (2012) estimated that insect pollinators contribute USD 2.69 billion to the agricultural economy in parts of the Hindu Kush Himalayas (HKH) in Bangladesh, Bhutan, China, India, and Pakistan. Realizing the contribution to agriculture and biodiversity, the Convention on Biological Diversity (CBD) recognized pollination as a key driver in the maintenance of biodiversity and ecosystem function.

Insects (bees, butterflies, flies, and others) are the most important pollinators of agricultural crops and thus play a fundamental part in maintaining food security for humans. However, a range of factors such as excessive application of chemical pesticides, habitat loss as a result of ongoing land use change, spread of pathogens, competition from alien species, and climate change, have led to an alarming decline in pollinator abundance and diversity, both globally and in the HKH region (Aizen & Feinsinger, 1994; Allen-Wardell et al., 1998; Partap & Partap 2002, 2009; Partap et al., 2001; Partap, 2003; 2011; Berezin & Beiko, 2002; Biesmeijer et.al., 2006; Eardley et al., 2006; Potts et al., 2010; Cameron et.al., 2011; Partap & Tang, 2012; IPBES, 2016). The serious implications of this decline were made clear in an extensive ICIMOD study by Partap & Partap (2002) in the apple farming regions of the Himalayas (Bhutan, China, India, Nepal, Pakistan), which showed a severe decline in apple yield and quality as a result of inadequate pollination.

Among the insects, honeybees have been reported to be one of the most efficient providers of crucial and high value pollination services and play an indispensable role in enhancing the production of many economic crops. Studies conducted in different parts of the world have proven the role of honeybee pollination in improving the yield and quality of various crops such as fruit and nuts, vegetables, pulses, oilseeds, spices, and fibre and forage crops (McGregor, 1976; Batra, 1985; Vithanage & Ironside 1986; Free, 1993; Partap & Verma, 1994; Verma & Partap, 1994; Singh et al., 2000; Sekita, 2001; Mattu et al., 2012). The role of domesticated bees is becoming increasingly important as the diversity and abundance of naturally occurring pollinators declines.

Large cardamom (*Amomum subulatum* Roxb.) is an economically valuable, ecologically adaptive, and agro-climatically suitable perennial cash crop grown in the eastern Himalayan region, especially Sikkim, parts of West Bengal, eastern Nepal, and southern Bhutan (Sharma et al., 2000; Awasthe et al., 2011; Government of Nepal (GoN), 2013). Cardamom is cultivated as an under-storey crop under forest cover in association with Himalayan alder (*Alnus nepalensis*) or other forest tree species as shade trees across a broad altitudinal range from 600 to 2,400 masl. On average, large cardamom contributes USD 500–1,600 (in extreme cases up to USD 16,000) to the annual household cash income of local farmers, depending on the landholding size and farm management efforts, and thus forms a substantial part of the livelihoods of mountain farming households (Sharma & Sharma, 1997; Sharma et al., 2000; Sharma et al., 2016).

Large cardamom is self-fertile but requires cross-pollination for crop production. Until recently, there was very little scientific information available on the pollination eco-biology of cardamom. However, the scientific community has become increasingly interested in large cardamom pollination in recent years and this has led to an increase in the information available on both pollination requirement, and pollinators, their efficiency, and the impact on production. Recent studies on large cardamom pollination such as Deka et al. (2011), Kishore et al. (2011), Sinu & Shivanna (2007), Singh et al. (2011), and Sinu et al. (2011) have reported bumblebees (*Bombus* spp.) to be the most efficient pollinators of the crop. Deka et al. (2010) reported two species of bumblebees – *Bombus haemorrhoidalis* Smith and *B. breviceps* Smith – to be the predominant pollinators in Sikkim, while Sinu et al. (2011) reported that *Bombus haemorrhoidalis*, *Apis cerana*, *Megachile lanata*, *Episyrphus ballatus* (hover fly), *Macroglossum stellatarum* (hawk moth), and *Aethopyga siparaja* (crimson sunbird) were common visitors to cardamom flowers.

There is still a dearth of scientific literature on the role of honeybees in pollinating large cardamom. Verma (1987) concluded that the indigenous honeybee *Apis dorsata* is an effective pollinator of large cardamom by directly relating an increase in crop production to an increase in the number of bee colonies in the vicinity of cardamom farms. However, the role of the indigenous hive bee *Apis cerana* as a pollinator of large cardamom is not well understood.

Studies conducted by Deka et al. (2011), Sinu and Shivanna (2007), and Sinu et al. (2011) concluded that *Apis cerana* is a 'pollen robber' and collects most of the pollen from large cardamom flowers without pollinating them. Their conclusions were based on the observation that due to their small size the bees could collect pollen from anthers without touching the stigma. Kishore et al. (2011) reported that *Apis cerana* pollinated only a small percentage of the flowers they visited, and Sinu and Shivanna (2007) that *Apis cerana* bees leave 70% of large cardamom flowers unpollinated while collecting all the pollen from the flower, thus depriving the flower of pollen for pollination by other pollinators and leading to a severe decline in crop production. However, these results are contradicted by other local observations. Beekeeping with *Apis cerana* is common in large cardamom farming areas in Sikkim and Nepal and the bee is a common visitor to large cardamom flowers, but farmers have not noticed any decline in yield due to *Apis cerana* visits. On the contrary, large cardamom farmers in Sikkim thought that *Apis cerana* was a good pollinator of the crop (Partap et al., 2014).

In light of these contradictory findings, we conducted a detailed investigation to gather scientific evidence on the role of *Apis cerana* in pollinating large cardamom flowers, the impact of bee visits on crop yield and quality, and whether *Apis cerana* is in fact a pollen robber or a pollinator of the crop. The investigation was conducted on large cardamom farms located at different sites in Sikkim, India. The effect of *Apis cerana* visits to large cardamom flowers on capsule yield and quality was investigated by placing a colony of *Apis cerana* in a large cardamom field, recording the key attributes of *Apis cerana* foraging, and measuring cardamom production and yield in fields with and without a colony, as well as in areas with all pollinators excluded.

Materials and Methods

Study Sites

On-farm experimental research on the pollination role of *Apis cerana* and its impact on the yield and quality of large cardamom (*Amomum subulatum* var. *ramsai*) was conducted on farmers' fields at three different sites: Hee-Martam in West Sikkim, and Lingee-Payong and Jaubari in South Sikkim (Figure 1). The sites were located at different elevations and aspects in areas dominated by large cardamom farming. Observations on pollinators were made in Hee-Martam on 3–8 May 2013, in Lingee-Payong on 14–18 May 2013, and in Jaubari on 26–30 May 2013, and observations on yields at the local harvest time. The major site characteristics are summarized in Table 1, more details are given in the following paragraphs.

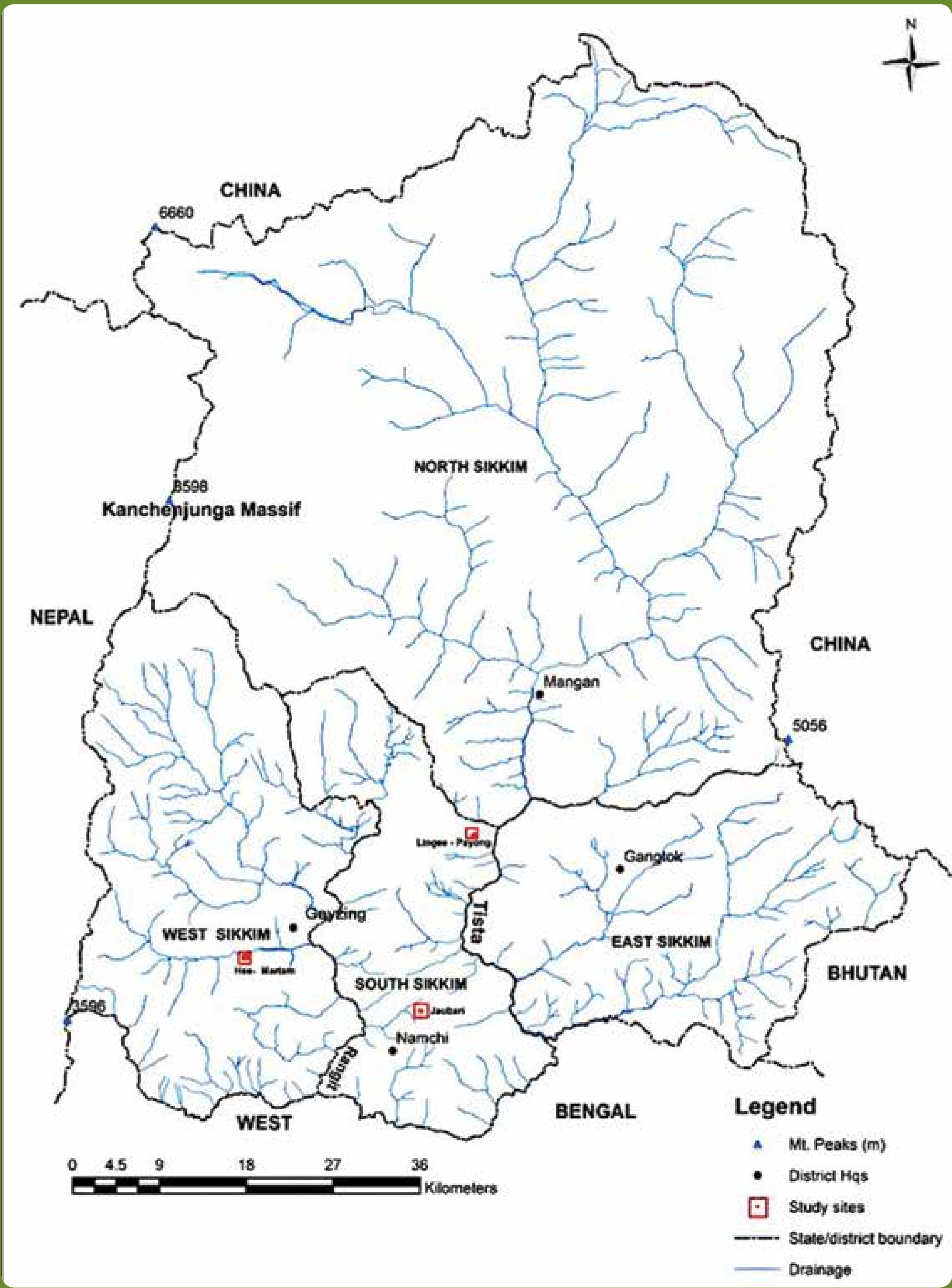
Lingee-Payong

Lingee-Payong is located at 1,100 masl in South Sikkim, near Maeman Wildlife Sanctuary. Around 280 households are engaged in cardamom farming in this area. Maize and vegetables are grown along with large cardamom. The main varieties grown are *ramsai*, *golsai*, *sauney*, and *bharlang*.

Hee-Martam

Hee-Martam is a group of villages located at 1,500 masl on the fringes of Barsey Rhododendron Sanctuary in West Sikkim. The area is dominated by large cardamom as the major local livelihood-cum-cash crop. Other crops include maize, oilseed, pulses, and vegetables, but most farmers in this area have replaced food-grain and vegetable crops with large cardamom, with around 400 households engaged in cardamom farming. The main varieties grown are *seremna* and *ramsai*.

Figure 1: **The location of the study sites within Sikkim**



Jaubari

Jaubari is located at around 2,000 masl in South Sikkim. Around 100 farmers are engaged in large cardamom farming. The main variety cultivated is *ramsai*. Farmers also grow ornamental plants and other trees such as dhokrey (*Brugmansia suaveolens*) in addition to large cardamom.

Table 1: **Characteristics of the study sites**

Site	Geographic coordinates	Elevation (masl)	Slope (°)	Aspect	Soil characteristics	Main variety of cardamom	No. of farmers engaged in cardamom production
Lingee-Payong	27° 21′ 40″ N 88° 27′ 54″ E	1,100	20–30	South facing	Mountain black loose soil	*Ramsai*	280
Hee-Martam	27° 15′ 09″ N 88° 13′ 15″ E	1,500	30–40	North facing	Mountain black loose soil	*Ramsai* *Seremna*	400
Jaubari	27° 11′ 46″ N 88° 23′ 31″ E	2,000	35–45	West facing	Red Soil	*Ramsai*	100

Experimental Design

Three experimental procedures were carried out at each of the three sites, the setup is shown in Figure 2.

Apis cerana pollinated

We placed a medium-sized colony of *Apis cerana* with approximately 10,000–12,000 bees free of any sign of disease in one plot. Flowers were accessible to all pollinators, but the *Apis cerana* foragers dominated throughout the flowering period due to the placement of the colony. Observations were made of the foraging behaviour of the *Apis cerana* bees in an area within 50 m radius of the hive; and the capsule yield and quality in the plot were recorded.

Naturally pollinated (bumblebees)

The results of natural pollination were observed in a plot about 250 metres away from the plot with the *Apis cerana* colony. Observations were made of the foraging behaviour of bumblebees (the major pollinator of large cardamom), and the capsule yield and quality were recorded. This plot was dominated by bumblebee foragers.

Control

Control data on the capsule yield and quality without pollinators was recorded from plants caged in a nylon net (3 x 3 x 3 m) which excluded bees and other insects.

Figure 2: **Experimental set up: top – colony of *Apis cerana* in a field of large cardamom (*Apis cerana* pollinated); bottom – plants caged with nylon net (control). Observations on naturally pollinated plants were made in another plot about 250 metres away**

Data Recording

Floral biology

Information on farming practices such as plant-to-plant and row-to-row distance of the crop and number of plants per hectare was collected from the farmers. The number of panicles (floral shoots) per plant, total number of flower buds per panicle, number of flowers per day per panicle, the time of flower opening and closing, and total flowering period of the crop (i.e. the floral biology) were recorded at each experimental site.

Foraging behaviour

Observations on the foraging behaviour of *Apis cerana* and bumblebees on large cardamom flowers were made on each of five days at three different times: in the morning at 09:00–11:00, at midday from 12:00–14:00, and in the afternoon from 15:00–17:00. They included the initiation and cessation of foraging, peak foraging hours, number of bees returning to hive in one minute and whether bringing pollen or nectar, and number of visits to a flower per hour and whether foraging for pollen or nectar. The key foraging attributes, i.e. the way foragers approached and landed on a flower, whether carrying pollen grains on their bodies (head, thorax, and abdomen), and whether touching the stigma, were recorded carefully. The visitation frequency on large cardamom flowers (expressed as the number of bee visits per flower per hour) was recorded in a 3 x 4 m transect in each plot (with and without an *Apis cerana* colony) during the peak hours of pollinator visits (09:00–11:00).

Impact of bee pollination

After the crop was ripe (in August in Lingee-Payong, late September/ October in Hee Martam, and November in Jaubari), 25 capsule-containing panicles of similar size were harvested from each plot (with and without an *Apis cerana* colony and control) using locally designed long-handled sharp knives. The effect of *Apis cerana* visits on quantity and quality of capsules was assessed by comparing counts of the number of capsules per panicle (fruit set), fresh and dry weight of capsules, and number of seeds in each capsule (seed set). Dry weight was measured at the GB Pant Institute of Himalayan Environment and Development laboratory at Pangthang. Capsules were dried to constant weight using a hot air oven (TestMaster ITC-902M) and weighed using a high precision electronic balance (Sartorius BP 121S)

Data Analysis

Data were analysed statistically using MS-Excel 2007 with SPSS 10 and Systat 6.0. The treatments within 50 m radius of the *Apis cerana* colony, 250 m away from the colony, and control were compared using a multifactor analysis of variance, independent samples t-test comparison, and regression analysis.

Results and Discussion

Floral Biology of Large Cardamom

The planting and floral biology results are summarized in Table 2; Figure 3 shows the typical appearance of cardamom in flower. The crop was planted at an average plant-to-plant distance of 1.5 m at all three sites (Table 2); the total flowering period varied from 20 to 25 days starting in mid-April at the lowest elevation site in Lingee-Payong, and ending in mid-July at the highest elevation in Jaubari. The mean number of panicles per plant varied from 6 to 10.

Flowers were borne on short compressed panicles, opening from the base (outer part of panicle) to the top (inside) of the panicle. Each panicle had 45–60 flower buds, however many aborted. The total number of flowers opening per panicle ranged from 25 to 28, with an average of one flower per panicle and 5–6 flowers per plant opening per day (Table 2). Flowers opened at around 06:00 to 07:00 in the morning, remained open throughout the day, and wilted in the late evening.

The flowers presented both pollen and nectar for the visiting pollinators. Large amounts of pollen were clearly visible along the sutures of the anthers as the flowers opened in the morning. Most of the pollen was collected by the bees by 12:00, after which they started collecting nectar. Similar observations have been made on small cardamom (Chandran et al. 1980).

Our observations on the floral biology of the crop agree with those of Gupta and John (1987), Sinu and Shivanna (2007), and Deka et al. (2011) who also reported that flowering starts in March/April at lower elevations and continues up to the end of June or July at higher elevations, depending on the weather conditions and type of cultivar. These authors also reported that flowers open in the morning and remain viable for 14 hours. However, the number of panicles per plant found in our study was much lower than the number of 20–45 reported by Sinu and

Table 2: **Planting distance and floral biology of large cardamom at the study sites**

Study site	Variety	Plant to plant distance (m)	Flowering season	No. of panicles per plant	No. of flowers per panicle	Number of flowers opening per plant per day
Lingee-Payong	*Ramsai*	1.5	mid-April to end May	8.1+ 2.8	28.0 ± 4.4	5.2
Hee-Martam	*Ramsai*	1.5	end April to end May	9.7 + 2.4	28.0 ± 4.0	5.8
Jaubari	*Ramsai*	1.5	mid-May to mid-July	5.9 + 1.4	25.4 ± 5.4	4.6

Figure 3: **Flowering large cardamom. The close up shows the three small corolla lobes and large labellum; the anther-stigma column and reddish-pink nectar guide are circled in red.**

Shivanna (2007). This may be due to the variety and/or age of the crop; we conducted our studies in five-year-old plantations. Sharma et al. (2002, 2009) reported that the productivity of large cardamom increases with age and was highest in 5–15 year-old stands. Productivity is related to the number of flowers and number of panicles per plant; the higher the number of panicles, the higher the number of flowers, and the higher the number of capsules produced per plant.

Flower Visitors

The main pollinators observed visiting cardamom flowers were two species of bumblebee and the indigenous honeybee *Apis cerana* (Figure 4). Hover flies (*Episyrphus balteatus*) and moths (*Udasees folus*) were also seen. Similar pollinators were observed at all sites. Deka et al. (2011), Kishore et al. (2011), Sinu and Shivanna (2007), Sinu et al. (2011) also reported the bumblebees *Bombus haemorrhoidalis* and *Bombus braviceps* as the main pollinators of large cardamom flowers, and *Apis cerana*, *Megachile lanata*, and *Aethopyga siparaja* (crimson sunbird) as additional visitors.

Figure 4: **Insect pollinators visiting large cardamom flowers: Bumblebee (left), *Apis cerana* bee (right)**

Foraging Behaviour of *Apis cerana* and Bumblebees on Large Cardamom

Foraging habits

The *Apis cerana* and bumblebee foragers had quite different foraging habits. The *Apis cerana* bees landed repeatedly on the anther-stigma column of a flower from different directions so that their pollen-covered bodies contacted the stigma, thus pollinating the flower; the bumblebees landed on the labellum and moved deep inside the flower column to collect nectar, contacting the stigma with their large pollen covered bodies as they moved in, also pollinating the flower.

The foraging activity of both bees was highest in the morning (09:00—11:00) on clear sunny days; their activity declined significantly (by about 30%) on rainy days. Both bees collected a significant amount of pollen in their pollen baskets (Figure 5), but the larger bumblebees collected nearly three times as much pollen as *Apis cerana* bees, which is in line with the observation by Verma and Dulta (1986) that the pollen carried by an insect is directly related to its body size. Both *Apis cerana* and the bumblebees brushed against the anthers so that their heads and thorax were covered with pollen grains, and pollinated the flowers by then coming into contact with the stigma. Our findings on the effectiveness of bumblebees in pollinating large cardamom were in strong agreement with those of Sinu et al. (2011), Deka et al. (2011), and Kishore et al. (2011) who reported that bumblebees are likely to come into contact with the stigma due to their large body size, and are thus excellent pollinators of large cardamom. But we also noted that *Apis cerana* bees were effective pollinators because they landed on the anther stigma column in order to collect pollen.

The returning *Apis cerana* foragers were observed within a one-minute period at the hive entrance during the peak hours of foraging (09:00–11:00); about 35% of the foragers returning to the hive in Lingee-Payong, 36% in Hee-Martam, and 38% in Jaubari had pollen loads. The proportion of bees bringing pollen declined in the afternoon when most foragers returned either with nectar or water. This is in line with our observation that large cardamom flowers offer pollen in the morning and nectar in the afternoon, and is also in agreement with earlier studies by Deka et al. (2011) and Sinu et al. (2011).

The foraging behaviour of both honeybees and bumblebees (i.e. time of commencement and cessation, trip duration, number of visits to flowers per hour) was different at different sites due to variations in elevation, ambient temperature, rainfall pattern, and humidity. This has also been observed in other studies on *Apis cerana* and *Apis mellifera* with different crops (Verma & Dulta, 1986; Verma & Rana, 1994; Partap & Partap, 2002; Tang et al., 2003; Mattu et al., 2012).

Figure 5: ***Apis cerana* bee (above) and bumblebee (below) collecting pollen from large cardamom flowers. Pollen loads are clearly visible in the corbiculae on the hind legs.**

Visitation frequency of the bees

Figure 6 shows the number of bee visits to cardamom flowers per hour (visitation frequency) recorded at different sites and times of day. More *Apis cerana* visits to flowers were observed in plots with a colony of *Apis cerana*, and more bumblebee visits in plots without an *Apis cerana* colony. Similar peak foraging hours have been reported for honeybees on various crops in other mountain areas such as apple in the Shimla hills of Himachal Pradesh, India (Verma & Dulta, 1986); cabbage and cauliflower in the Kathmandu valley, Nepal (Verma & Partap 1994); and radish (Partap & Verma, 1994); and for bumblebees on apple in Himachal Pradesh (Verma & Chauhan, 1985).

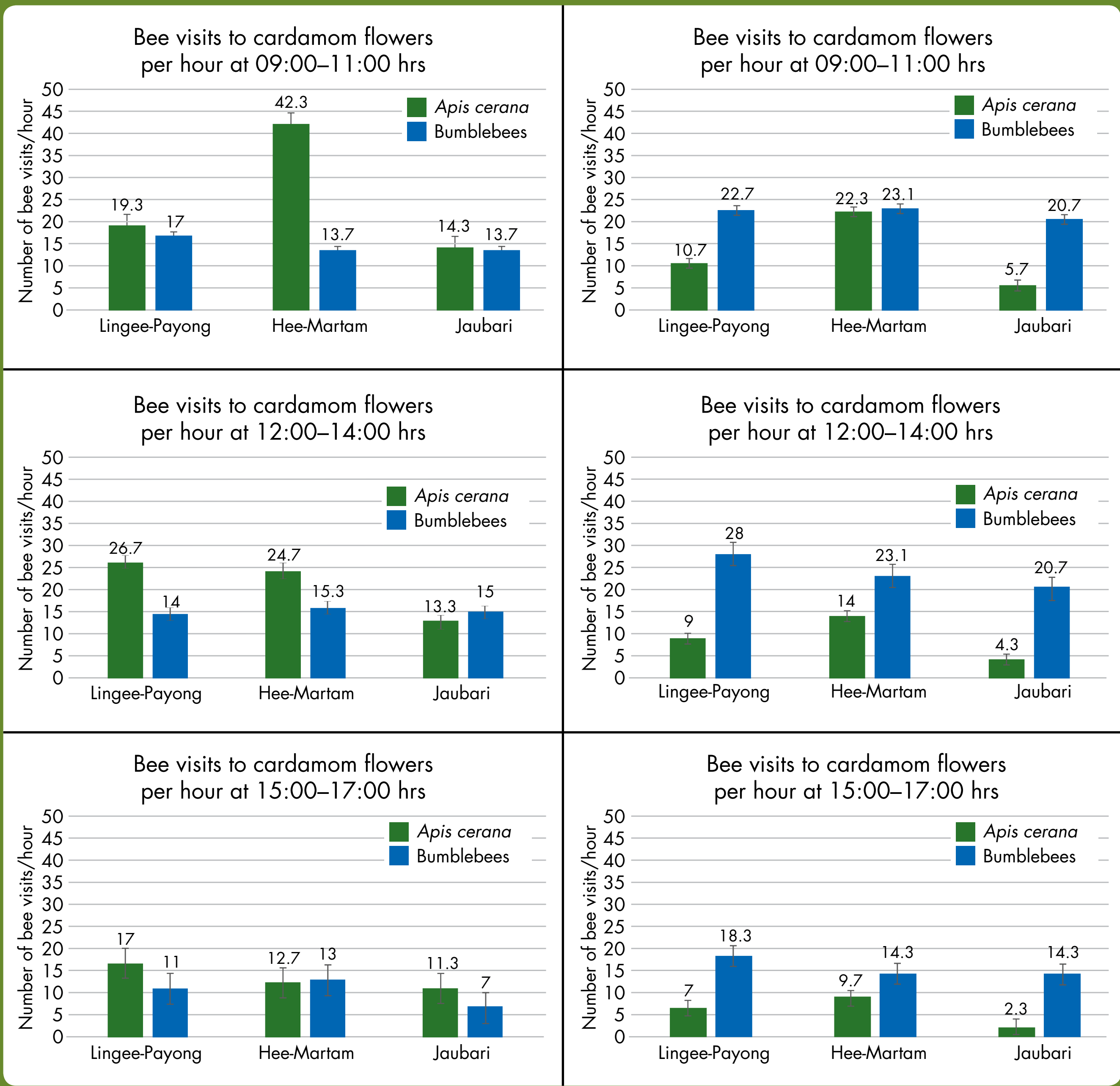
A large number of bees of both species were recorded visiting flowers on bright sunny days, but the number was negligible on cold mornings and evenings and on rainy days. These observations are in close agreement with observations of *Apis cerana* foraging on cabbage and cauliflower in Kathmandu valley, Nepal (Verma & Partap, 1994), and on apple in the Shimla hills of Himachal Pradesh (Verma & Dulta, 1986).

The visitation frequency of both *Apis cerana* and bumblebees was significantly lower at Jaubari than at Lingee-Payong and Hee-Martam (Figure 6). This is probably due to the generally low ambient air temperature (9–12°C) under the bushes, cold breeze, and high elevation of the area. Frequent rain, low temperatures, and cloudy weather during the cardamom flowering season, as observed at Jaubari, has previously been reported to affect pollination by natural pollinators (Partap & Partap, 2002; Tang et al., 2003). Fitter and Fitter (2002) and Miller et al. (2007) showed that weather is the key factor influencing crop pollination. Temperature has been reported to have a large impact on pollinator interactions and is also known to affect flowering in crops and plants (Arft et al., 1999; Inouye et al., 2003). An air temperature of 17–18oC is considered optimal for apple flower blooming.

The statistical analysis showed that the number of floral visits by all bees (*Apis cerana* and bumblebees together) was significantly higher in plots with an *Apis cerana* colony than in those without (t = 4.398, $P \leq$ 0.001); the number of

Figure 6: **Number of bee visits to cardamom flowers per hour in plots with (left column) or without (right column) an *Apis cerana* colony, in the morning (top row), midday (middle row), and afternoon (bottom row) (values are mean ± SE, n = 27)**

Plots with an *Apis cerana* colony **Plots without an *Apis cerana* colony**



floral visits by *Apis cerana* alone also differed significantly between plots with and without an *Apis cerana* colony ($t = 8.177$, $P \leq 0.001$), as did floral visits of bumblebees alone (*Bombus braviceps* and *B. haemorrhoidalis*) ($t = 6.262$, $P \leq 0.001$).

Density of pollinators (bees)

Figure 7 shows the number of honeybees and bumblebees observed visiting flowers between 09:00 and 11:00 in a 3 x 4 m transect at the different study sites. Table 3 shows the proportion at the different sites and overall. The number of *Apis cerana* bees was higher in the plots with an *Apis cerana* colony (62% overall), while the number of bumblebees was higher in the open pollinated plots without an *Apis cerana* colony (74% overall). This indicates both that there were more *Apis cerana* pollinators in the vicinity of the colony, and that bumblebees avoided areas with higher competition and more limited availability of nectar/pollen (due to collection by the *Apis cerana* foragers), and preferred to forage in places with less competition from *Apis cerana* bees.

Figure 7: **Number of bees observed in a 3 x 4 m transect in the morning (09:00–11:00) at the different study sites: a) in plots with an *Apis cerana* colony; b) in plots without an *Apis cerana* colony**

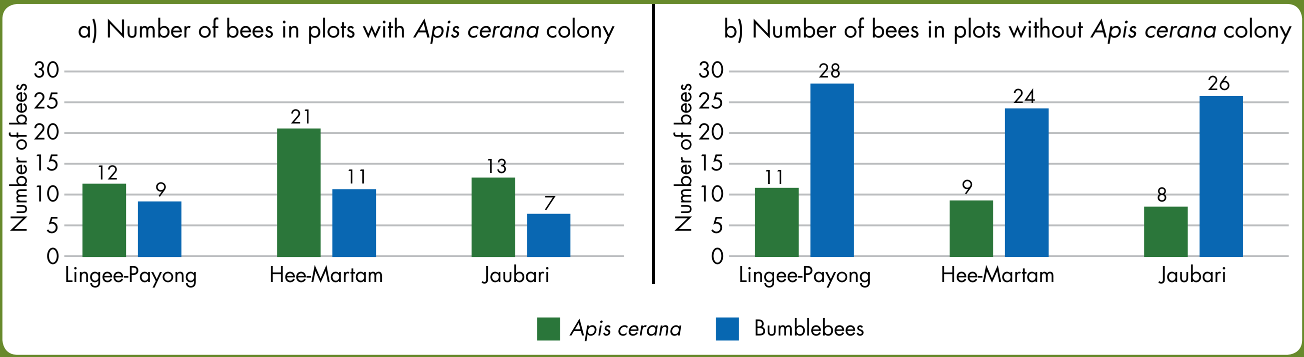


Table 3: **Proportion of bee visits to flowers in sites with and without an *Apis cerana* colony (%)**

Site	Site with *Apis cerana* colony (%)		Site without *Apis cerana* colony (%)	
	Apis cerana	Bumblebee	*Apis cerana*	Bumblebee
Lingee-Payong	57.1	42.9	28.2	71.8
Hee-Martam	65.6	34.3	27.3	72.7
Jaubari	65.0	35.0	23.5	76.5
Average	63.0	37.0	26.3	73.7

The number of *Apis cerana* bees observed visiting flowers in the transects in the plots with colonies was higher in Hee-Martam than in Lingee-Payong or Jaubari, while the number of bumblebees was similar at all sites (Fig. 7a). This may have been related to the strength (number of bees) of the colony; the colony used in Hee Martam was stronger than that used in Jaubari.

Regression analysis showed a linear relationship between the number of flowers and the number of pollinators (bees) visiting flowers in the plot. Bee visits (honeybees and bumblebees) increased with increasing number of cardamom flowers (Figure 8).

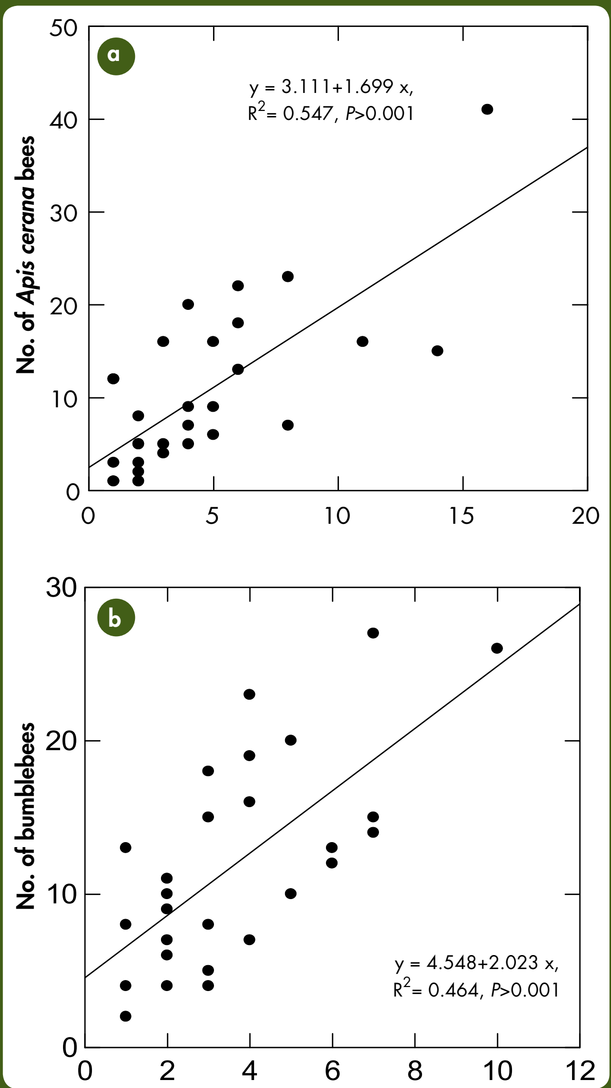
Number of flowers visited per minute

Table 4 shows the number of flowers visited per minute by bees at the different study sites at different times of day. The number of flowers visited by *Apis cerana* was lower in the morning at Hee-Martam and in late afternoon at Jaubari, but at all other times and sites was fairly constant at around four visits per minute in plots with an *Apis cerana* colony, and four to five visits

Figure 8: **Relationship between number of cardamom flowers and number of bees visiting**

a) *Apis cerana* visiting flowers in colony containing plots within the site

b) bumblebees visiting flowers in non-colony containing plots within the site



per minute (Lingee Payong) or three visits per minute (Hee-Martam and Jaubari) in plots without an *Apis cerana* colony. At Lingee-Payong and Jaubari, bumblebees visited a similar number of flowers per minute to *Apis cerana* in the plots with an *Apis cerana* colony, and more per minute (4-6) in the plots without a colony at all times of day. At Hee-Martam, bumblebees visited fewer flowers per minute than *Apis cerana* in the plot with an *Apis cerana* colony, and a similar number or slightly more in the plot without a colony at all times of day.

Overall, there was no significant difference between *Apis cerana* and bumblebees in the number of flowers visited per minute during different hours of day and in plots with or without an *Apis cerana* colony (Table 4). However, one-way analysis of variance (one-way ANOVA) showed significant differences across sites ($F_{2,284} = 9.581$, $P<0.001$) in the number of flowers visited by *Apis cerana* and bumblebees together in plots with and without an *Apis cerana* colony. Further analysis using Tukeys pairwise comparison probabilities, also showed significant differences ($P<0.001$) in the number of flowers visited by (i) *Apis cerana* in plots with an *Apis cerana* colony and bumblebees in plots without an *Apis cerana* colony; (ii) *Apis cerana* and bumblebees in plots without an *Apis cerana* colony; and (iii) bumblebees in plots with and without an *Apis cerana* colony.

Table 4: **Number of flowers visited per minute by bees at different times of day** (values show mean ± SE, n = 18)

Time	Number of flowers visited per minute			
	Site with an *Apis cerana* colony		Site without an *Apis cerana* colony	
	Apis cerana	Bumblebees	*Apis cerana*	Bumblebees
Lingee-Payong				
09:00–11:00	4.0±0.4	3.9±0.3	5.1±0.7	6.6±0.7
12:00–14:00	3.6±0.5	4.9±0.5	4.3±0.6	6.0±0.9
15:00–17:00	3.6±0.7	4±0.6	4.4±0.3	4.8±0.4
Hee-Martam				
09:00–11:00	2.9±0.8	2.4±0.3	2.1±0.30	2.9±1.2
12:00–14:00	4.1±0.3	2.9±0.3	3.1±0.30	3.6±0.4
15:00–17:00	4.3±0.4	2.4±0.3	3.4±0.30	3.1±0.3
Jaubari				
09:00–11:00	4.1±0.1	4.6±0.7	2.8±0.4	5.9±0.30
12:00–14:00	3.8±0.2	3.6±0.5	3.1±0.3	6.1±0.99
15:00–17:00	2.9±0.2	3.1±0.5	2.4±0.3	4.3±0.42

Pollen collection

Both *Apis cerana* and bumblebees were observed collecting pollen from large cardamom flowers. The number of *Apis cerana* foragers returning with or without pollen was recorded at the hive entrance from 09:00–11:00 (numbers were not recorded for bumblebees). Just over one-third of foragers were seen to return to the hive with pollen loads; the remaining foragers probably carried nectar or water (Table 5).

Sinu and Shivanna (2007), Deka et al. (2011), and Kishore et al. (2011) reported that *Apis cerana* only collected pollen without pollinating the large cardamom flowers. Our observations of pollen loads showed that the bees carried pollen. Further investigations would be needed to discover whether the pollen was from large cardamom flowers or other flowers in the vicinity, however, the observations of bees actually foraging provide a clear indication

Table 5: **Number of *Apis cerana* foragers per minute returning to the colony with and without pollen loads (09:00–11:00)** (values are mean ± SE, n = 20)

Study site	No. of bees with pollen loads	No. of bees either empty or with nectar/water	Total No. of bees
Lingee-Payong	14.7±4.8 (35.5%)	26.7±5.8 (64.5%)	41.4±7.9
Hee-Martam	19.3±3.6 (36.0%)	34.3±5.8 (64.0%)	53.6±9.2
Jaubari	11.9±3.4 (38.1%)	19.3±6.5 (61.9%)	31.2±6.8

that the *Apis cerana* bees do pollinate the flowers as 36–46% of *Apis cerana* bees observed on the cardamom flowers carried pollen grains on their bodies (head, thorax, abdomen) and also touched stigma (Table 6). Verma (1987) reported that *Apis cerana* was an abundant visitor to cardamom flowers in India and observed a much lower fruit set when the proportion of *Apis cerana* was low. Chandran et al. (1980) observed in small cardamom that *Apis cerana* pollen gatherers passed over both anther and stigma and so ensured pollination. The foraging behaviour and visitation frequency observed in our study suggest that *Apis cerana* is an effective pollinator of large cardamom.

The observations indicated that the most favourable conditions for foraging were on warm sunny days with temperatures of 13–15°C or more and relatively low humidity. On a warm sunny day with a temperature above 15°C and relative humidity around 60%, about 90% of *Apis cerana* foragers observed from 11:00 to 13:00 returned with pollen loads, while the number was greatly reduced on cold rainy days and in the morning and late afternoon. Bumblebees continued foraging even during cold weather and are thus likely to be efficient pollinators of large cardamom under such conditions, as reported previously (Sinu and Shivanna 2007).

Table 6: **Number of *Apis cerana* foragers carrying pollen grains on their bodies and touching stigma (morning)**

Study site	Number of plants observed	Number of bee visits to flowers	Number of bees with pollen grains on their bodies and touching stigma
Lingee-Payong	14	71	32 (45%)
Hee-Martam	17	234	108 (46%)
Jaubari	14	44	16 (36%)

Impact of *Apis cerana* Pollination on Cardamom Yield and Quality

The effect of *Apis cerana* pollination on cardamom yield and seed quality was investigated by comparing the results of harvested cardamom (Fig. 9) from the three types of plot: with an *Apis cerana* hive, without a hive, and with pollinators excluded.

Fruit and Seed Set

The number of capsules, fruit set (number of capsules compared to number of flowers), and number of seeds per capsule in large cardamom plants from the different plots is shown in Table 7. The effect of *Apis cerana* was evaluated by comparing the values from plots with an *Apis cerana* colony with those from plots without a colony (naturally pollinated) and with pollinators excluded by a net cage (control). All panicles produced capsules in all plots both with and without *Apis cerana* colonies, but less than half produced capsules in the control plots (43%, 47%, and 39% in Lingee-Payong, Hee-Martam, and Jaubari, respectively). Plants in plots with an *Apis cerana* colony at the different sites produced between 175 and 400% more capsules than control plants.

Statistical analysis showed that overall plants in plots with an *Apis cerana* colony produced a significantly higher number of capsules per panicle than those in plots without a colony (t=3.65; 1.25; 1.93; $P \leq 0.05$, Lingee-Payong,

Figure 9: **Harvesting large cardamom from experimental plots**

Table 7: **Effect of *Apis cerana* pollination on capsule and seed set (values are mean ± SE, n=15)**

	Plot with *Apis cerana* colony[a]	Plot without *Apis cerana* colony[a]	Control (pollinators excluded)	Increase due to *Apis cerana* pollination (%)	
				Increase over naturally pollinated[b]	Increase over control
Lingee-Payong (average number of flowers per panicle 28.0)					
Number of capsules per panicle	8.8 + 0.6; n=15	5.3 + 0.2; n=15	2.9 + 0.3; n=9	65.9	199.7
Fruit set (%)	31.5	19.0	10.5	12.6	20.6
Number of seeds per capsule	36.4 + 1.6; n=15	34.0 + 1.3; n=15	14.4 + 2.2; n=15	7.1	152.8
Hee-Martam (average number of flowers per panicle 28.0)					
Number of capsules per panicle	12.1 + 0.6; n=15	7.6 + 0.4; n=15	2.4 + 0.3; n=9	59.5	406.7
% Fruit set	43.1	27.0	8.2	16.1	34.9
Number of seeds per capsule	34.5 + 0.9; n=15	32.2 + 1.8; n=15	18.1 + 1.6; n=15	7.1	90.6
Jaubari (average number of flowers per panicle 25.4)					
Number of capsules per panicle	10.1 + 0.2; n=15	5.3 + 0.3; n=15	3.7 + 0.3; n=9	91.6	175.6
Fruit set (%)	39.7	20.7	14.4	19.0	25.3
Number of seeds per capsule	35.8 + 1.7; n=15	32.2 + 1.8; n=15	12.2 + 2.5; n=15	11.8	193.4

[a] Open plots also visited by bumblebees and other natural pollinators, including free-ranging *Apis cerana*
[b] Mainly bumblebees, but also free-ranging *Apis cerana*

in Hee-Martam, and Jaubari, respectively), or caged with a net (control) (t=4.34; 6.27; 8.89; $P \leq 0.001$). One-way ANOVA showed a significant difference in the effect of treatments on fruit set ($F_{2, 36}$=8.60; 18.57; 10.81; $P \leq 0.01$). The number of seeds per capsule was also significantly higher in plots with an *Apis cerana* colony than in controls (t=2.74; 7.84; 1.12; $P \leq 0.5$; $F_{2,36}$=224.65;173.48; 246.34; $P \leq 0.01$), but the difference between plots with and without an *Apis cerana* colony was not significant.

Capsule Quality

The average dry weight (and wet weight) of capsules from large cardamom plants from the different plots is shown in Table 8. The dry weight of the capsules harvested from plots with an *Apis cerana* colony was significantly higher than that of capsules harvested from plots without an *Apis cerana* colony (t = 5.724, $P \leq 0.001$) or from control plots (t = 12.969, $P \leq 0.001$). The weight of capsules harvested from plots without an *Apis cerana* colony was also significantly higher than those from control plots (t = 8.669, $P \leq 0.001$). These findings were confirmed by one-way ANOVA which showed a significant difference ($F_{2,447}$ = 93.115, $P < 0.001$) between and within treatments.

The capsule weight (fresh and dry weight) was low in the capsules collected from the control plots and most of the capsules were rotten. This indicates that many of the flowers in the control were unpollinated and failed to set fruit.

Table 8: **Effect of *Apis cerana* pollination on capsule weight (values are mean ± SE, n=50; weight of fresh capsule in parentheses)**

Site	Average capsule weight (g)			Increase due to *Apis cerana* pollination (%)	
	Plot with *Apis cerana* colony[a]	Plot without *Apis cerana* colony[a]	Control (pollinators excluded)	Increase over naturally pollinated[b]	Increase over control
Lingee-Payong	1.3 ± 0.0 (4.0 ± 0.1)	0.8 ± 0.1 (3.6 ± 0.1)	0.8 ± 0.04 2.9 ± 0.1)	65.4 (9.9)	63.4 (38.2)
Hee-Martam	1.4 ± 0.0 (4.9 ± 0.2)	1.1 ± 0.02 (4.7 ± 0.2)	0.9 ± 0.0 (2.8 ± 0.1)	33.3 (4.9)	59.1 (70.3)
Jaubari	1.0 ± 0.04 (4.0 ±0.1)	1.0 ± 0.1 (3.7 ± 0.1)	0.9 ± 0.04 (2.6 ± 0.1)	7.3 (7.0)	12.0 (50.2)

[a] Open plots also visited by bumblebees and other natural pollinators, including free-ranging *Apis cerana*
[b] Mainly bumblebees, but also free-ranging *Apis cerana*

Effect of *Apis cerana* Pollination on Overall Yield

The impact of *Apis cerana* pollination on overall crop yield was estimated using the values observed in the experimental plots. *Apis cerana* pollination increased the fruit set, seed set, and fruit and seed weight (capsule quality), all of which contribute to increased production. The large cardamom yield per hectare was calculated for plots with and without an *Apis cerana* colony, and controls (Table 9). The yield was calculated by taking the average number of plants per hectare as given by KIRAN (2016), and the number of flowering shoots (panicles) per plant, number of capsules per panicle, and dry weight per capsule from our research findings. The yield per hectare was nearly 45% higher in plots with an *Apis cerana* colony than in those without.

The variety of large cardamom (*ramsai*) planted by farmers in the fields used for the study has 30–40 seeds per capsule. This means that only 30–40 pollen grains are required to fertilize all ovules to produce the seeds (Spices Board of India, 2013). The plots with an *Apis cerana* colony had more pollinators overall because they also had naturally occurring bumblebees, thus the pollination rate was higher than in the naturally pollinated plots. The increased pollination of the flowers in plots with an *Apis cerana* colony led to the higher fruit set, heavier capsules, and overall increase in yield of large cardamom. Previous studies have also shown that supplementing natural pollinators by placing honeybee colonies in a field enhances fruit set, seed set, fruit weight, and seed weight in various crops, including small cardamom (Gupta et al., 1993; Dulta & Verma, 1987; Partap & Verma, 1994; Verma & Partap, 1994; Gupta et al., 2000; Partap 2000a; b; c; Singh et al., 2000; Mayfield & Belawadi, 2012; Sheffield, 2014), but this is the first time it has been shown for large cardamom.

Table 9: **Effect of *Apis cerana* pollination on large cardamom yield**

Parameter	Plot with *Apis cerana* colony	Plot without *Apis cerana* colony	Control (pollinators excluded)
Average number of large cardamom plants per hectare	4,000 (2016)		
Average number of panicles per plant	8		
Average number of panicles per hectare	32,000		
Average number of capsules per panicle	16	14	3
Average number of capsules per hectare	512,000	448,000	96,000
Dry weight of a capsule (g)	1.2	0.95	0.73
Dry weight of capsules in one hectare (g)	614,400	425,600	70,800
Yield (kg/ hectare)	614	425	71
Increase in yield due to managed *Apis cerana* pollination over natural pollination in kg/hectare*	189 (44.5%)		

* The increase in yield over caged plots was not calculated as these were only used to demonstrate the importance of pollinators for crop yield; the method is never used by farmers.

Apis cerana is an Effective Pollinator of Large Cardamom

Our study shows that *Apis cerana* is an important pollinator of large cardamom. The foraging attributes that make *Apis cerana* an effective pollinator for these flowers include foraging throughout the day, visiting all flowers on a panicle and then moving on to another, the unique pattern of approaching the anther-stigma column in flowers from either side, passing over both anthers and stigma while collecting pollen – brushing against the anther to capture pollen grains and touching the stigma in the process. The studies by Sinu and Shivanna (2007), Deka et al. (2011), and Kishore et al. (2011) reported that due to their small size *Apis cerana* bees did not touch the stigma while visiting cardamom flowers and hence did not pollinate the plants, but the observations described here show that this is not the case. The other important foraging attribute of *Apis cerana* is that it visits the same flower many times, which increases the chance for pollination (the more visits to a flower, the more chances of touching the stigma). Sinu and Shivanna (2007) also observed that while bumblebees seldom revisit flowers on the same panicles on a given day, *Apis cerana* visits the same flowers repeatedly. The effect of the increased visits was shown in the significantly higher fruit set, higher number of seeds per capsule, and higher capsule and seed weight in plots supplemented by an *Apis cerana* colony at all three sites.

The results of the present studies prove that *Apis cerana* is an effective pollinator of large cardamom and not a pollen robber as reported earlier by Sinu and Shivanna (2007), Deka et al. (2011), and Kishore et al. (2011). The findings also show that *Apis cerana* and bumblebees complement each other in pollinating large cardamom and when used together increase crop yield and quality. Fruit set, fruit weight, seed set and seed weight was significantly high in the plots where both *Apis cerana* and bumblebees visited the flowers. In areas where bumblebee populations and other natural pollinators are scarce, *Apis cerana* can be used to pollinate large cardamom to ensure a reasonable harvest and better quality capsules. Similar complementarity between different pollinators in pollinating crops, such as apples, cauliflower, cabbages, and mustard have been reported in earlier studies made in mountain areas (Verma and Rana 1994; Verma and Partap 1994).

Conclusion

"*Mauri le ta alainchi lai dherai fayeda gardo rahechha. Mauri ta rakhnu hi parchha ho*". "*Apis cerana* really benefits large cardamom. We must use bees for its pollination," said Mr Mahendra Singh Kunwar, one of our research farmers, impressed by the yield and quality of capsules as a result of *Apis cerana* pollination. Another farmer, Mr Bir Bahadur Kunwar, who had planted the *seremna* variety on his farm, had a similar reaction.

Although large cardamom is a self-fertile crop, it requires cross-pollination for reasonable production due to its flower structure. So far, there has been only limited scientific research on pollination and pollinators of large cardamom. Studies have reported bumblebees to be the main (and most effective) pollinators of large cardamom, but the present on-farm experimental research clearly shows that *Apis cerana* helps enhance the yield and capsule quality in large cardamom through provision of pollination services.

Apis cerana and bumblebees are important pollinators of large cardamom because of their foraging patterns. While bumblebees remain the most effective pollinators of large cardamom due to their large body size and preference for nectar collection, supplementing their numbers by placing *Apis cerana* colonies in the large cardamom fields greatly enhances the yield and quality of capsules. Thus, bumblebees and *Apis cerana* complement each other in achieving sufficient and efficient pollination of large cardamom and improving its yield and quality. If, as seems likely, natural pollinator populations decline in coming years as a result of various factors, domesticated honeybees such as *Apis cerana* can play a great role in pollination of crops to maintain yield and quality. While conservation and protection of the major cardamom pollinators (bumblebees) is important, in areas where bumblebees are scarce, using *Apis cerana* as a supplement will help farmers to obtain a reasonable harvest and better quality capsules.

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International Centre for Integrated Mountain Development
GPO Box 3226, Kathmandu, Nepal
Tel +977 1 5003222 **Fax** +977 1 5003299
Email info@icimod.org **Web** www.icimod.org

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