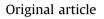
Journal of Asia-Pacific Biodiversity 9 (2016) 17-21

Contents lists available at ScienceDirect

Journal of Asia-Pacific Biodiversity

journal homepage: http://www.elsevier.com/locate/japb



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Variations in pollinator density and impacts on large cardamom (*Amomum subulatum* Roxb.) crop yield in Sikkim Himalaya, India

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ARTICLE INFO

Article history: Received 22 September 2015 Received in revised form 29 December 2015 Accepted 31 December 2015 Available online 8 January 2016

Keywords: Bombus sp. large cardamom pollination pollinator Sikkim Himalaya

ABSTRACT

Large cardamom (*Amomum subulatum* Roxb.), a perennial cash crop, cultivated under an agroforestry system in the eastern Himalaya of India, is well recognized as a pollination-dependent crop. Observations on pollinator abundance in Mamlay watershed of Sikkim Himalaya were collected during the blooming season to evaluate the pollinator abundance across sites and time frames, and impact of pollinator abundance on crop yield from 2010 to 2012. The results revealed that the bumblebees and honeybees are most frequent visitors of large cardamom flowers. The abundance of honeybees, however, varied between sites for the years 2010-2012, while that of bumblebees varied for the years 2011 and 2012. The abundance of honeybees resulted in a variation within time frames for 2010 and 2011, while that of bumblebees varied for 2010 and 2012 (p < 0.01). The density of pollinators correlated positively with the number of flowers of the target crop. The impact of pollinator abundance revealed that the increasing bumblebee visitation resulted in a higher yield of the crop (i.e. 17-41 g/plant) and the increasing abundance of all bees (21-41 g/plant) was significant (p < 0.03). Therefore, the study concluded that the large cardamom yield is sensitive to pollinator abundance and there is a need for adopting the best pollinator conservation and management practices toward sustaining the yield of large cardamom.

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Introduction

The large cardamom (*Amomum subulatum* Roxb.), family Zingiberaceae, is one of the major perennial cash crops in the eastern Himalaya of India, Nepal, and Bhutan. In particular, Sikkim, a Himalayan state of India, contributes nearly 57% of world's total production (Sharma et al 2000). The species is cultivated between an elevation of 600 m and 2000 m above sea level (asl) under different agroforestry systems (Sharma et al 2009). In addition, cultivation of large cardamom is considered cost effective compared with other crops as it is a less labor-intensive and nonnutrient exhaustive crop (Singh et al 2005; Singh 2008). The capsule and seeds are used as condiments for culinary and other preparations due to their pleasant aromatic odor; the seeds contain 3% essential oil with a high cineole content having

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medicinal properties (Sharma et al 2000; Sinu and Shivanna 2007; Singh 2008; Kishore et al 2012). The species has subterranean rhizomes that give rise to leafy shoots with spikes. The flowers emerge during the months of April–June, and the numbers of inflorescences produced on each bunch range from 20 to 45 depending on the age of the clump (Sinu and Shivanna 2007). Each inflorescence produces ~ 30-50 yellowish flowers (4.5–8 cm in length). The most prominent part of the flower (i.e. yellowish labellum/lip) provides a platform for the visiting insects (Sinu and Shivanna 2007).

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A. subulatum is a pollinator-dependent crop (Sharma et al 2000), and its quality and quantum of yield is determined by effective pollination services. In spite of its proven value, pollination studies on this species have been neglected until recently, when Sinu and Shivanna (2007) explored pollination biology of this species in Sikkim part of the Indian Himalaya. Subsequently, other workers have contributed to the knowledge base on pollinators and pollination biology of *A. subulatum* (Deka et al 2011; Sinu et al 2011; Kishore et al 2012). Although Kishore et al (2011) have studied the pollination efficiency and nectar production in large cardamom, several issues, which pertain to pollinator

http://dx.doi.org/10.1016/j.japb.2015.12.010

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diversity, frequency, habitat dependence, and more importantly impact of pollinator activity on the yield of the crop, have not yet been resolved. Realizing this and considering its importance, the present study targets *A. subulatum* in Sikkim Himalaya for exploring the following: (1) variations in pollinator abundance during the blooming period across diverse site conditions (different levels of naturalness as described under the "Materials and methods" section); (2) interaction of pollinators with flowering frequency; and (3) impact of pollinator abundance on crop yield.

Materials and methods

Study areas and observational sites

The Mamlay watershed (from $27^{\circ}12'3''$ to $27^{\circ}16'4''N$ latitude, and from $88^{\circ}19'2''$ to $88^{\circ}23'30''E$ longitude), situated in the southern part of Sikkim in India, forms the study area and covers an area of 31.89 km^2 (Figure 1). Watershed experiences diverse landuse patterns (i.e. agricultural land 11.22 km^2 , open mixed forest and scrub land 10.41 km^2 , reserved forest 8.63 km^2 , and settlement 2.61 km^2) along an extensive altitudinal range (300-2650 m asl) that represents a wide range of traditional agricultural practices including agroforestry.

Observational sites were identified in the large cardamomgrowing areas traditionally cultivated by farmers. The sites were selected to represent three distinct landscape features: Site 1—within village boundary (human inhabited area where interventions in natural conditions are frequent in diverse form); Site 2—under forest canopy (villagers frequently harvest forest products—leaves for fodder, wood for fuel, etc.); and Site 3—close to reserved forest, i.e. 200 m (villagers seldom use forest products). The minimum distance between any two sites was 1.5 km. Four subplots of $20 \times 20 \text{ m}^2$ were established at each site for this study.

Data collection

Data were recorded in the observational plots from the onset of the main blooming period of the target crop (3rd week of May onward). Pollinator abundance was measured by scan sampling (Levin et al 1968) in four different time intervals (1st, May 23–31; 2^{nd} , June 2–10; 3^{rd} , June 12–20; and 4^{th} , June 22–30). These time intervals were chosen to capture the overall changes in flowering abundance across the flowering season. The recording hours [i.e. morning hours (0800-1200 hours)] were same for each site. During the recording time, weather conditions, i.e. sunny or overcast, were also observed. A day was considered sunny when the cloud cover varied between 0% and 50% and overcast when the cloud cover varied between 50% and 100%. Scan sampling process was a slow walk along a set path or row, and recording of the insect visitors/pollinators seen on individual floral parts (i.e. simultaneously count the 100 open flowers and visit of insects/pollinators to open flowers). The pollinator abundance was recorded per 100 open flowers with a similar process in each plot of the three observational sites. Moreover, the recording time in each plot was logged (count the insects/pollinators within 15 minutes). Considering the covariance effect on pollinator diversity, flowering observation was recorded for five randomly selected plants in individual plots (i.e. 20 plants in each site). The number of fullbloomed flowers from each plot was counted for a given period of time. For estimating the deficit of pollination in the target crop, vield data (fruit weight per plant) of the crops of the selected plots were measured during harvesting (i.e. mid-October). The mean of randomly selected five plants per plot was considered for measurement of the yield per plant.

Data analysis

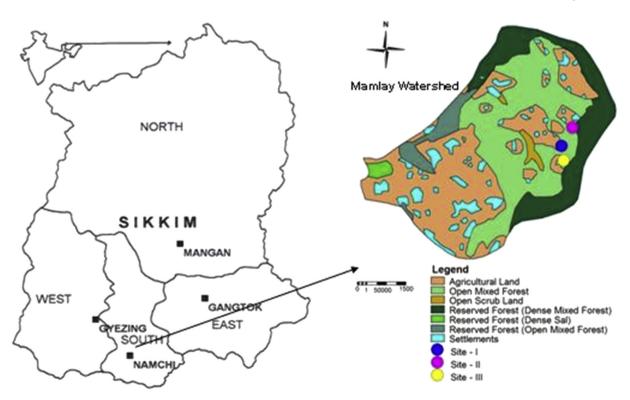


Figure 1. Land use pattern of study area (Mamlay watershed).

The mean \pm standard error of pollinator abundance was calculated. Based on the observational data structure, generalized linear



Figure 2. Major visitors/pollinators of large cardamom. A, honeybee (A. cerana); B, bumblebee (Bombus sp.).

Table 1. Average frequency of insect visitors per 100 flowers of Amomum subulatum per year.

Year	Apis cerana	Bombus sp.	Other bees
2010	2.4	2.8	0.1
2011	0.9	5.1	0.1
2012	0.5	2.6	0.4
$\text{Mean}\pm\text{SE}$	1.3 ± 0.6	$\textbf{3.5}\pm\textbf{0.8}$	$\textbf{0.2}\pm\textbf{0.1}$

SE = standard error.

mixed model analysis was performed to test the effects of sites, time frames, and their interaction on pollinator abundance using the SPSS 21 statistical package (© IBM Corporation, 2011). For comparing the mean of abundance between sites and time frames for the 3 consecutive years, the least significant difference (LSD) was calculated. After combining the observational data of different subplots for 3 consecutive years to ensure the appropriate sample size, Spearman rank correlation analysis was applied to find the interaction between flowering frequency and abundance of pollinators. Moreover, a linear regression model was applied to estimate the impact of pollinators on crop yield.

Results

Pollinator abundance across sites, time, and flower phenology

Among the insect visitors, *Apis cerana* and *Bombus* sp. were observed as the most frequent visitors of large cardamom

Table 2. Generalized linear mixed model analysis to find the effect of sites, time frames, and their interaction on the abundance of *Apis cerana* and *Bombus* sp. (per 100 open flowers of large cardamom) in 3 consecutive years.

Source	df	A. cerana		Bombus sp.	
		F _{value}	p_{value}	F _{value}	p_{value}
Year 2010					
Intercept	1	108.624	< 0.001	213.762	< 0.001
Site (S)	2	4.000	0.025	0.869	0.426
Time frame (T)	3	6.294	0.001	22.651	< 0.001
$S \times T$	6	1.415	0.228	4.758	0.001
Year 2011					
Intercept	1	85.263	< 0.001	134.179	< 0.001
Site (S)	2	8.463	0.001	7.143	0.002
Time frame (T)	3	28.441	< 0.001	1.118	0.351
$S \times T$	6	4.870	0.001	1.373	0.245
Year 2012					
Intercept	1	38.629	< 0.001	174.727	< 0.001
Site (S)	2	4.343	0.018	6.045	0.005
Time frame (T)	3	1.600	0.202	6.909	0.001
$S \times T$	6	1.600	0.168	1.227	0.309

df = degree of freedom.

(Figure 2). The average abundance of the *Bombus* sp. (3.5 ± 0.8) was more than that of the *A. cerana* (1.3 ± 0.6) and other bees (0.2 ± 0.1) (Table 1).

Considering the site variation, it was, however, revealed that the abundance of the identified important visitors (*Bombus* sp. and *A. cerana*) did not follow uniform patterns across different sampling years (Table 2). These variations were significant in 2010 ($F_{cal} = 4.00$), 2011 ($F_{cal} = 8.463$), and 2012 ($F_{cal} = 4.343$) (p < 0.05) for *A. cerana*, and between 2011 ($F_{cal} = 7.143$) and 2012 ($F_{cal} = 6.045$) for *Bombus* sp.

While considering the abundance of pollinators across observation times, the abundance of *A. cerana* varied significantly (p < 0.01) in 2010 ($F_{cal} = 6.294$) and 2011 ($F_{cal} = 28.441$), whereas for bumblebees, significant variations in abundance with time frames were observed in 2010 ($F_{cal} = 22.651$) and 2012 ($F_{cal} = 6.909$). Effects of interactions of sites and time frames on pollinator abundance were significant in the year 2011 for honeybees and in 2010 for bumblebees (Table 2).

A significant positive correlation exists between the number of flowers and the density of *A. cerana* (r = 0.50; p < 0.01) and *Bombus* sp. (r = 0.37; p < 0.05), and the total number of bees (r = 0.46; p < 0.01).

Impact of landscape features on pollinators

Considering the effects of landscape features on the abundance of pollinators (i.e. *A. cerana* and *Bombus* sp.) in large cardamom flowers, the results showed (Figure 3) that the sites located within villages had significantly higher visitation by bumblebees in the years 2010 (LSD = 2.505; p < 0.05) and 2012 (LSD = 1.161; p < 0.05). The honeybee visitation pattern indicated that a significantly higher visitation rate was observed for sites close to natural habitats in the year 2010 (LSD = 1.273; p < 0.05), those within villages in 2011 (LSD = 0.551; p < 0.05), and those under forest canopy in 2012 (LSD = 0.496; p < 0.05). In this context, it can be concluded that honeybee visitation to large cardamom did not follow a uniform pattern across the years.

Impact of pollinator density on yield

A linear regression model estimated that the increased density of pollinators per 100 flowers results in an increase in the yield of large cardamom (Figure 4). The model projected, on average, an increase of 17–41 g/plant in the yield of large cardamom with an increase in the number of bumblebees, which was significant (p < 0.03). However, considering an increase in the number of total bees, a significant (p < 0.01) increase of 21–41 g/plant was

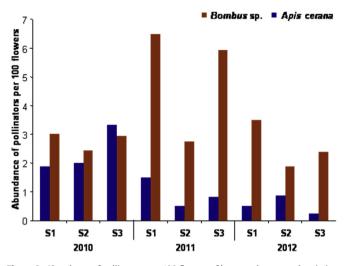


Figure 3. Abundance of pollinators per 100 flowers of large cardamom and variations across sites (S1: within village boundary; S2: under forest canopy; and S3: close to natural forest).

estimated (Table 3), which may be due to that honeybees and other bees also contribute to pollination services in large cardamom crop.

Discussion

Pollinator abundance across sites, time, and flower phenology

Observations from the present study corresponded with earlier reports (Sinu and Shivanna 2007), which identified honeybees (*A. cerana*) and bumblebees (*Bombus haemorrhoidalis*) as the most frequent visitors of large cardamom, of which bumblebees emerged as the effective pollinators (Kishore et al 2011). Another study from Sikkim further supported this fact, reporting 100% pollination efficiency of bumblebees in large cardamom (Kishore et al 2012). In support of the earlier reports of bumblebees as the predominant visitors of large cardamom (Deka et al 2011; Sinu et al 2011), we report a greater frequency of bumblebee visitations than that of honeybee visitations (Table 1).

Results of the current study indicate that the abundance of pollinators varied across years. Reasons for such inconsistent results would call for explanations from in-depth investigations, especially emerging from studies on the (1) interaction between abundance of pollinators and other foraging resources and (2) relationships of pollinators with annual variations in weather.

The positive correlation between the frequency of flowers and abundance of pollinators revealed that the high frequency of forage availability attracts both *A. cerana* and *Bombus* sp. Similarly, Tchuenguem et al (2009) indicated a positive relationship between the number of honeybee visits and the number of open flowers.

Impact of landscape features on pollinators

Impact of landscape features indicates that honeybees can frequently visit the flowers of large cardamom in different landscapes. However, bumblebee visitations were higher mostly in sites located within village boundaries. Heard et al (2007) investigated the bumblebee abundance in different landscape features and concluded that the impacts of landscape characteristics were distinct due to its foraging resources.

These results are indicative of the fact that the abundance patterns of bumblebees as pollinators of large cardamom are dependent on landscape features (i.e. closeness of natural habitats).

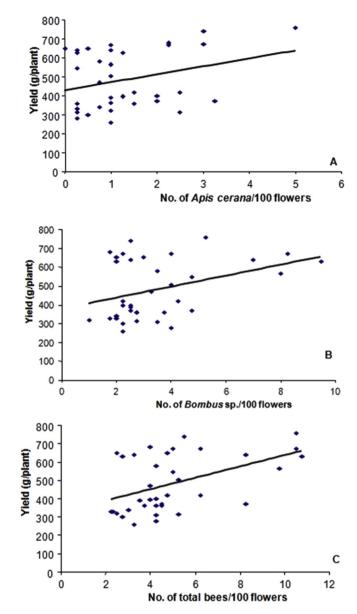


Figure 4. Impact of pollinator abundance on the yield of large cardamom crop. A, impact of the abundance of *A. cerana* ($t_{cal} = 1.856$; p > 0.05); B, *Bombus* sp. ($t_{cal} = 2.307$; p < 0.05); C, total bees ($t_{cal} = 3.161$; p < 0.01).

However, it is also important to mention that forests and foraging resources are predominant in the landscape and form a very closeknit mosaic of different forest canopy features (Figure 1), and it is not possible to have a very clear demarcation of natural forests, forest canopies, and village sites.

Effect of weather conditions on pollinators

More convincing explanations for pollinator abundance variations may be obtained through patterns across different time

Table 3. Linear regression model analysis to assess the impact of pollinator's abundance on the yield of large cardamom.

Pollinators	Ν	β	SE	R^2	t _{value}	р
Apis cerana	36	22.94	22.94	9.20	1.856	0.07
Bombus sp.	36	28.98	12.13	13.54	2.307	0.03
Total bees	36	30.94	9.78	20.44	3.161	0.01

 β = regression coefficient; *N* = total number of observations; *R*² = coefficient of determination; SE = standard error.

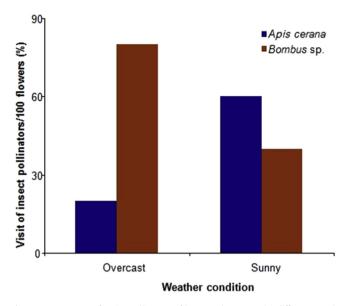


Figure 5. Frequency of major pollinators of large cardamom under different weather conditions, i.e. overcast (9 days) and sunny (3 days).

frames that suggest weather-dependent variations. In this context, the performance of the two pollinators (*A. cerana* and *Bombus* sp.), under different weather conditions, can be summarized as follows: more frequent visits of *A. cerana* to large cardamom flowers during sunny days and a prevalence of *Bombus* sp. in overcast conditions (Figure 5). Maximum flowering of large cardamom occurs in the early rainy season (May–June), which means that rain with overcast conditions would remain prevalent during its flowering. As bumblebees are more active during such conditions, it is safe to indicate that bumblebees are most suitable pollinators of large cardamom crop, compared with *A. cerana*. This fact has also been reported in several other studies (Deka et al 2011; Sinu and Shivanna 2007; Kishore et al 2011).

Impact of pollinator density on yield

Impact of pollinator abundance on large cardamom yield suggests that the yield of large cardamom would definitely decline if an adequate number of suitable pollinators were not available. This observation has wide-ranging implications in explaining the overall declining trends of large cardamom production in Sikkim. The reasons for decline are most often attributed to various factors such as soil nutrient dynamics, disease and pests, climatic variability, and farming practices (Sharma et al 2000), without even indicating inadequate pollination as a possible reason. Therefore, findings of the present study call for attention toward conservation and management of bumblebees under the farming practices of large cardamom. In this context, it is important to mention that the study by Garibaldi et al (2009) predicts that, without pollination by animal pollinators, the yield would reduce, on average, by 95% globally. In addition, Allen-Wardell et al (1998) concluded that a yield decline in California almond orchards in 1995 could be attributed to a combination of weather and pollinator loss. Such inferences drawn from other studies should not be ignored while analyzing the overall decline in large cardamom yield in Sikkim.

The present study concludes the following points: (1) bumblebees are the most common pollinators of large cardamom compared with honeybees (*A. cerana*); (2) pollinator abundance in large cardamom sites and different time frames varies significantly, and no definite patterns are discernible with site characteristics; (3) flower abundance of the target crop significantly influences pollinator abundance; and (4) pollinator abundance impacts significantly the yield of large cardamom, thereby highlighting the need for adopting suitable pollinator conservation and management practices for sustaining the yield of large cardamom.

Acknowledgments

We thank Dr P.P. Dhyani, Director of G. B. Pant Institute of Himalayan Environment & Development, for providing necessary facilities and encouragement. The financial support received from GEF/UNEP/FAO (Global Pollination Project) is thankfully acknowledged. The critical comments and suggestions from anonymous reviewers helped us in improving the manuscript. We would also like to thank Dr Triloki Pant for statistical support.

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