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Impact of Variation in Air Quality, Humidity and Temperature on Four Nest Entrance Activities of *Hypotrigona gribodoi*

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ABSTRACT

Variability in ambient climate and weather conditions greatly influences the survival and distribution of much important flora or faunal species, especially bee pollinating species. In this study, the impact of variation in two air pollutants (Carbon monoxide in parts per million (CO_PPM) and Ozone in parts per million (O₃_PPM), as well as humidity and temperature on foraging, guarding, flying in and flying out activities of 19 *Hypotrigona gribodoi* bee nest entrances, were assessed. Generally, positive correlations were observed between increasing levels of air pollutants, climatic variables and the number of bees involved in nest entrance activities. The guarding behavior of these bees seems to be controlled by other factors more than the measured conditions in this study. This study contributes to stakeholders' knowledge on how climatic conditions including air pollutants influence on bees' nest entrance activities.

Keywords: Carbonmonoxide PPM, Ozone PPM, Temperature, Humidity, Bees, Foraging, Guarding, Flight.

INTRODUCTION

Many organisms and their ecosystems have been affected directly or indirectly by changes in climatic variables, even posing potential threats of endangering the survival of many species of organisms on earth [1-3]. Ecosystem services such as pollination services are provided largely by animal pollinators which contribute as much as the US \$235-577 billion annually to the total economic value of crop pollination worldwide [4-7]. Animal pollinators also provide multiple benefits to people, beyond food production; contributing directly to medicine, biofuels, fibres, construction materials, musical instruments, arts, crafts, recreational activities and as sources of inspiration for art, music, literature, religion, traditions, technology and education. Reports indicate that various environmental pressures including pesticide use, agriculture intensification, invasive species, and land-use changes threaten the sustainability of animal pollination of both natural and agriculturally important plant species [8].

Change in climate variables was initially regarded to be a further threat to pollination services, thus earlier empirical studies rarely focused on the effects of climate change on wild plant-pollinator interactions [9-11]. However, within the last decade, the focus of several studies is shifted to include species interactions when analyzing the ecological effects of climate [12,13]. Indirect assessment of the potential effects of climate change on crop plants and their wild and managed pollinators and studies on wild plant-pollinator systems are becoming more relevant as many important pollinator populations face declines in the current changing climates [2,14]. While climate change may provide opportunities and threats for pollinators, changes to the composition, extent, and configuration of habitat in the

landscape are likely to pose a challenge to many pollinator species as climate change progresses [15,16]. For instance, the biological impacts of rising temperatures are known to depend upon the physiological sensitivity of organisms to temperature change [14,15]. Based on patterns in warming tolerance, climate change is predicted to be most deleterious for insects in tropical zones [17]. Climate change is currently considered as one of the primary drivers causing declines in the pollinator species [2].

The most important animal pollinators of pollinator-dependent crops are known to be mainly bees and some species of flies, birds, beetles/weevils, butterflies/moths, and wasp [18]. Bee pollinator species such as bumblebees, honey bees and stingless bees, like other insects are affected by ambient temperatures and are ectothermic, with activities such as flight depending on environmental temperatures to increased body temperatures [3]. Other bee species, however, are known to undergo endothermic heating. This is commonly found in many bee species with a body mass ranging between 35 mg-50 mg [19-21]. Hence in different taxa of bees, variations in endothermic abilities and thermal requirements exist, with most bee species having upper critical body temperatures (UCT) ranging from 45°C to 50°C [3,22]. A significant correlation was observed between forage activity and environmental factors in *Hypotrigona sp.* indicating that these bees are more likely to forage in low humidity and high-temperature conditions. It further revealed that a favorable range for peak foraging is temperatures above 29°C with relative humidity below 70% for the same bee species [23].

So far, it is known that the most plausible and important effect of climate change on plant-pollinator interactions can be expected to result from an increase in temperatures and relative humidity [24].

Currently, most empirical studies on elevated levels of ozone and carbon monoxide focus more on plant-herbivorous insect interaction in increased pollutant environments and are yet to focus on pollinating species such as *Hypotrigona* bee species. Studies on pesticide residues on pollinating insects in agroecosystems have also increased in many parts of the world [25], but yet to consider the effects of these air pollutants on bee activities. Responses to air pollution are reported to differ markedly within many animal and plant groups. In their report to WWF Dudley and Stolton, [26] identified the effects of air pollutants on 1,300 species, including 11 mammals, 29 birds, 10 amphibians, 398 higher plants, 305 fungi, 238 lichens, and 65 invertebrates, providing the most detailed survey to date. They added that key taxa examined in these studies mainly looked at the link between air pollution and wildlife-focused on the so-called "charismatic megafauna", ie on large and "colorful" species of animals. Sirk [27] alluded to the fact that air quality is likely one of a variety of environmental factors that influences the success of pollinators and bee managers should be aware of ozone pollution around their colonies and adjust some of their practices in the case of pollution events. An increase in the levels of tropospheric ozone has been observed to destroy floral aromas that bee pollinators use to detect pollen sources, thus affecting efficiency in locating potential foraging plant resources [28]. Beyond these studies, research is yet to be extended to other pollinating species. Hence, research linking air pollutants and many lower organisms such as other non-*Apis* bees needs to be evaluated.

This present study therefore aimed at assessing the combined effects of two air pollutants (CO₂-PPM and O₃-PPM) and ambient temperature and humidity, on nest entrance activities of bee species *Hypotrigona gribodoi*. This is a new contribution to data on the effect of climate change variables on bees' activities beyond temperature and relative humidity in field experimentations.

Hypotrigona stingless bees of the tribe Meliponini and is commonly found in the tropics and subtropical regions parts of the world. They are characteristically small-sized bees (4 mm), often found nesting in almost any small cavities, in tree bark or tree cavities, or small spaces in manmade structures with translucent protruding nest entrance tubes. In complex spaces, cell clusters are often divided, connected only by passageways [29-32]. These pollinating bees occur in the tropics and subtropical regions of the world and are important contributors to the pollination of crops such as chilli, eggplant, cowpea etc.

MATERIALS AND METHOD

Study area

This study took place at the Science Botanical Garden of the School of Biological Science, University of Cape Coast, Ghana between November 2018 and January 2019. This garden harbors diverse flora and fauna for academic research. The garden is bordered on the west by commercial taxi station and market, south-east by human settlement and north by College of Agriculture and Natural Sciences building complex.

Hypotrigona nest entrance assessments

A total of 19 *Hypotrigona* bee nests within 5m radius were selected and assessed for entrance characteristics categorized as the number of bees foraging (foragers), guarding (guards), flying in (returning bees) and flying out (exiting bees). The count of bees categorized as foragers included bees entering nest with pollen loads, guarding bees considered bees constantly guarding or maintaining the nest entrance. The numbers of bees observed to be entering or exiting each nest were categorized as a fly in and fly out respectively. Air pollutant and climatic variables of CO_PPM, O₃_PPM, temperature, and humidity were measured throughout nest assessments using a low-cost air monitor that was mounted at the central perimeter of the 19 nests. An ASE sampling box equipped with specific sensors including MICS_2614_O₃_1-0034, MICS_5524_CO_1-0044, and Temp/RH_DHT22_1-0074 detected and recorded continuous data on CO_PM, O₃_PPM, temperature and relative humidity variables within 50 m radius.

A 2 minutes video recording was taken thrice a week at each nest entrance during the morning (9:00 pm), afternoon (1:00 pm) and evening (5:00 pm). The starting nest was rotated to minimize possible error due to sampling times per nest. All recordings per nest were assessed and bee counts were entered into Microsoft Excels sheets.

Measurements for air pollutants and climatic variables were imported from a memory drive of the air pollutant sensor and all readings were also entered into Microsoft Excels sheets for analysis.

Data analyses

The data were analysed using Minitab Version 17 and SPSS Version 22. Correlation analyses were assessed to ascertain the probable relationships between several bees involved in each nest entrance activity (fly-in, fly-out, foraging and guarding) and the air pollutants and climatic variables (CO, O₃, Temperature and, humidity) under study. After which the extent of these relationships was estimated using a linear regression model. The fitted line of best-fit graphs was plotted to help illustrate the various predictive models.

Besides, scatterplot graphs were used to illustrate how the bee activities (fly-in, fly-out, forager and guarders) within each nest vary with the respect to various levels of the air pollutants and climatic variables (CO, O₃, Temperature and, humidity) under study.

Subsequently, ANOVA was computed to compare the levels of bee activities (fly-in, fly-out, foraging and guarding) within the nests at specific periods of the day (morning, afternoon and evening). A Post-Hoc test (Tukey) was used to assess how specific bee activities within each nest vary at specific periods of the day.

RESULTS

Effects of ambient temperature on the number of bee foragers, guards, fly in and fly out

The temperature was found to affect the number of bees involved in foraging activities at the nest entrance of *H. gribodoi* was significant ($p=0.000$). However, a negative correlation was observed resulting in higher temperatures causing a decrease in the number of foragers (Figure 1A). The predictive regression model showed foragers = $760-25.02 \text{ Temp } (^{\circ}\text{C})$. Generally, the temperature did not affect the number of guards at the nest entrances ($p=0.306$). A positive correlation between temperature and number of guarding bees was observed, indicating higher temperatures causing deployment of more nest guards (Figure 1B). The predictive linear regression model showed guards = $70.9+1.78 \text{ Temp } (^{\circ}\text{C})$. The number of bees flying in significantly ($p\text{-value}=0.005$) decreased with increasing temperatures. Similar trends were observed for bees flying out, with the number of bees flying out of nest significantly ($p\text{-value}=0.000$) decreasing with increasing temperatures. Negative correlations were observed in both temperature effects on many bees flying in and out of nests (Figures 1C and 1D). Predictive regression model indicated Fly-In = $961-30.19 \text{ Temp } (^{\circ}\text{C})$ and fly-out = $1466-46.9 \text{ Temp } (^{\circ}\text{C})$.

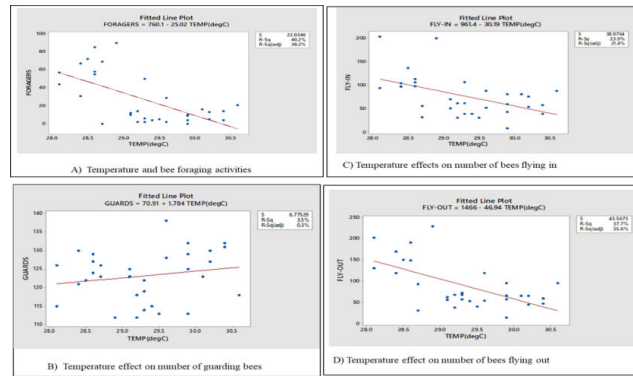


Figure 1. Effect of temperature on the behaviour of *H. gribodoi* at the nest entrance

It was observed that increasing temperature beyond 29.5°C seems to decrease the number of foraging bees with all the 19 colonies exhibiting reduced foraging activities (Figure 2A). In all the 19 nests, the number of guarding bees at each nest entrance seems not to vary much even with increasing temperatures (Figure 2B). Only one nest showed marked fluctuations in the number of guards at the entrance as temperature increased. The numbers of bees per nest involved in-flight activities were affected by increasing temperature. Decreasing ambient temperatures below 30°C increase flight activities of *Hypotrigona* bees than higher temperatures (Figure 2C and 2D).

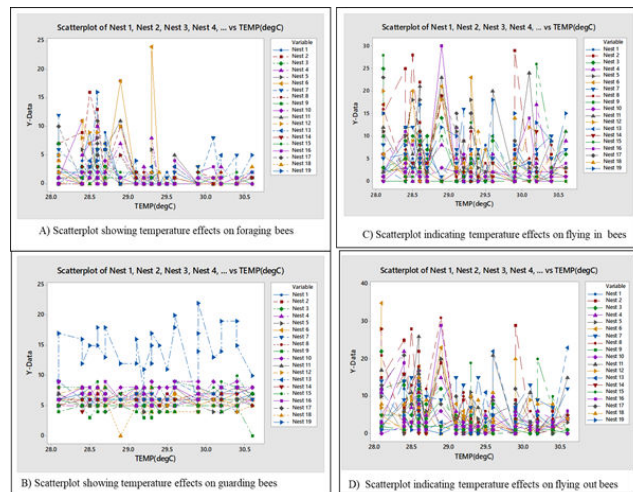


Figure 2. Effect of temperature on A) Foraging, B) Guarding, C) Fly in and D) Fly out activities of *H. gribodoi* colonies at the nest entrance

Assessment of humidity on foraging, guarding, fly in and fly out

Generally, Pearson correlation assessments of humidity against all four nest entrance activities showed non-significant relationships (Figure 3A-3D). A positive correlation was observed between humidity and number of foragers, though non-significant (p-value=0.073), while a negative correlation was observed for humidity against the number of guarding bees (p-value=0.564). Further, positive correlations were observed between humidity and the number of bees flying in and fly out, though effects are non-significant (p-values=0.502 and 0.717 respectively). Predictive linear regression models obtained for the number of bee foragers, guards, fly in and fly outs were foragers = -214 + 3.33 humidity (%), guards = 142.4 - 0.266 humidity (%), fly-In = -67 + 1.99 humidity (%) and fly-Out = -6 + 1.34 humidity (%) respectively.

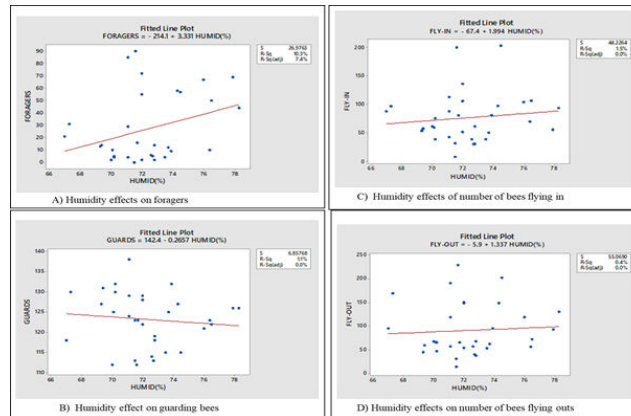


Figure 3. Correlation graphs of Humidity effects on a number of bees A) Foraging, B) Guarding, C) Flying in, D) Flying out

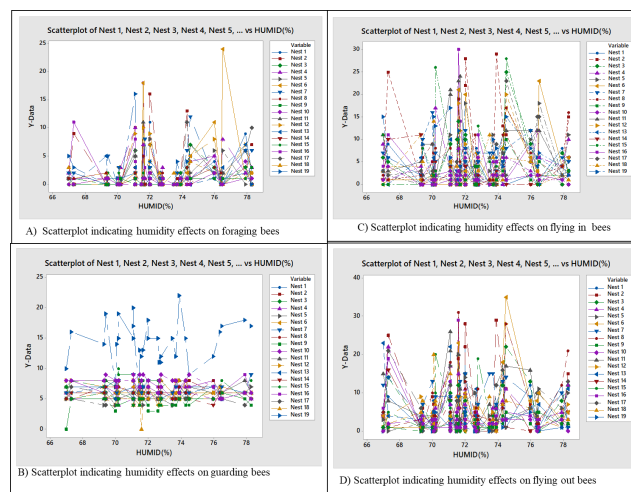


Figure 4. Scatterplots of humidity effects on bees activities in all nests A) Foraging, B) Guarding, C) Flying in, D) Flying out

Generally, changes in relative humidity did not significantly vary the number of bees involved in foraging, guarding, flying in and fly out of each of the 19 nests studied in this research (Figure 4A-4D). There seemed to be an optimum humidity (>70%), under which all activities took place.

Assessment of CO_PPM on foraging, guarding, fly in and fly out

Generally, it was observed that increasing CO_PPM significantly affected the population of *H. gribodoi* involved in foraging (p-value=0.000), flying in (p-value=0.000), and flying out=0.000 at the nest entrance. However, no effect was observed between CO_PPM and the number of guarding bees (p-value=0.567). Positive correlations were observed between CO_PPM and foragers, fly in and fly out, while a negative correlation was observed on the number of guarding bees (Figure 5A-5D). Predictive linear regression models obtained for number of bee foragers, guards, fly in and fly out were foragers=13.96+5.34 CO_PPM, guards=123.74-0.228 CO_PPM, fly-in=51.91+10.30 CO_PPM and fly-out=59.31+13.03 CO_PPM.

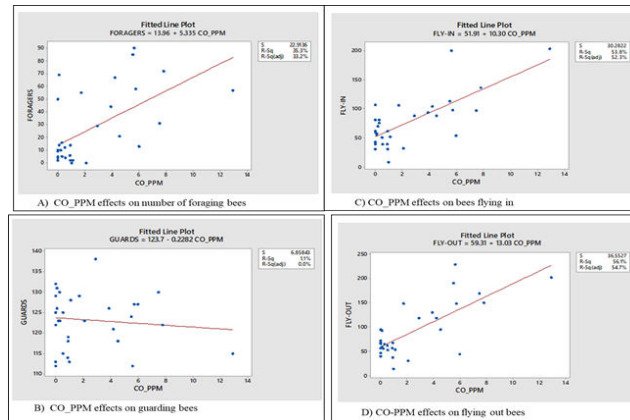


Figure 5. Correlations of CO_PPM effects on the number of bees A) Foraging, B) Guarding, C) Fly-in, D) Flyout

Assessments done per nest showed that the number of bees involved in foraging, fly in and fly out increased with increasing CO_PPM. At all nest entrances, more bees were active in low CO_PPM than in high CO_PPM. Several guards slightly decreased with an increase in CO_PPM (Figure 6A-6D).

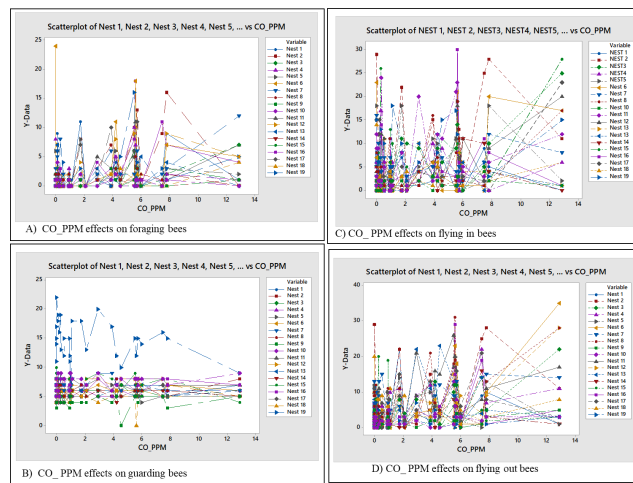


Figure 6. Scatterplots of CO_PPM effects on the number of bees in each nest involved in A) Foraging, B) Guarding, C) Flying-in, D) Flying-out

Assessment of O₃_PPM on foraging, guarding, fly in and fly out

Assessment for variation in ozone from Pearson correlations indicated that increasing O₃_PPM had highly significant effects on several bees involved in foraging (p-value=0.002), flying in (p-value=0.000), and flying out=0.000. However, a non-significant effect was observed between O₃_PPM and the number of guarding bees (p-value=0.646). Negative correlations were observed between ozone and foragers, fly in and fly out, while a positive correlation was observed on the number of guarding bees (Figure 7A-7D). Predictive linear regression models obtained for several bee foragers, guards, fly in and fly out were foragers =158.3-1.528 O₃_PPB, guards=118.2+0.058 O₃_PPB, fly-in =313.9-2.756 O₃_PPB and fly-out =413.1-3.747 O₃_PPB respectively.

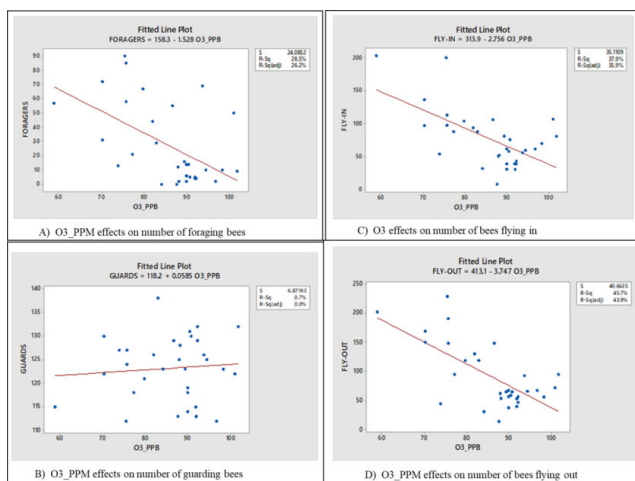


Figure 7. Correlation graphs indicating O₃_PPM effects on number of bees involved in A) Foraging, B) Guarding, C) Flying in D) Flying out

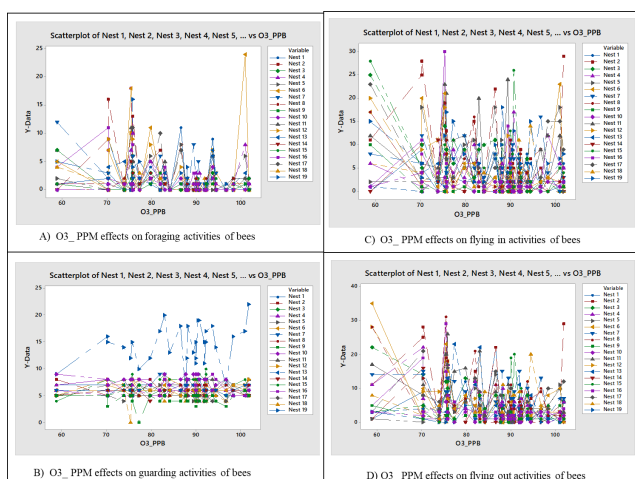


Figure 8. Scatterplots of O₃_PPM effects on the number of bees in all nests involved in A) Foraging, B) Guarding, C) Flying-in D) Flying-out

Assessments done per nest showed that the number of bees involved in foraging, fly-in and fly out decreased with increasing O₃_PPM. At all nest entrances, less number of bees were active in high O₃_PPM than in high O₃_PPM. Several bees involved in guarding slightly increased with an increase in O₃_PPM (Figure 8A-8D).

Results obtained for the assessments of bee activities during mornings, afternoons and evenings in both ANOVA (Tables 1-4) and multiple comparisons in Turkey HSD showed variable levels of significance in most in all nest entrance activities except guarding activities.

Bee foragers' activities during morning, afternoon and evening differed significantly in levels in several nests, with nests 2, 11, 12, 16 and 17 recording significantly high levels at the mean difference of significance at the 0.05 level (Table 1).

The number of bees involved in guarding activities during morning, afternoon and evening was observed to be non-significant in all 19 nests studied (Table 2).

Flying in and out activities at nest entrances during the morning, afternoon and evening recorded variable levels of significance among the nest with nest 3 showing highly significant flying in activities. Further, nests 3 and 4 showed highly significant variation in the flying out activities during different times of the day (Tables 3 and 4).

Further, Tukey’s test to assess how specific bee activities within each nest vary at specific periods of the day (morning, afternoon and evening) showed nests 3 and 4 recorded significantly high levels of activities in the mornings than afternoons and evening.

Table 1. ANOVA results for foraging activities during mornings, afternoons and evenings

		Sum of Squares	df	Mean Square	F	Sig.
NEST_1	Between Groups	92.551	2	46.275	8.212	0.001
	Within Groups	163.418	29	5.635		
	Total	255.969	31			
NEST_2	Between Groups	373.310	2	186.655	23.043	0.000
	Within Groups	234.909	29	8.100		
	Total	608.219	31			
NEST_3	Between Groups	34.248	2	17.124	8.058	0.002
	Within Groups	61.627	29	2.125		
	Total	95.875	31			
NEST_4	Between Groups	29.066	2	14.533	2.542	0.096
	Within Groups	165.809	29	5.718		
	Total	194.875	31			
NEST_5	Between Groups	48.528	2	24.264	6.328	0.005
	Within Groups	111.191	29	3.834		
	Total	159.719	31			
NEST_6	Between Groups	107.978	2	53.989	1.928	0.164
	Within Groups	811.991	29	28.000		
	Total	919.969	31			
NEST_7	Between Groups	68.639	2	34.319	5.492	0.009
	Within Groups	181.236	29	6.250		
	Total	249.875	31			
NEST_8	Between Groups	13.964	2	6.982	5.323	0.011
	Within Groups	38.036	29	1.312		
	Total	52.000	31			
NEST_9	Between Groups	3.614	2	1.807	5.061	0.013
	Within Groups	10.355	29	0.357		
	Total	13.969	31			
NEST_10	Between Groups	5.278	2	2.639	4.094	0.027
	Within Groups	18.691	29	0.645		
	Total	23.969	31			
NEST_11	Between Groups	85.911	2	42.956	10.384	0.000
	Within Groups	119.964	29	4.137		
	Total	205.875	31			
NEST_12	Between Groups	193.002	2	96.501	23.945	0.000
	Within Groups	116.873	29	4.030		
	Total	309.875	31			
NEST_13	Between Groups	10.641	2	5.321	1.921	0.165
	Within Groups	80.327	29	2.770		
	Total	90.969	31			

NEST_14	Between Groups	2.884	2	1.442	2.989	0.066
	Within Groups	13.991	29	0.482		
	Total	16.875	31			
NEST_15	Between Groups	7.827	2	3.914	5.237	0.011
	Within Groups	21.673	29	0.747		
	Total	29.500	31			
NEST_16	Between Groups	203.673	2	101.836	10.535	0.000
	Within Groups	280.327	29	9.666		
	Total	484.000	31			
NEST_17	Between Groups	115.157	2	57.578	10.520	0.000
	Within Groups	158.718	29	5.473		
	Total	273.875	31			
NEST_18	Between Groups	1.137	2	0.568	0.488	0.619
	Within Groups	34.827	29	1.201		
	Total	36.000	31			
NEST_19	Between Groups	56.664	2	28.332	3.525	0.043
	Within Groups	233.055	29	8.036		
	Total	289.719	31			
*The mean difference is significant at the 0.05 level						

Table 2. ANOVA results for guarding activities during mornings, afternoons and evenings

		Sum of Squares	Df	Mean Square	F	Sig.
NEST_1	Between Groups	0.710	2	46.275	8.212	0.001
	Within Groups	10.509	29	5.635		
	Total	11.219	31			
NEST_2	Between Groups	0.066	2	186.655	23.043	0.000
	Within Groups	13.809	29	8.100		
	Total	13.875	31			
NEST_3	Between Groups	0.045	2	17.124	8.058	0.002
	Within Groups	9.955	29	2.125		
	Total	10.000	31			
NEST_4	Between Groups	2.673	2	14.533	2.542	0.096
	Within Groups	14.827	29	5.718		
	Total	17.500	31			
NEST_5	Between Groups	1.105	2	24.264	6.328	0.005
	Within Groups	14.364	29	3.834		
	Total	15.469	31			
NEST_6	Between Groups	1.232	2	53.989	1.928	0.164
	Within Groups	21.736	29	28.000		
	Total	22.969	31			
NEST_7	Between Groups	0.014	2	34.319	5.492	0.009
	Within Groups	31.955	29	6.250		
	Total	31.969	31			
NEST_8	Between Groups	0.728	2	6.982	5.323	0.011
	Within Groups	19.991	29	1.312		

	Total	20.179	31			
NEST_9	Between Groups	1.139	2	1.807	5.061	0.013
	Within Groups	33.736	29	0.357		
	Total	34.875	31			
NEST_10	Between Groups	0.128	2	2.639	4.094	0.027
	Within Groups	39.591	29	0.645		
	Total	39.719	31			
NEST_11	Between Groups	1.187	2	42.956	10.384	0.000
	Within Groups	25.783	29	4.137		
	Total	26.969	31			
NEST_12	Between Groups	1.384	2	96.501	23.945	0.000
	Within Groups	27.491	29	4.030		
	Total	28.875	31			
NEST_13	Between Groups	0.932	2	5.321	1.921	0.165
	Within Groups	12.536	29	2.770		
	Total	13.469	31			
NEST_14	Between Groups	0.327	2	1.442	2.989	0.066
	Within Groups	15.673	29	0.482		
	Total	16.000	31			
NEST_15	Between Groups	0.873	2	3.914	5.237	0.011
	Within Groups	7.127	29	0.747		
	Total	48.000	31			
NEST_16	Between Groups	6.687	2	101.836	10.535	0.000
	Within Groups	20.282	29	9.666		
	Total	26.969	31			
NEST_17	Between Groups	2.509	2	57.578	10.520	0.000
	Within Groups	15.491	29	5.473		
	Total	18.000	31			
NEST_18	Between Groups	0.741	2	0.586	0.488	0.619
	Within Groups	32.727	29	1.201		
	Total	33.469	31			
NEST_19	Between Groups	10.457	2	28.332	3.525	0.043
	Within Groups	429.418	29	8.036		
	Total	439.875	31			
*The mean difference is significant at the 0.05 level						

Table 3. ANOVA results for fly-in activities during mornings, afternoons and evenings

		Sum of Squares	Df	Mean Square	F	Sig.
NEST_1	Between Groups	35.557	2	17.778	1.922	0.165
	Within Groups	268.18	29	9.252		
	Total	303.875	31			
NEST_2	Between Groups	628.839	2	314.41	5.427	0.10
	Within Groups	1680.036	29	9		
	Total	2308.875	31	57.932		

NEST_3	Between Groups	524.491	2	262.24	16.854	0.000
	Within Groups	451.227	29	6		
	Total	975.719	31	15.506		
NEST_4	Between Groups	10.537	2	5.268	0.319	0.729
	Within Groups	478.682	29	16.506		
	Total	489.219	31			
NEST_5	Between Groups	331.105	2	165.55	5.466	0.010
	Within Groups	878.364	29	3		
	Total	1209.469	31	30.288		
NEST_6	Between Groups	241.293	2	120.64	3.256	0.053
	Within Groups	1074.582	29	7		
	Total	1315.875	31	37.055		
NEST_7	Between Groups	29.205	2	14.603	1.088	0.350
	Within Groups	389.264	29	13.423		
	Total	418.469	31			
NEST_8	Between Groups	225.702	2	112.85	5.471	0.010
	Within Groups	598.173	29	1		
	Total	823.875	31	20.627		
NEST_9	Between Groups	15.537	2	7.768	1.237	0.305
	Within Groups	182.182	29	6.282		
	Total	197.719	31			
NEST_10	Between Groups	4.730	2	2.365	1.424	0.257
	Within Groups	48.145	29	1.660		
	Total	52.875	31			
NEST_11	Between Groups	379.432	2	189.71	4.415	1.021
	Within Groups	1246.036	29	6		
	Total	1625.469	31	42.967		
NEST_12	Between Groups	175.323	2	87.662	3.805	0.034
	Within Groups	668.145	29	23.039		
	Total	843.469	31			
NEST_13	Between Groups	.801	2	0.400	0.066	0.936
	Within Groups	175.918	29	6.066		
	Total	176.719	31			
NEST_14	Between Groups	42.755	2	21.378	2.918	0.070
	Within Groups	212.464	29	7.326		
	Total	255.219	31			
NEST_15	Between Groups	51.066	2	25.533	0.547	0.585
	Within Groups	1353.809	29	46.683		
	Total	1404.875	31			
NEST_16	Between Groups	140.855	2	70.428	2.213	0.127
	Within Groups	922.864	29	31.823		
	Total	1036.719	31			
NEST_17	Between Groups	18.364	2	9.182	0.332	0.720
	Within Groups	802.355	29	27.667		
	Total	820.719	31			

NEST_18	Between Groups	4.591	2	2.295	0.219	0.804
	Within Groups	303.409	29	10.462		
	Total	308.000	31			
NEST_19	Between Groups	50.945	2	25.473	0.833	0.445
	Within Groups	887.055	29	30.588		
	Total	938.000	31			

*The mean difference is significant at the 0.05 level

Table 4. ANOVA results for fly-out activities during mornings, afternoons and evenings

		Sum of Squares	df	Mean Square	F	Sig.
NEST_1	Between Groups	284.491	2	142.245	7.301	0.003
	Within Groups	565.009	29	19.483		
	Total	849.500	31			
NEST_2	Between Groups	628.839	2	314.419	5.427	0.010
	Within Groups	1680.036	29	57.932		
	Total	2308.875	31			
NEST_3	Between Groups	714.191	2	357.095	40.801	0.000
	Within Groups	253.809	29	8.752		
	Total	968.000	31			
NEST_4	Between Groups	434.569	2	217.284	15.429	0.000
	Within Groups	408.400	29	14.083		
	Total	842.969	31			
NEST_5	Between Groups	342.873	2	171.436	8.614	0.001
	Within Groups	577.127	29	19.901		
	Total	920.000	31			
NEST_6	Between Groups	412.164	2	206.082	4.585	0.019
	Within Groups	1303.555	29	44.950		
	Total	1715.719	31			
NEST_7	Between Groups	124.509	2	62.255	2.530	0.097
	Within Groups	713.491	29	24.603		
	Total	838.000	31			
NEST_8	Between Groups	694.314	2	347.171	8.823	0.001
	Within Groups	1141.127	29	34.349		
	Total	1835.469	31			
NEST_9	Between Groups	8.182	2	4.091	2.589	0.092
	Within Groups	45.818	29	1.580		
	Total	54.000	31			
NEST_10	Between Groups	7.710	2	3.855	1.978	0.157
	Within Groups	56.509	29	1.949		
	Total	64.219	31			
NEST_11	Between Groups	574.778	2	287.389	8.917	0.001
	Within Groups	934.691	29	32.231		
	Total	1509.469	31			
NEST_12	Between Groups	390.184	2	195.092	6.604	0.004
	Within Groups	856.691	29	29.541		

	Total	1246.875	31			
NEST_13	Between Groups	197.464	2	98.732	3.473	0.044
	Within Groups	824.536	29	28.432		
	Total	1022.000	31			
NEST_14	Between Groups	124.091	2	62.046	6.588	0.004
	Within Groups	273.127	29	9.418		
	Total	397.219	31			
NEST_15	Between Groups	54.414	2	27.207	1.261	0.298
	Within Groups	625.555	29	21.571		
	Total	679.969	31			
NEST_16	Between Groups	258.384	2	129.192	4.561	0.019
	Within Groups	821.491	29	28.327		
	Total	1079.875	31			
NEST_17	Between Groups	260.460	2	130.230	5.701	0.008
	Within Groups	662.509	29	22.845		
	Total	922.969	31			
NEST_18	Between Groups	2.432	2	1.216	0.057	0.945
	Within Groups	622.036	29	21.450		
	Total	624.469	31			
NEST_19	Between Groups	1.657	2	0.828	0.179	0.837
	Within Groups	134.218	29	4.628		
	Total	135.875	31			

*The mean difference is significant at the 0.05 level

DISCUSSION

In this present research, four key components of climate variables predictably impact the activities of *Hypotrigona* bees at their nest entrances: mean temperature, humidity and air pollutants such as CO and O₃.

The number of bees involved in foraging, flying in and out activities at all nest entrances decreased significantly in response to increasing temperatures. Temperature generally poses diverse effect on insects, especially pollinators, whereby increasing environmental temperature greatly influences the fitness of pollinator species and even the pollinator-dependent plants. The timing of both plant flowering and pollinator activity is reported to be strongly affected by temperature [11]. According to Kingsolver et al. [33], both tropical and temperate ectotherms may suffer declines in mean fitness during the growing season in response to climate change. Changes in environmental temperature cause frequent heatwaves and may predictably have an impact on plant fitness by decreasing reproductive output of flowering plants [3,34]. Further, increasing environmental temperatures is implicated in the increase in the length of plant growing season, and the frequency with which insect species experience temperatures close to their optimal temperatures [33]. Thus, the responses observed on *Hypotrigona* bees in this present study possibly follow a similar reason for bee fitness as a driving indicator for bees to reduce environmental activities such as foraging and flight to stay fit. Kjølhl et al. [15] indicated that as temperature increases, pollinators are at risk of overheating, particularly in regions where current ambient temperatures are high. The number of bees involved in guarding behaviour in this study remained fairly constant even with increasing temperatures, possibly for security purposes. In most cases, the number of bees guarding nest entrances remains constant, because, their numbers provide the required space-size cover to prevent non-colony members and potential invaders from intruding or attacking. Guarding the nest entrances seems to be controlled by another inherent mechanism other than temperature. The predictive model generated in this study can suggest the number of bees that will possibly be involved in foraging, guarding, flying in and out of nest entrances of *Hypotrigona* bees as temperature changes.

Generally, humidity is regarded as an important climate component that influences insects' activities. The relation of a particular insect to atmospheric moisture is often very precise. If low humidity is unfavourable, then the higher the humidity the better, up to the point where elimination becomes impossible: in fact, the optimum is just below the

point of danger [35]. A recent study on *Hypotrigena* bees observed a positive correlation between humidity and foraging activities of this species, and also found humidity of below 70% as the optimum percentage for peak bee foraging activities [23]. This study also revealed a positive correlation between humidity and number of foragers, however, the relationship was non-significant and peak foraging activities were found at humidity percentages above 70. Further, the number of bees guarding nest entrances slightly decreased as humidity increases, though non-significantly. Flight activities involving bees flying in and out of nest entrances showed a correlation indicating that, possibly, as humidity increases the number of bees involved in flight may slightly increase, though non-significantly. In all nests, optimum humidity exerts similar influences on all activities where effects are not so variable. As optimum humidity is attained, it may be possible to predict the number of bees that will possibly be involved in foraging, guarding, flying in and out of nest entrances of *Hypotrigena* bees in a known humidity. Foraging economics and survival rates of pollinators are directly influenced by hydro and thermodynamic processes of the weather [36,37].

Even though the mean CO₂-PPM recorded in this study was below the WHO minimum standards, all bee activities of foraging, guarding, flying in and flying out occurred in lower levels of CO₂-PPM in all nests. Generally, increasing CO₂-PPM levels had significantly high effects on the number of bees involved in foraging, flying in, and flying out. However, a non-significant effect was observed between CO₂-PPM and number of guards. In all the nests, higher numbers of bees' activities of foraging, flying in and flying out occurred at lower levels of CO₂-PPM. Insect populations are directly or indirectly affected by air pollutants such as CO₂-PPM [38]. Carbon monoxide poisoning is the most common type of fatal air poisoning in many countries and can react chemically with other atmospheric constituents (primarily the hydroxyl radical, OH₂) that would otherwise destroy methane. Many biogenic processes release carbon-containing compound such as CO and CO₂ which can react with Volatile Organic Compounds (VOCs) to cause ozone pollution [39]. A high tropospheric CO/CO₂ concentration also promotes stratospheric ozone loss, which results in elevated UV-B radiation. Both elevated temperatures and enhanced UV-B radiation are the results of increased levels of gaseous pollutants in the atmosphere. These factors can have significant effects on plant-feeding insects including bee pollinators [40]. The type of pollutant determines whether the effect of that pollutant is expected locally or on a wider scale [41].

Air pollutant such as O₃, is one of many climatic variables that greatly influence bee populations. As O₃ levels increases, the number of bees involved in foraging, flying in and out of the nest entrances significantly decreased. This phenomenon was reversed in the observation between increasing ozone levels and the number of guarding bees. The number of guarding bees increased with increasing O₃ levels. According to Fuentes et al. [28], increasing levels of tropospheric ozone destroy floral aromas that bee pollinators use to detect pollen sources. This invariably imposes a negative impact on how a potential bee pollinator navigates its flight to forage on a plant and subsequently, their pollination efficiency on crops. As bees are unable to locate good forage resources, fewer numbers possibly will be recruited into these vital bee activities. Even though the study area is a botanical garden, it is bordered by human settlements, commercial taxi station and a market center. These are potential sources of pollutants that might influence air pollutant levels in the area, hence influence nest entrance activities even in a vegetation rich environment. According to Sirk [27], industrial regions where human activities are high with activities such as combustion of fuel leads to high levels of Nitrogen Oxides (NO_x) and subsequently, highest ranges of ozone levels. Further, other studies have also shown a significant decline in plant health with increasing ozone levels. Thus, when a pollinator collects unhealthy forage resources, there is the likelihood of collecting low-quality food resource which may eventually affect its health [42,43].

Bee activities during mornings, afternoons and evenings vary significantly among the nests as colony needs may vary from one nest to another. The age of the colony, colony capacity, food resource stores may play key roles in influencing how and when activities in and around the nest are conducted. Generally, many pollinating bee species are known to be active in the mornings in synchrony to high plant resources of pollen and nectar produced over the night. In this study, most nests did record non-significant levels of bee activities probably due to other inherent factors other than those measured in this work.

SUMMARY AND CONCLUSION

Nest entrance activities of bees such as foraging, flying in and flying out are significantly affected by temperature, CO₂-PPM and O₃-PPM generally. Increasing ambient temperatures decrease the number of bees involved in foraging, flying in and flying out of nests. Bee activities also seem to occur at an optimum humidity above 70%. The number of guarding bees at a nest entrance is influenced more by factors other than air pollutants and climatic

conditions. Predictive linear regression model could suggest more data on numbers of bees involved in nest entrance activities in changing climatic conditions and air quality variables.

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