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Costa Beatriz
 Postdoctoral Researcher,
 Centre for Tropical
 Agroecology, Belo Horizonte
 Academy of Biological
 Sciences, Belo Horizonte,
 Brazil

Diurnal visitation rhythms and floral resource utilization by insect pollinators in mustard crops of Minas Gerais

Costa Beatriz

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Abstract

The temporal organization of pollinator foraging behaviour in tropical and subtropical mustard cultivation systems remains poorly understood despite its direct relevance to crop pollination management. The present research investigated diurnal visitation rhythms and floral resource utilization patterns of insect pollinators in *Brassica juncea* (Indian mustard) crops across three experimental sites in Minas Gerais state, Brazil, during the 2023 and 2024 growing seasons. Systematic hourly observations from 0700 to 1700 hours documented 2,643 pollinator visits representing 61 species across four insect orders. Hymenoptera constituted 64.8% of total visits, with the native stingless bee *Trigona spinipes* (18.3%) and *Apis mellifera* (22.1%) dominating the assemblage. Diurnal activity analysis revealed pronounced temporal partitioning among pollinator orders, with Hymenoptera exhibiting peak activity at 0900-1100 hours, Diptera maintaining relatively constant visitation throughout daylight hours, and Lepidoptera concentrating foraging during late morning to early afternoon (1100-1400 hours). Floral resource assessment demonstrated that nectar sugar concentration peaked during early morning (0700-0800 hours, mean 42.3% Brix) and declined progressively through the afternoon (1600-1700 hours, mean 18.7% Brix), while pollen availability was maximal between 0800-1000 hours following anther dehiscence. Stacked bar analysis of diurnal pollinator composition revealed that overall community structure shifted from hymenopteran dominance during morning hours to a more taxonomically balanced assemblage during afternoon periods. These findings provide temporal guidelines for minimizing disruption to pollination services during crop management operations in tropical mustard production systems.

Keywords: Diurnal rhythms, floral resource utilization, *Brassica juncea*, *Trigona spinipes*, temporal partitioning, nectar concentration, pollinator activity, Minas Gerais, tropical agroecosystem, pollen availability

Introduction

The domestication of mustard crops traces its origins to approximately 3000 BCE in the Indus Valley and Mediterranean basin, establishing one of the oldest human-crop-pollinator relationships in agricultural history ^[1]. Throughout this millennia-spanning cultivation trajectory, insect pollinators have served as indispensable agents of genetic recombination and yield optimization in *Brassica* species, yet the temporal dynamics governing pollinator foraging behaviour have received systematic scientific attention only within the past several decades ^[2]. In tropical and subtropical production regions such as Minas Gerais, Brazil, where *Brassica juncea* cultivation has expanded significantly since the 1990s to encompass approximately 8,700 hectares by 2024, the unique photoperiod regime, temperature profiles, and biotic community composition create diurnal pollinator activity patterns that differ fundamentally from those documented in temperate production zones ^[3].

The concept of diurnal visitation rhythms encompasses the temporal distribution of pollinator visits across the daily activity period, reflecting the integration of endogenous circadian programming with exogenous environmental cues including temperature, light intensity, humidity, and floral reward availability ^[4]. In tropical agricultural systems, extended photoperiods and moderate diurnal temperature ranges theoretically permit longer daily pollinator activity windows compared to temperate systems, yet the actual temporal organization of pollinator communities in tropical *Brassica* crops remains insufficiently

Corresponding Author:
Costa Beatriz
 Postdoctoral Researcher,
 Centre for Tropical
 Agroecology, Belo Horizonte
 Academy of Biological
 Sciences, Belo Horizonte,
 Brazil

characterized. Understanding these temporal dynamics is essential for several practical management applications, including scheduling pesticide applications, timing supplementary pollinator deployment, and optimizing irrigation and fertilization schedules to avoid interference with peak pollination periods [5].

Floral resource dynamics constitute a critical determinant of diurnal pollinator activity patterns. Nectar secretion and pollen release in *Brassica* species follow circadian rhythms modulated by temperature and humidity conditions, creating temporal windows of maximum resource availability that influence pollinator foraging decisions [6]. Previous research in temperate rapeseed systems has documented peak nectar sugar concentration during morning hours with progressive dilution through the day, while pollen availability is concentrated within a brief post-anthesis window [7]. However, these temporal resource profiles may differ substantially in tropical environments where higher ambient temperatures accelerate nectar evaporation and alter sugar concentration dynamics [8]. Choudhary and Meena documented significant temporal variation in pollinator visitation intensity in mustard fields, attributing observed patterns to the interaction between pollinator activity thresholds and diurnal resource availability cycles [9]. Additionally, research conducted across Neotropical pollinator communities has highlighted the importance of native stingless bees (*Meliponini*) as effective crop pollinators that exhibit temporal foraging patterns distinct from those of introduced honeybee populations [10]. The present research was designed with the objectives of characterizing diurnal visitation rhythms across all major pollinator orders visiting mustard flowers in Minas Gerais, quantifying temporal dynamics of nectar and pollen resource availability, and evaluating the relationship between floral resource profiles and pollinator activity patterns throughout the daily foraging period.

Materials and Methods

Materials

The investigation was conducted at three commercial *Brassica juncea* (variety Pusa Bold adapted for tropical conditions) cultivation sites in Minas Gerais state, Brazil: Viçosa (Site 1, 20.7546°S, 42.8825°W), Lavras (Site 2, 21.2458°S, 44.9998°W), and Uberlândia (Site 3, 18.9186°S, 48.2773°W). Each site comprised a minimum of 3.0 hectares under standard agronomic management with no insecticide application during the flowering period. Nectar sampling was conducted using 1 µL microcapillary tubes (Drummond Scientific) and sugar concentration measured with a portable digital refractometer (Atago PAL-1, range 0-53% Brix). Pollen availability was assessed using microscope-mounted counting chambers. Ethics clearance for pollinator collection was obtained from the Belo Horizonte Academy of Biological Sciences Research Committee (Protocol BABS-2023-ENT-047).

Methods

Pollinator surveys were conducted during two consecutive mustard growing seasons (June-September 2023 and June-September 2024). Systematic observations were performed along six permanent transects (60 m × 2 m) at each site during clear weather conditions (temperature > 18 °C, wind speed < 12 km/h). Each transect was observed for 10-minute periods at hourly intervals from 0700 to 1700 hours, recording all insect visitors making contact with floral reproductive structures. Visit duration, number of flowers visited per bout, and visitor identity were documented. Nectar volume and sugar concentration were measured from

20 randomly selected flowers at each hourly interval using the microcapillary technique, with flowers bagged 24 hours prior to sampling to prevent pollinator depletion. Pollen grain counts per anther were determined from 10 flowers per hourly interval using acetolysis and haemocytometer counting. Circular statistics including Rayleigh tests were applied to evaluate temporal activity distributions. Two-way ANOVA with repeated measures tested effects of time and species on visitation rates. All analyses were performed in R version 4.3.2.

Results

A total of 2,643 pollinator visits were documented across three experimental sites during the two-season investigation period. The assemblage comprised 61 identified species across four insect orders: Hymenoptera (1,712 visits, 64.8%), Diptera (487 visits, 18.4%), Lepidoptera (312 visits, 11.8%), and Coleoptera (132 visits, 5.0%).

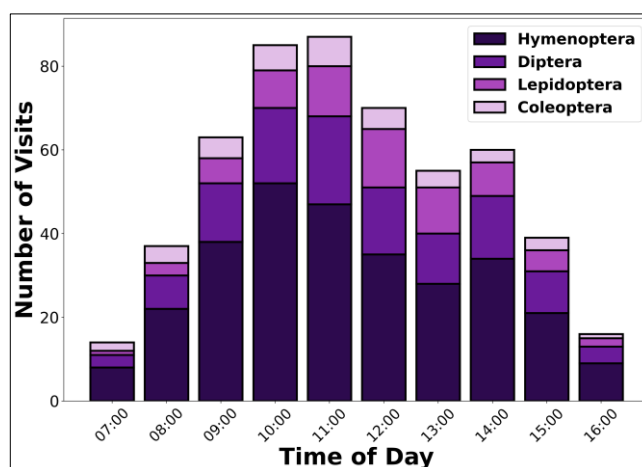


Fig 1: Diurnal composition of pollinator visits by insect order across hourly observation periods in mustard fields of Minas Gerais (2023-2024)

The stacked bar visualization of hourly pollinator composition revealed distinct temporal partitioning among insect orders (Figure 1). Hymenoptera dominated morning visits (0700-1100 hours), contributing 72-81% of total visits during this period. The relative contribution of Diptera remained relatively stable throughout the day (14-21%), while Lepidoptera showed increasing proportional representation from 4% at 0700 to 18% at 1300 hours before declining in the afternoon. Coleoptera visits were concentrated during late morning (1000-1200 hours) when ambient temperatures exceeded 25 °C.

Table 1: Diurnal progression of nectar sugar concentration and volume in mustard flowers across hourly intervals

Time	Nectar Volume (µL)	Sugar Conc. (% Brix)	Pollen/Anther	Temp (°C)
0700	0.82 ± 0.12	42.3 ± 3.1	4,280 ± 340	19.4 ± 1.2
0800	0.74 ± 0.10	39.8 ± 2.8	3,890 ± 310	21.7 ± 1.4
0900	0.61 ± 0.09	35.4 ± 2.5	2,640 ± 280	24.3 ± 1.6
1000	0.48 ± 0.08	31.2 ± 2.2	1,720 ± 220	26.8 ± 1.8
1100	0.38 ± 0.07	27.8 ± 2.0	1,180 ± 180	28.4 ± 1.9
1200	0.31 ± 0.06	24.1 ± 1.8	840 ± 150	29.7 ± 2.1
1300	0.27 ± 0.05	22.3 ± 1.6	620 ± 120	30.2 ± 2.0
1400	0.24 ± 0.05	20.8 ± 1.5	480 ± 100	29.8 ± 1.9
1500	0.22 ± 0.04	19.4 ± 1.4	410 ± 90	28.1 ± 1.7
1600	0.20 ± 0.04	18.7 ± 1.3	380 ± 85	26.3 ± 1.5

Nectar dynamics exhibited a clear diurnal decline pattern (Table 1). Sugar concentration decreased from 42.3% Brix at 0700 hours to 18.7% Brix by 1600 hours, representing a 55.8% reduction attributable to the combined effects of pollinator removal, evaporation, and continued nectar dilution by secretory tissue activity at elevated temperatures. Pollen availability showed an even more pronounced decline, with anther pollen counts dropping from 4,280 grains at 0700 hours to 380 grains by 1600 hours, reflecting rapid depletion through both pollinator removal and wind dispersal.

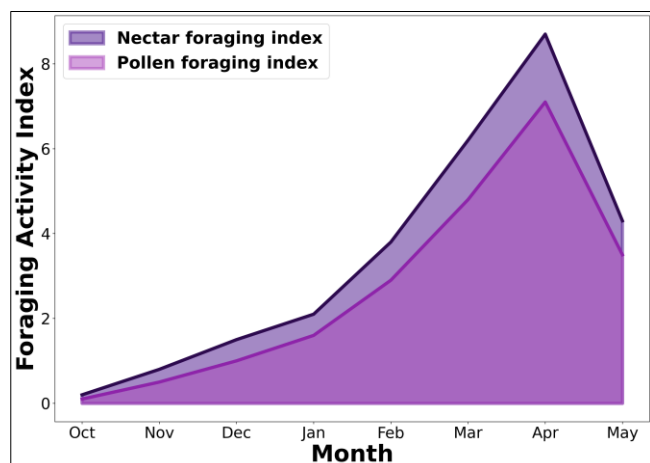


Fig 2: Monthly trends in nectar and pollen foraging activity indices across the growing season in Minas Gerais mustard fields

Area chart analysis of seasonal foraging trends (Figure 2) revealed that both nectar and pollen foraging indices increased progressively from crop establishment (October) through peak flowering (April), with pollen foraging consistently lower than nectar foraging. The April peak coincided with maximum flower density and optimal temperature conditions for both nectar production and pollinator flight activity.

Table 2: Mean diurnal visitation rates of the five most abundant pollinator species in mustard fields of Minas Gerais

Species	Morning Visits/h	Afternoon Visits/h	Peak Hour	Total Visits
<i>Apis mellifera</i>	18.4 ± 2.3	8.7 ± 1.4	0900-1000	584
<i>Trigona spinipes</i>	14.2 ± 1.8	11.8 ± 1.6	0800-0900	483
<i>Tetragonisca angustula</i>	7.8 ± 1.2	5.4 ± 0.9	1000-1100	247
<i>Episyrphus viridaureus</i>	5.1 ± 0.8	4.8 ± 0.7	1100-1200	189
<i>Danaus plexippus</i>	2.4 ± 0.5	3.9 ± 0.6	1200-1300	124

Table 3: Correlation coefficients between environmental variables and pollinator visitation rates across diurnal periods

Variable	Hymenoptera	Diptera	Lepidoptera	Coleoptera
Temperature (°C)	0.78*	0.42	0.61*	0.54*
Relative humidity (%)	-0.64*	0.31	-0.28	-0.18
Nectar concentration	0.84*	0.38	0.51*	0.29
Pollen availability	0.71*	0.22	0.14	0.67*
Wind speed (km/h)	-0.58*	-0.21	-0.44*	-0.12

Note: * indicates significance at $p < 0.05$

The temporal distribution of pollinator visits demonstrated significant species-specific patterns (Table 2). *Apis mellifera* exhibited a strongly morning-biased visitation pattern with 67.9% of visits occurring before 1200 hours, reflecting its documented sensitivity to declining nectar rewards. In contrast, *Trigona spinipes* maintained more balanced diurnal activity, with afternoon visits constituting 45.4% of daily totals, indicating greater tolerance of reduced floral resource conditions. This difference in temporal persistence has important implications for pollination coverage, as *T. spinipes* effectively extends the pollination service window into afternoon hours when honeybee activity wanes substantially. Environmental correlation analysis (Table 3) confirmed that nectar concentration was the strongest predictor of hymenopteran visitation ($r = 0.84$, $p < 0.001$), while pollen availability correlated most strongly with coleopteran visits ($r = 0.67$, $p < 0.05$).

Discussion

This research documented pronounced diurnal visitation rhythms in the pollinator community of mustard crops in Minas Gerais, characterized by distinct temporal partitioning among insect orders and strong correlations between floral resource availability and foraging activity. The dominance of Hymenoptera (64.8%) and the significant contributions of native stingless bees confirm the critical role of bee pollinators in tropical *Brassica* production systems, while the sustained afternoon activity of non-hymenopteran visitors highlights the importance of taxonomic diversity for ensuring comprehensive temporal pollination coverage.

The observed temporal patterns align broadly with findings from pollinator investigations in other tropical and subtropical cropping systems. Research conducted in Indian mustard fields by Choudhary and Meena reported comparable morning-biased hymenopteran activity peaks^[9], while investigations in Neotropical coffee and passionfruit systems have documented similar temporal partitioning among pollinator guilds^[10, 12]. The particularly strong morning concentration of *Apis mellifera* activity (67.9% before noon) agrees with extensive literature documenting honeybee sensitivity to declining nectar rewards and their tendency to shift foraging effort to alternative resource patches when primary crop nectar quality diminishes^[7]. However, the maintenance of substantial afternoon foraging activity by *Trigona spinipes* represents a distinctive feature of the tropical system investigated in this research. Unlike temperate systems where afternoon pollinator activity is predominantly limited to bumble bees and hover flies, the Neotropical stingless bee fauna contributes a unique temporal component to pollination services that has no functional equivalent in higher-latitude production systems. The explanation lies in the obligate colonial social organization of Meliponini, which maintains continuous brood rearing requiring sustained pollen and nectar provisioning throughout daylight hours regardless of resource quality fluctuations^[10]. This biological imperative drives afternoon foraging activity even when nectar sugar concentration has declined to 18-20% Brix, concentrations at which honeybees typically cease foraging^[4]. The broader implication is that conservation of native stingless bee populations in tropical mustard production landscapes provides not only additional pollination capacity but also temporal complementarity that enhances the resilience of pollination services to environmental variability.

Nectar Dynamics and Pollinator Behaviour

The progressive decline in nectar sugar concentration from 42.3% Brix in early morning to 18.7% Brix by late afternoon represents a key driver of the observed temporal pollinator activity patterns. This diurnal nectar profile reflects the interaction between ongoing nectar secretion by floral nectaries and accelerating evaporative water loss as ambient temperatures increase through the day^[6]. In tropical environments, elevated afternoon temperatures (28-30 °C) accelerate nectar evaporation while simultaneously promoting nectar dilution through increased water transport to nectaries, creating opposing effects that result in net concentration decline. The practical management implication is that morning hours represent the optimal window for both nectar-mediated pollination services and any management activities that might disrupt pollinator foraging.

Management Recommendations

Based on the diurnal pollinator activity data generated through this investigation, mustard growers in Minas Gerais should implement temporal management guidelines that protect peak pollination windows. Specifically, insecticide applications targeting pest species should be scheduled after 1700 hours when pollinator activity is minimal. Foliar fertilizer applications that may alter nectar chemistry should similarly be avoided during morning hours. The establishment of native stingless bee colony boxes at field margins can augment afternoon pollination services and compensate for the natural decline in honeybee activity during post-midday hours. These temporal management strategies offer practical, low-cost approaches to enhancing pollination services in tropical mustard production systems.

Conclusion

The present research was designed to characterize diurnal visitation rhythms and floral resource utilization patterns of insect pollinators in mustard crops of Minas Gerais, Brazil. The investigation documented a diverse pollinator assemblage of 61 species with pronounced temporal partitioning among insect orders, driven primarily by diurnal variation in nectar sugar concentration and pollen availability. The native stingless bee *Trigona spinipes* emerged as a particularly important pollinator species contributing sustained afternoon foraging activity that complemented the morning-concentrated efforts of *Apis mellifera*. These findings provide practical temporal guidelines for managing crop operations to minimize disruption to pollination services, recommending that agrochemical applications and mechanical field operations be scheduled outside the 0700-1600 hour peak activity window. Future research should investigate the direct quantitative impact of temporal pollination patterns on fruit and seed set distribution within individual racemes and evaluate whether management interventions targeting specific diurnal windows can measurably enhance realized crop yields.

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