rence at 50 μg/sq. cm. Historically, the essential oils and individual compounds from medicinal and aromatic plants have been known to exhibit antifeedant properties against a number of insects[15,16]. Several essential oil compounds demonstrated feeding deterrence in a dose-dependent manner, but were less active (3–4 fold) than azadirachtin[17]. However, no reported data are available for geijerene and pregeijerene. These results suggest that the present oils and the isolates served as effective toxicants and anti-feedant deterrents. Nevertheless, a mixture comprising these compounds may be more effective towards the total insecticidal and deterrent activity of the oil of *C. swietenia* with major phytochemical performance by sesquiter-penes, which agrees with published reports[18,19], however, the synergistic action of other phytoconstituents of the oil cannot be disregarded.

The present study indicates that the essential oils and sesquiterpenes of *C. swietenia* exhibit insecticidal properties and compare favourably with the commercial insecticide, monochrotophos. Moreover, these results may lead to the development of newer and more selective natural products, particularly geijerene and pregeijerene as effective insecti-cides, especially with respect to synergy.

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Pollination biology of large cardamom (*Amomum subulatum*)

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Amomum subulatum* Roxb. (family Zingiberaceae) is the large cardamom of commerce cultivated in tropical wet evergreen forests of the Eastern Himalayas of India, Nepal and Bhutan. This study seeks to identify floral visitors and pollinators, examine floral adaptations for pollination and evaluate pollination efficiency. Studies were carried out in two flowering seasons (2005, 2006) in a 6-ha plantation located adjacent to a degraded reserve forest in the Sikkim part of the Himalayas. Only two flower visitor species, a bumble-bee (*Bombus haemorrhoidalis* Smith) and a honey bee species (*Apis cerana* F.) were recorded. The bumble-bee was the effective and only pollinator, but *A. cerana

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was the pollen robber. **Major flower adaptations for pollination by the bumble-bee are the length of the nectar tube, which is not accessible to short-tongued bees and a narrow passage in the fresh flower between the anther–stigma column and the labellum. The narrow passage forces the bumble-bee to push the anther–stigma column to enter the flower, which brings the body of the bumble-bee in contact with the anther and the stigma, and effects pollination. *A. cerana* does not come in contact with the stigma during pollen foraging and hence is unable to bring about pollination. Thus, structural features of the flower of *A. subulatum* differentiate the pollinator and the pollen robber. Pollination efficiency in the plantation was low due to the low population density of wild native pollinator, *B. haemorrhoidalis*.**

Keywords: *Amomum subulatum, Apis cerana, Bombus haemorrhoidalis*, pollination efficiency.

EFFECTIVE pollination is a major limitation for the stability of the yield of several economically important crop species[1]. This is particularly true in the tropics where a larger proportion of species depends on animals as pollinators[2]. Among many other factors, population density of pollinators, their diversity, and visitation frequency, and quantity and quality of the pollen that reaches the stigma are important biotic components affecting pollination success in animal-pollinated crop species[3–5]. Unlike the neotropics where considerable data are available on the details of pollination biology on a large number of species, only limited information is available on species of tropical Asia[6]. The large cardamom (*Amomum subulatum* Roxb., family Zingiberaceae) is one of the major cash crops cultivated between elevations of 600 and 2000 m in tropical wet evergreen forests of Eastern Himalayas in India (Sikkim and Darjeeling areas), Nepal and Bhutan. The seeds have properties similar to those of true cardamom (*Elettaria cardamomum*, family Zingiberaceae) and are used as a condiment for culinary and other preparations. The seeds contain 2–3% essential oils, which possess medicinal properties and are used as adjuncts to various medicinal preparations[7]. As the fruits and seeds are the economic products, effective pollination is a prerequisite for fruit and seed set, and to a large extent determines the yield. In spite of its economic importance, there are hardly any studies on the pollination biology of this species, except for a preliminary report implicating honey bees as pollinators[8]. In this study, we investigated pollination biology of large cardamom in the Sikkim part of Eastern Himalayas. The focus of our study was to document all floral visitors and pollinators, assess pollination efficacy of different pollinators, and understand floral adaptations to achieve effective pollination in large cardamom. We also estimated the pollination efficiency of the plantation under open field conditions to understand whether the plantation was pollinator-limited or not.

The study was conducted in the *A. subulatum* Roxb. plantation (27°24.282′N, 88°37.316′E, elevation 1594 m asl) of the Spices Board of India, located at Kabi about 20 km away from Gangtok, Eastern Himalayas. The 6-ha plantation is located adjacent to a degraded forest and is bordered on one side by a public road with heavy traffic. The farm has scattered shade trees along with some patches of native bamboo thickets, and is intensively managed by regular manual weeding and watering. Investigations were carried out during two flowering seasons (May and June) in 2005 and 2006. We used a widely adapted cultivar 'Sowney' for our studies.

For studying floral phenology, oldest flower buds that would open the next day were tagged in the evening and monitored from 04.00 h the next morning until the flowers senesced. To estimate the amount of nectar, the flowers were selected randomly from different clumps and bagged just before opening to prevent floral visitors. They were excised at hourly intervals ($N = 15$) and the amount of nectar was estimated using calibrated microcapillary tubes (50 µl Drummond Microcaps). Quantitative studies on floral visitors were carried out by selecting a group of 20–25 flowers from 3 to 4 clumps, which were clearly visible from an observation spot. We monitored floral visitors continuously from 06.00 h (soon after opening of the flowers) until 13.00 h (when the flowers cease to attract any visitors) for six days (a total of over 50 h) in the peak of the flowering period. We recorded the frequency of each flower visitor species, the approach, details of foraging and foraging period. Visitors were trapped using either a net or an aspirator. Soon after trapping, the visitors were immobilized by transferring them to a bottle containing a piece of filter paper dipped in ethyl acetate. Immobilized insects were observed under a stereo-microscope to study pollen load on their body.

Preliminary studies showed that bumble-bees (*Bombus haemorrhoidalis*) and honey bees (*Apis cerana*) were the only flower visitors. Bumble-bees seldom revisit the flowers of the same clumps on a given day. We therefore switched the study spot every 15 min. However, *A. cerana* visited the same flowers repeatedly and the same observation spot selected in the early morning was used throughout the observation period on the day. For statistical analyses data of each 2 h time interval were pooled across dates and the difference in the mean visitation frequency and average foraging time of flower visitors across time intervals was compared using one-way ANOVA. Spearman rank correlation coefficient r was used to understand the relationship between available nectar amount and bumble-bee visitation frequency and foraging time. For studying pollination efficiency of bumble-bees, flowers were excised immediately after the first visit and examined for pollen deposition on their stigmas under a stereo-microscope. For *A. cerana*, the bee-visited flowers were harvested at the end of the observation period (12.00 h) and the stigma was scored for the presence of pollen.
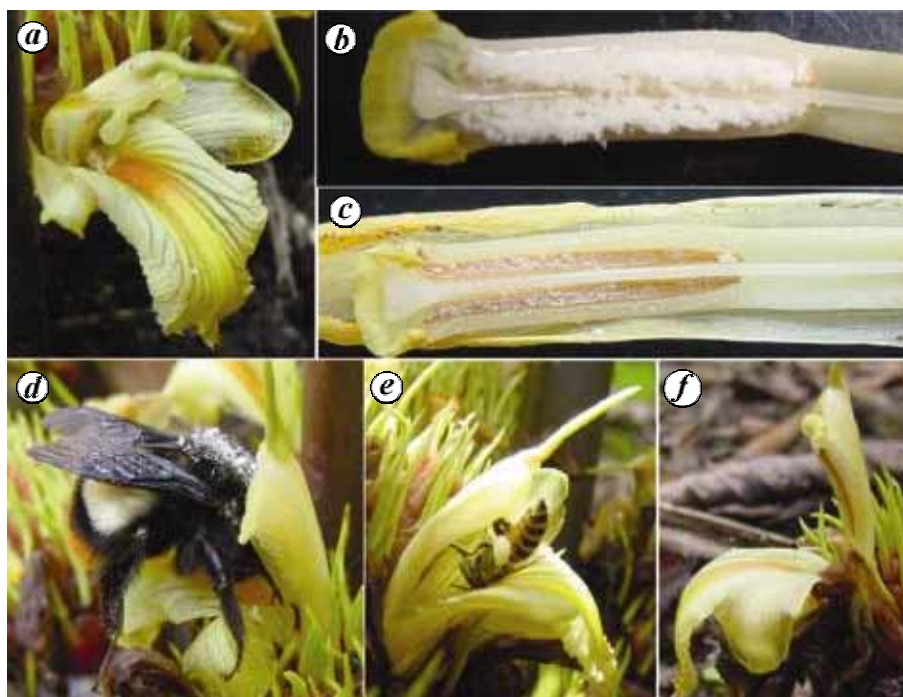
Figure 1. ***a***, An individual flower of *Amomum subulatum*. ***b***, Anther–stigma column photographed from the lower side before honey bee visit to show massive amount of pollen on the anther. The hood is also seen at the tip (left). ***c***, Similar to (***b***), but photographed at 12.00 h after repeated visits by the honey bee. There is hardly any pollen left on the anther. A petal is seen below the anther–stigma column. ***d***, The bumble-bee, *Bombus haemorrhoidalis* engaged in foraging nectar from the flower. Pollen load on its body is apparent. ***e***, The honey bee, *Apis cerana* collecting pollen from the flower. One of the pollen baskets is clearly seen. The distance between the anther–stigma column and the labellum is narrow. ***f***, A flower photographed at 14.00 h to show significant increase in the distance between the anther–stigma column and labellum.

A. subulatum is a perennial herb having subterranean rhizomes which give rise to leafy shoots and spikes. The flowers are borne on shortly peduncled spikes of about 5–6 cm in diameter. The number of inflorescences produced on each clump ranged from 20 to 45 depending on the age of the clump. Each inflorescence produces 30–50 flowers. Flowers are yellowish and measured 7.03 ± 0.26 cm in length ($N = 20$). The most conspicuous part of the flower is the yellowish labellum/lip, which provides a platform for the visiting insects. The basal parts of the petals and the labellum are fused to form a corolla tube/nectar tube (3.07 ± 0.19 cm long, $N = 20$). The terminal-expanded part of the labellum is 3.52 ± 0.12 cm long and 1.4 ± 0.17 cm wide ($N = 20$). The midrib region of the labellum is deeper yellow and the veins are translucent (Figure 1 *a*).

The anther is solitary, borne on about 1.0 cm long filament, originating from the tip of the corolla tube, and measures 10.6 ± 0.74 mm ($N = 15$) in length (Figure 1 *b*). The stamen extends beyond the anther in the form of a rolled-up leafy hood/crest (7 mm long and 8 mm wide; Figure 1 *b* and *c*). The pistil is solitary; the ovary is 5.25 ± 0.57 mm long ($N = 15$), and contains an average of 106.8 ± 5.03 ovules ($N = 15$). The style is long (5.03 ± 1.5 cm, $N = 15$) and delicate; it passes through the groove present between the two pollen sacs. The stigma is cup-shaped (1.0 mm deep and 1.5 mm wide) and slightly flattened (Figure 1 *b* and *c*) with a row of unicellular, non-receptive hairs on its margin. Only the inner surface of the cup is receptive. The stigma cup is pointed distally and the inner wall is lined with a viscous exudate. The stigma extends 1.5–2.0 mm beyond the level of the anther and is covered with the rolled-up extension of the stamen (crest) in the form of a hood. Two yellowish nectaries (3.93 ± 0.32 mm long, $N = 15$) are located at the base of the style and fill the entire space in the lower part of the corolla tube.

A. subulatum flowers during April and May; it may extend up to the end of June in higher altitudes. At the Kabi farm, flowering is initiated during early May and extends for about 6–8 weeks, reaching a peak by the end of May. The flowers open early in the morning, between 04.30 and 06.30 h. The anther dehisces longitudinally just before flower opening and a large mass of pollen grains is visible along the two dehiscing sutures in freshly opened flowers (Figure 1 *b*). The flowers offer both nectar and pollen to the visitors. There is hardly any nectar in the corolla tube soon after anthesis, but it accumulates gradually and reaches a maximum of about 25 μl by 12.00 h. The nectar does not fill the nectar cup completely; its level is invariably 7–10 mm below the mouth of the cup.

The flowers last only for a day; senescence sets in around 14.00 h on the day of anthesis and the flower fades by 18.00 h. Flower senescence is independent of pollination.

The flowers were visited by bumble-bees (*B. haemorrhoidalis*) and the honey bees (*A. cerana*). Bumble-bee visits started early in the morning and continued until 12.00 h. The visit of the bumble-bee was infrequent; after it visited freshly opened flowers of a clump, the bee seldom revisited the clump again on the same day. Thus, most of the flowers receive only one visit of the bumble-bee. The foraging time (24.12 s/flower) was almost uniform in all the time intervals. The differences in foraging time per flower (one-way ANOVA, $F_{5,18} = 0.237$, $P = 0.6$) across the three 2-h time periods were not statistically significant. No significant relationship was found between the nectar volume and average bumble-bee visits (Spearman, $r = 0.13$) or foraging time ($r = 0.18$).

As the size of the bumble-bee was larger than the space between the downwardly curved anther–stigma column and the labellum (Figure 1 *a*), the bee had to push the anther–stigma column upwards to enter the flower. During this process the upper surface of its body came in contact with the anther and the stigma. The bee pushed its head into the nectar tube for foraging nectar, but never collected pollen. The abdomen of the bumble-bee showed rhythmic movement during sucking of nectar. The white pollen coating was clearly visible on the head and thorax of the bumble-bee when it emerged from the flower (Figure 1 *d*). The bumble-bee brought about pollination by pushing the pollen, loaded on its head and thorax, into the distally oriented stigma cup when the bee entered the flower.

A. cerana also visited flowers during morning hours. The frequency of honey-bee visits was more during the early morning (06.00–08.00 h), and gradually reduced over time, but the difference was not statistically significant (one-way ANOVA, $F_{2,23} = 0.82$, $P = 0.45$). Unlike bumble-bees, honey bees collected only the pollen (Figure 1 *e*) and visited the flowers repeatedly. Each flower received an average of 32.3 (± 2.60 SE) visits per day. Unlike their frequency, the foraging time of *A. cerana* increased as the day progressed, although the difference was statistically not significant (one-way ANOVA, $F_{2,23} = 0.49$, $P = 0.61$). Foraging of pollen by *A. cerana* was thorough; there was hardly any pollen left on the anther column in honey bee-visited flowers (Figure 1 *c*). Apart from two pollen baskets on the hind legs (Figure 1 *e*), pollen load was also found throughout the surface of the honey bee.

Pollination efficiency of *B. haemorrhoidalis* and *A. cerana* was determined by direct examination of the stigma of visited flowers (Table 1). One visit of the bumble-bee resulted in pollination of 78% of the flowers. The distance between the anther–stigma column and the labellum on which the pollinator lands steadily increases over time, from 0.5 cm in fresh flower to nearly 2 cm by 16.00 h (compare Figure 1 *a* and *f*; one-way ANOVA, $F_{5, 81} = 83.55$, $P = 0.0001$). Thus, the bee is likely to be less effective in touching the anther–stigma column as the day progressed. To test whether this increase affects pollination efficiency of the bumble-bee over time, we marked all bumble-bee-visited flowers, harvested them at hourly intervals and scored their stigma for pollination. As expected, the bumble-bee-visited flowers showed a steady decline in pollinated flowers and an increase in unpollinated flowers as the day progressed (Figure 2).

A. cerana-visited flowers ($N = 52$) were also excised and observed for pollination. Although most of these flowers had received multiple visits by the bee, and the bees invariably had heavy pollen load, none of the flowers were pollinated; only five of the flowers showed a few pollen grains on the outer non-receptive surface of the stigma cup or the non-receptive hairs on the margin of the stigma cup.

Pollination efficiency under open field conditions was low (Table 1). Flowers collected randomly from the plantation revealed pollination in only about 30% of the flowers.

Three pollination guilds – spiderhunters (family Arachnothera), *Amegilla* bees (family Anthophoridae) and halictid bees (family Halictidae) – have been reported in members of Zingiberaceae, including several species of *Amomum*, in old world tropics[9]. Humming bird and euglossine bee-pollinated guilds have been reported in the Neotropical Zingiberaceae[10,11]. Studies carried out on *Curcumophora longifolia* in the monsoon rainforests of China have shown that *Bombus* sp. and honey bee (*Apis florea*) are the pollinators[12]. A preliminary report on *A. subulatum* in the Himalayas has implicated (but not documented) honey bees as pollinators on the basis of an increase in the yield in the presence of honey bee hives in the plantation[8]. However, this study did not investigate pollination efficiency of honey bees and also did not monitor the visits of any other insects, particularly bumble-bees to the flowers. Our present study, however, clearly demonstrates, on the basis of pollen load on the stigma of flowers visited by the bees that *B. haemorrhoidalis* is the only pollinator and *A. cerana* is not the pollinator, but pollen robber of *A. subulatum*.

Table 1. Pollination efficiency of floral visitors and under open field conditions

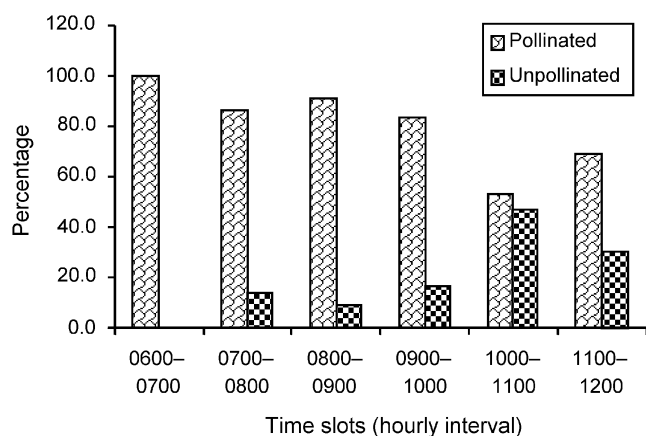| Mode of pollination	Number of flowers examined	Percentage pollination efficiency	
		Pollinated	Unpollinated
After bumble-bee visits	228	78	22
After honey bee visits	52	0	100
Open	170	30	70

Figure 2. Percentage of pollinated and unpollinated flowers visited by bumble-bees in increasing time slots of the day.

The flower is well adapted to bumble-bee pollination. Although pollen grains are exposed and accessible to any visitor, the nectar is protected in the nectar tube of about 3 cm and is accessible only to those visitors with long proboscis. The size of the bumble-bee and the space between the anther–stigma column and the labellum through which the bee has to enter the flower, are critical for successful pollination. In the morning hours, the gap is narrow (ca. 5 mm), and the large-bodied bumble-bee pushes the anther–stigma column upwards to enter the flower to access nectar and effects pollination only if it carried pollen grains from the previously visited flowers. The bee leaves, the flower with pollen deposited on its body but without any contact with the distally pointed receptive stigmatic cup. Thus, the shape and orientation of the stigma have a role to prevent autogamy. It, however, does not prevent geitonogamy as the bumble-bee visits several other flowers of the same clump one after another. There was no significant correlation between the amount of nectar present in the flower and the frequency of bumble-bee visits or their foraging time. These features indicate that the pollination guild in *A. subulatum* is not optimized.

A. cerana collected only pollen seemingly due to inaccessibility of nectar with its short probosis. The position and structure of the stigma and pollen-collecting behaviour of this bee suggest that the latter has no role in pollination and simply acts as a pollen robber, depriving the plant of pollen availability for pollination by bumble-bees.

Many of the early investigators on pollination biology have not distinguished flower visitors and pollinators[13]. Several studies have identified pollinators on the basis of pollen load on the visitor. Our present study clearly emphasizes the importance of distinguishing flower visitors and pollinators on the basis of stigmatic pollen loads. On the basis of our studies, the pollination syndrome of *A. subulatum* can be considered as specialized as there is only one effective pollinator (see Johnson and Steiner[13],

and Waser *et al.*[14] for a discussion on generalization vs specialization).

The pollination efficiency of the plantation used for the present investigation was low, as about 70% of the flowers remained unpollinated. This is obviously because of lower population density of the wild pollinator species, *B. haemorrhoidalis* in the plantation. The present study advocates the need for similar systematic studies in other locations subjected to different degrees of habitat degradation, to have a better understanding of the key factors that affect bumble-bee population density and pollination efficiency.

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