



Environment, foraging instinct and food attractiveness promote indoor foodborne pathogens carrying capacity of *Camponotus maculatus* (Hymenoptera: Formicidae) in Nigeria

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Received: 11 November 2024 / Accepted: 17 March 2025 / Published online: 31 March 2025
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Abstract

Camponotus maculatus ranks among the largest African ant species, displaying remarkable surface activity when foraging for food. This research was carried out in the Entomology laboratory of the Federal University of Technology, Akure and surrounding settlements in Akure metropolis with the objectives of providing insight on foraging behaviour, infestation and potential to vector foodborne pathogenic microorganisms to food in kitchens by ants. Observation stations were set up with different food attractants (sugar, honey, cola drink, milk and candy) dry and moistened soaked in cotton wool. Microbial analyses of ants placed to forage on contaminated food and introduced to fresh food showed the ants have vector potential to transmit foodborne pathogens. Majority of *C. maculatus* ants exhibited a distinct preference for sugary foods over other tastes, and moist food over dry food. The relative abundance of the ants attracted in different location using similar food attractants were significantly different ($p < 0.05$). In the microbial evaluation of fresh food exposed to infected ants from contaminated food, microbial colony counts of *Aspergillus* spp. (11.22 ± 0.66 SFU/ml) and *Staphylococcus aureus* (11.11 ± 0.72 CFU/ml) are significantly different ($p < 0.05$) from that of *Bacillus* spp. (20.78 ± 0.85 CFU/ml). *C. maculatus* selective and potential transmission capacity for pathogen is driven by the environment and the food sources.

Keywords Colony · Foraging · Transmission · Pathogen · Colony forming unit

Abbreviations

CFU	Colony Forming Units
LDZ	Low Density Zone
MDZ	Medium Density Zone
HDZ	High Density Zone
FUTA	Federal University of Technology
PDA	Potato Dextrose Agar
SFU	Spore forming unit

Introduction

Ants, belonging to the order Hymenoptera, are social insects that exhibit a diverse range of behaviour and occupy a wide array of ecological niches across the globe (Chang et al. 2021). They are highly abundant insects that play crucial roles in ecosystem services particularly, nutrient recycling and seed dispersal (Kass et al. 2022). *C. maculatus* (Fabricius, 1782) belongs to the *Camponotini* tribe within the Formicidae family, widely distributed across tropical and subtropical Africa, Asia, and parts of Australia, this species has adapted to diverse environments. The ants are known for their diverse habitats and foraging behaviour (Andersen and Hoffmann 2003). They inhabit a wide range of ecosystems, displaying diverse feeding behaviour and interactions with other organisms, especially plants and insects (Hölldobler and Wilson 1990). Their colonies exhibit a wide spectrum, from small groups residing in natural crevices to large, intricately structured territories, with populations ranging from just few individuals to millions (Perfecto and Snelling 1995). *C. maculatus* ranks among the largest African ant species, displaying remarkable surface activity when

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foraging for food (Philip et al. 2016). This species exhibits its defense against any threats or aggression it encounters. Its taxonomic classification places it within an intriguing ecological niche, showcasing several behavioural adaptations that have evolved over millennia. Its peak of activity levels is typically observed during the evening and night time hours. The presence of ants, including *C. maculatus* in urban surroundings is significant due to their potential to act as carriers or vectors of pathogenic microbes (Oliveira et al. 2016; 2017).

Pathogen carrying capacity is a phenomenon that can be expressed in mathematical equation to model pathogen transmission. Pathogens are microbes (bacteria, viruses, protozoans and fungi) capable of causing host damage or a static or unchanging entity believed to possess an inherent capacity to cause disease in hosts which was absolutely distinct from other types of microbes (Pierre-Olivier 2014). The ants are not the host of this micro-organisms but they are only carriers, vectors or transmitters of the pathogens to a specific or susceptible host. What determines organism's pathogen carrying capacity involves abiotic factors, the environment (Onyango et al. 2020; Corbin et al. 2021) some limiting factors such as available supplies of food or water or material resources containing the pathogens, nesting areas, space, or the amount of waste that can be absorbed without degrading the environment and decreasing carrying capacity and biotic factors such as the host and the microbial genetics (Rocha et al. 2016; Stathopoulos et al. 2014).

Insects act as primary or intermediate hosts or carriers of animal and plant diseases pathogens by transmitting protozoa, bacteria, viruses, and helminths such as tapeworms, flukes, and roundworms (Sanchez-Contreras 2008; Sarwar 2015). These insect-vector-borne pathogens pose the greatest threats to man, plant and animal on a global scale (Heck 2018). and according to Sanchez-Contreras (2008) insects are the reservoirs for emerging human pathogens and particularly those linked to bacteria-associated with vertebrates are known for the spread of deadly bacteria diseases such as *Yersinia pestis* responsible for bubonic plague (Waterfield et al. 2007; Sanchez-Contreras 2008). Insects vectoring or carrying pathogens cut across the entire class insecta. Generally, these insects have established one form of relationships or interactions with the pathogens, which may be pathogenic (Maximo et al. 2014) or mutualistic/ symbiotic relationship (Moreau 2020). The symbiotic associations between the insects and the pathogens can be classified as commensalism, mutualism and parasitism (Moya et al. 2008; Lv et al. 2024). This interactions, either symbiotic or pathogenic have been developed as a result of coevolution between the insects and the pathogens. There are some evolutionary selective pressures on pathogens which facilitate their mode of transmission and dispersal ability by some insects (Powell 2019). The pathogen-vectoring efficiency

in insect is dependent on many factors which are facilitated by the interactions between the invading parasites and pathogens and the vectoring insects (Ratcliffe et al. 2024) this include the host fitness, reproductive success, feeding, influence of other symbionts and the environmental circumstances (Sanchez-Contreras 2008).

It has become a subject of contention on why many invertebrates are vectors of pathogens and why they are not susceptible to the virulence. Insects among the invertebrates have been the most incriminated as transmitter or vector of pathogens or microbes due to inadequate control practices and improper storage of food that might aggravate their infestation (Santos et al. 2009; Zarzuela et al. 2002) The ants as the most common indoor pests have high capacity for carrying microorganisms (Pereira 2008). Thus, it can be inferred that ants may be one of the agents responsible for disseminating microorganisms in hospital environments. Ants, bacterial and fungi have been found to form mutualistic associations, raising concerns about the possible role of ants as a disease vectors, microbial and pathogenic fungi dispersal in houses (Akinwande et al. 2024) and in hospitals (Lima et al. 2013 and Oliveira et al. 2016). The black ant *Achycondyla senaarensis*, the fire ant *Solenopsis* spp. carpenter ant *Camponotus* spp. \ *Monomorium pharaonis* L. and *Monomorium minimum*, ghost ant *Tapinoma melanocephallum* and other species pose threats to residential apartments (Akinwande et al. 2024) and may cause food poisoning to hospital environment (Alharbi et al. 2019).

Ants associations with pathogens in different forms facilitate pathogen transmission, some pathogens are carried by ants through trophylaxis, the ant vulnerability to the pathogen is the consumption of contaminated or poisoned food, Akinwande et al. (2024) and Bruna et al. (2017) claimed pathogenic diseases transmitted via food poses a public health problem all over the world resulting in high mortality and morbidity rates. Some insects vector pathogens which are commonly associated with them, Moreira et al. (2005) have shown a mutualistic relationship between ants and the bacteria and fungal pathogens found on its exoskeleton. Some bacteria transmitted in this manner are responsible for hospital-acquired (nosocomial) infections and associated with potentially serious medical problems (Moreira et al. 2005; Máximo et al. 2014). Habitat modification caused by increase in temperature and environmental pollution and anthropization or urbanization support high densities of indoor or domestic insect vectors associated with pathogens which are trophically linked to man or domestic animals. (Kilpatrick 2011; Santiago-Alarcon 2022). The class insecta as a very successful group of animals in terms of number, diversity, abundance and distribution and driven by climatic factors. Insects are one of the most available and accessible group of organisms in the course of indoor vector–host–pathogen interaction (de angel Dutra et al. 2022;



Pérez-Rodríguez et al. 2014), and transmitting pathogens that impact human population putting their lives at risk (Shaw and Catteruccia 2019).

In the tropics, there are few research studies on *C. maculatus* in vector-borne pathogens transmission during indoor foraging on domestic food and the risk factors associated with it. Owing to this limited understanding, this research is aimed to (i) provide insight on knowledge and foraging behaviour and infestation of domestic kitchen by ants; (ii) identify pathogenic microorganisms that are vectored by *C. maculatus* which regularly infest kitchens; and other premises in human residential apartments in the study area (iii) investigate *C. maculatus* potential to vector the identified foodborne pathogenic microorganisms onto ready-to-eat food.

Materials and methods

Study locations

This study was carried out in the Federal University of Technology (FUTA), Akure, Akure South local government area of Ondo state (Fig. 1a,b,c) (7.3037°N 5.1388°E), and surrounding neighborhoods in Akure city. Akure city is hypothesized to have a pattern of most cities in SW Nigeria, with settlements pattern described by a map that appeared like three concentric rings classified into Low-Density Zone (LDZ), Medium-Density Zone (MDZ), and High-Density Zone (HDZ). The outermost concentric circles encompassing the forested boundaries, labeled as a low-density zone (LDZ), characterized by limited human habitation impact and often referred to as preserved (Fig. 1a,b,c). The middle concentric zone is identified as the medium-density zone (MDZ), experiencing minimal anthropization. It is situated at a considerable distance from the large boundary covered with vegetation. The central core is the high-density zone (HDZ), it is characterized by high population and low vegetation. Communities within this Akure metropolis used for the study were selected on the basis of this zonation.

The study locations are Ibule (LDZ), FUTA (MDZ) and Orita Obele to City Center (HDZ). The study areas were divided into three sites (1, 2 and 3) for experimental replications, each site to capture the *C. maculatus* population and carry out all the investigations.

Study duration

The study location was carried out in this evergreen forest area for a period of 8 months (March–October, 2024), which is a wet season. The study involved field and laboratory.

Ant collection and identification

Ant population were drawn to the sample collection bottles with a protein food source consisting of dead cockroach and cricket meals. Cotton wool soaked in 75% sugar-water solution in the plastic bottles were used to trap the ants, after about 250 ants were trapped, they were transported to the Entomology laboratory of the Department of Biology, Federal University of Technology, Akure for identification. The ants collected were identified and preserved life for the laboratory study.

Foraging observations

Observation was carried out on the field (natural habitat) and in the Entomology laboratory of the Federal University of Technology, Akure (Fig. 2). Observation chambers were set up that mimics the ant's natural environment, the observation station is made up of 4 arms rubber tubes connected to a central core chamber (insect release chamber). The rubber tubes represent tunnels / channels leading to different food attractants. In all the sites where the tests were carried out, the ants were allowed to adapt to the observation station for 7 days.

In the laboratory, the ants in test tubes were kept and fed with the 75% sucrose solution which served as their carbohydrate source. The sucrose solution soaked in cotton wools were to prevent the ants from drowning. During the observation on the field and in the laboratory, conditions of temperature and relative humidity were recorded.

Domestic food preferences of *C. maculatus*

To determine the food preferences of *C. maculatus*, a range of tests were conducted using food of different tastes: sugary, salty, sour, and bitter foods. Also the specific food items in dry and moist forms (soaked in cotton wool) were selected for further investigation, which included sugar, honey, cola drink, milk and candy; and other foods of different nature. These preliminary tests were chosen to determine the food that are appealing and of potential significance for the ants. The response of ants to these food samples were studied in the laboratory and on the field.

Rate of attraction of *C. maculatus* to some domestic food items

Ant population were collected in each locations with a bait of protein food source consisting of dead cockroach and cricket meals. The ants were attracted into many bottles. Another location on the site was chosen, observation chambers with different food baits at the outlets were set up on the field. About 250 ants collected were

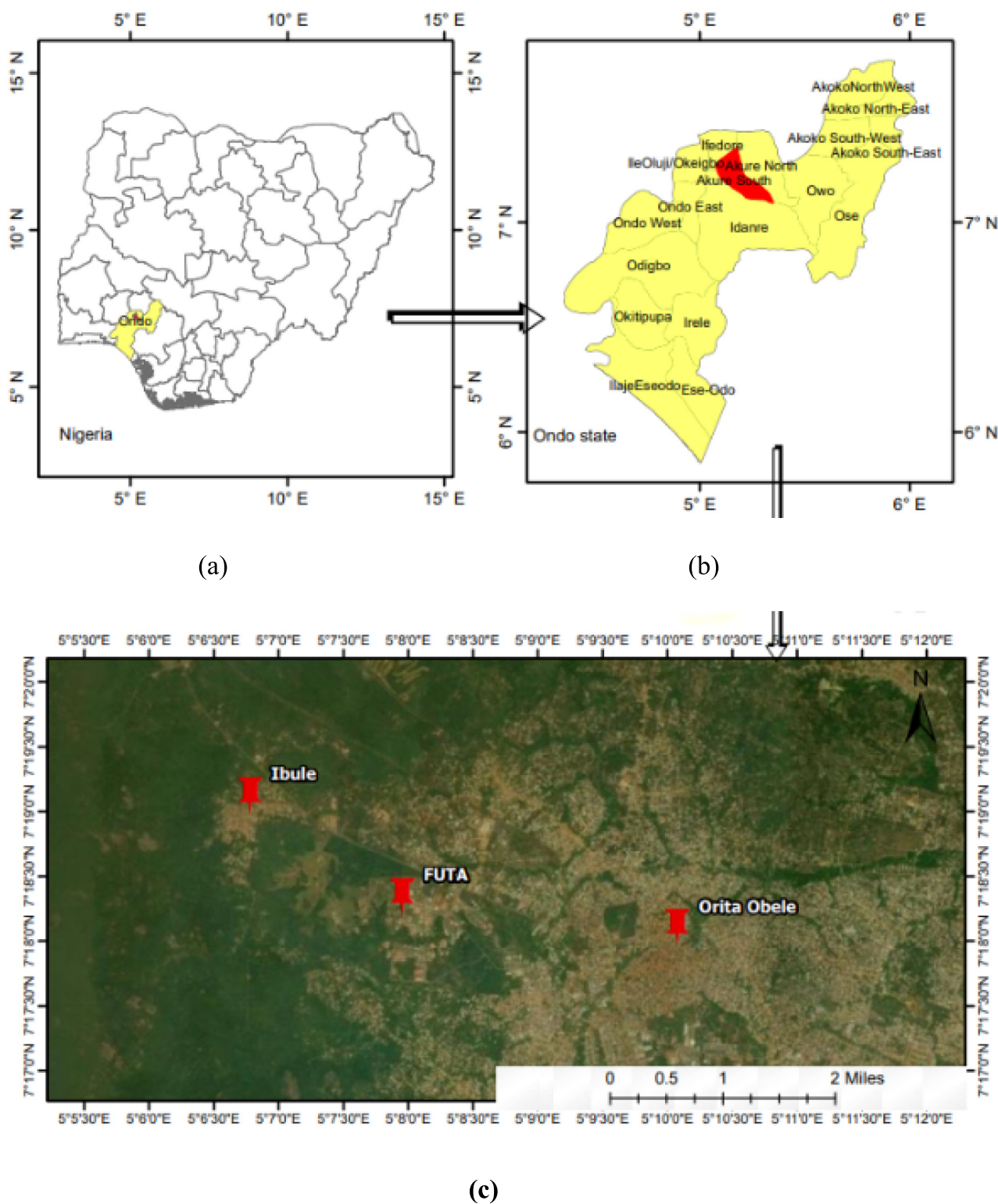


Fig. 1 a, b and c Map showing the study area

introduced into the insect release-chamber of the observation tool. Once an ant reached the food source, it will determine if the food is palatable and it will go back to

recruit others or get repelled. At the expiration of testing time. An entrance to the food chambers were blocked, the ants were killed and numbers counted on the sites. Similar



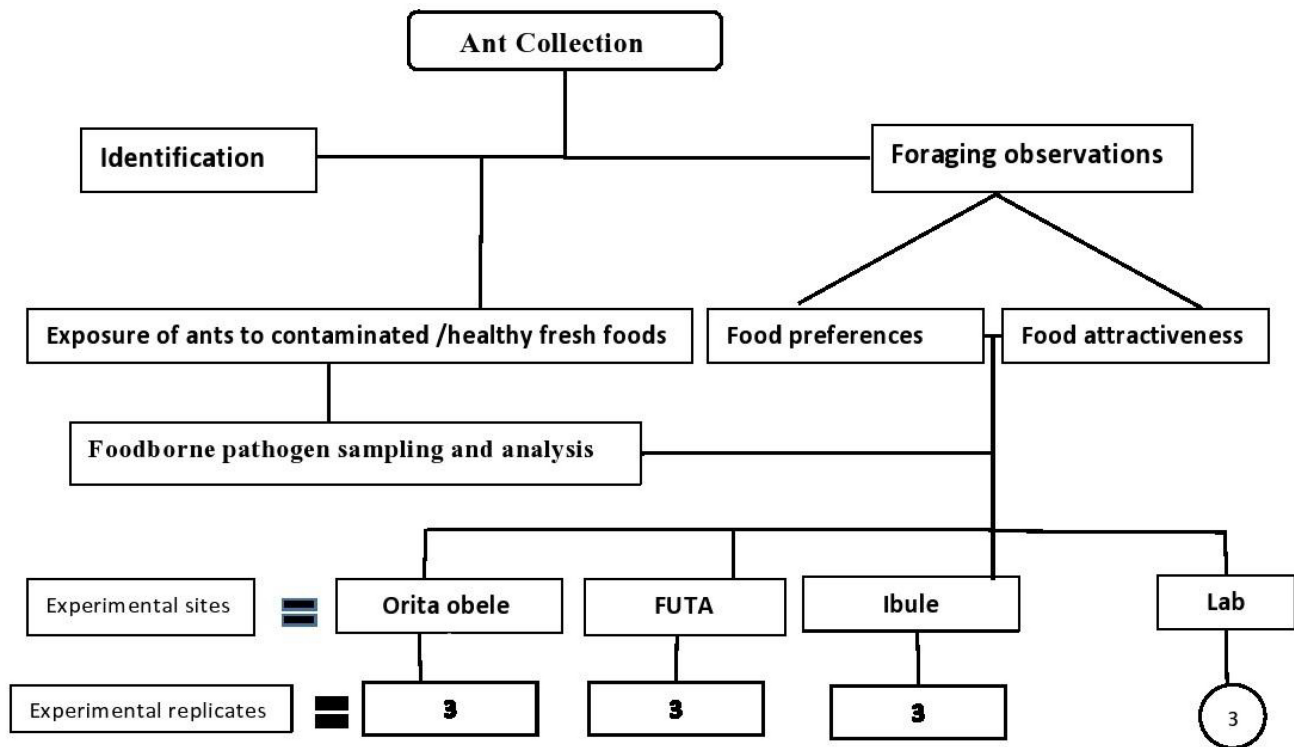


Fig. 2 Flow chart of the experimental design

observation experiment was carried out in the laboratory. Evaluation of the ants foraging, food and conditions that promote the foraging were carried out on the site and in the laboratory under different conditions with food attractants placed at each bowl in the tube outlets. Variation in conditions of temperature and relative humidity were recorded.

The rate of attraction of *C. maculatus* to the food substances was investigated by counting the number of the ants that locate, gather and forage on the food substances, after the appearance of the first scouting ants in stipulated time of 1 h, 2 h and 3 h consecutively. The ants were removed hourly and cumulative numbers recorded,

Rate of attraction of *C. maculatus* to moist and dry sugar food in different locations

Again, 4 ($n = 24$) plastic containers with perforated cover lid each containing 50 g of different food attractants (dry and moist sugar) were introduced in each study location for a period of 4 h during the peak foraging activity in the late evenings. The number of ants to gather and forage on the food in the containers were recorded.

Exposure of the ants to contaminated and healthy fresh foods

Some of the ants from the study sites were exposed to spoilt food for a period of 24 h, after which their microbial compositions were evaluated. Subsequently, a portion of the ants introduced to foodborne microbes were allowed to forage on healthy fresh foods for 24 h. Following this interaction, microbial analysis was carried out to determine whether the ants have successfully transported the food-borne pathogens from the spoiled food to the healthy food (Fig. 2).

Foodborne pathogen sampling and analysis

To investigate the potential of *C. maculatus* to carry food-borne pathogens, common household cooked food was used such as cooked rice, beans, bread, banana, and beef. Microbial analyses of the freshly cooked food samples and the ants were conducted using five types of agar media, specifically Nutrient Agar (NA), Potato Dextrose Agar (PDA), MacConkey Agar, *Salmonella Shigella* Agar, and Mannitol Salt Agar. The food samples were allowed to decay in transparent plastic containers over a period of three to four days, ensuring protection from external influences that might

impact the results. Following this, a serial dilution process was performed, involving the introduction of one gram of the food sample into a test tube containing 9 ml of distilled water, and subsequent dilution of 1 ml of the solution into successive test tubes, each containing 9 ml of distilled water. Subsequently, 1 ml of the fourth dilution was pipetted to petri dishes for each of the five agar types. These dishes were incubated for 24 h to estimate the bacterial colony forming unit (CFU).

However, for PDA, a 72-h incubation period was applied to assess fungal growth. A parallel procedure was repeated to ascertain the microbial composition on *C. maculatus* under aseptic conditions, random sampling of the ants was performed, followed by microbial analysis.

Data analyses

All data collected from the three study locations were pooled together and analyzed using both inferential and descriptive statistics. Data regarding foraging behaviour were analyzed using a one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$. In cases where there was significant difference in the observation, post hoc analysis was conducted using Tukey's test. The analyses were carried out using the SPSS 27 software package. Graphical representations of the data were drawn using Microsoft Excel 2016.

Results

Domestic foraging food preferences of *C. maculatus*

C. maculatus exhibited a distinct preference for sugary foods over other tastes. Among the tested food items, these ants showed a strong inclination towards sugar, consistently favouring it as their primary food choice while the ants were poorly attracted to salty, sour, and bitter foods.

Attractiveness to food

Attraction of *C. maculatus* to various food samples across the different study locations HDZ (Orita Obele), MDZ (FUTA) and LDZ (Ibule) showed sugar exhibited greater attraction with high relative abundance of $240 (43.96 \pm 1.67)$, $190 (38.69 \pm 1.09)$ and $156 (39.80 \pm 0.79)$ N (Mean R.A $\pm$ S.E %) in high, middle and low density zones respectively (Table 1) compared to other food substances. While milk exhibited the lowest attraction to the ant species, with attracted ants having a relative abundance (%) of $22 (5.61 \pm 0.25)$, $38 (7.69 \pm 0.29)$ and $31 (5.68 \pm 0.39)$ in low, middle and high density zones respectively (Table 1). The order of the food attracting the ants significantly decreases from sugar > honey > candy > cola drink > milk across the zone. LDZ ($F = 342.72$, Sig. = 0.001, $p < 0.05$), MDZ ($F = 349.55$, Sig. = 0.001, $p < 0.05$) and HDZ ($F = 270.79$, Sig. = 0.002, $p < 0.05$) (Table 1). Similarly, the relative abundance of ants attracted to similar foods in different locations were significantly different ($p < 0.05$). The high relative abundance of the ants in the HDZ and MDZ compared to the LDZ is attributable to factors driving the ant population

Table 1 Attractiveness of *C. maculatus* to food samples on the field

Food samples	Number (Mean Relative abundance) of <i>C. maculatus</i> attracted to the food substances within 1 h				P -value
Density Zonation/ Location	LDZ (Ibule) N (R.A $\pm$ S.E)	MDZ (FUTA) N (R.A $\pm$ S.E)	HDZ (Orita Obele) N (R.A $\pm$ S.E)	Laboratory N (R.A $\pm$ S.E)	
Sugar ^c	156 (39.80 ± 0.79)	190 (38.69 ± 1.09)	240 (43.96 ± 1.67)	108 (43.20 ± 1.20)	$F = 4.987$ Sig. = 0.010
Honey ^b	89 (22.70 ± 0.99)	114 (23.08 ± 0.60)	138 (25.27 ± 1.01)	50.0 (20.0 ± 1.05)	$F = 6.396$ Sig. = 0.003
Cola drink ^b	62 (15.82 ± 0.67)	77 (15.61 ± 0.38)	63 (12.45 ± 0.54)	35 (14.0 ± 0.98)	$F = 8.020$ Sig. = 0.001
Milk ^a	22 (5.61 ± 0.25)	38 (7.69 ± 0.29)	31 (5.68 ± 0.39)	16 (6.80 ± 0.45)	$F = 7.533$ Sig. = 0.001
Candy ^b	63 (16.07 ± 0.45)	73 (14.83 ± 0.44)	69 (12.64 ± 0.40)	41 (16.4 ± 0.99)	$F = 7.668$ Sig. = 0.001
Total	392 (100%)	492 (100%)	546 (100%)	250 (100%)	
P value	$F = 342.72$ Sig. = 0.001	$F = 349.55$ Sig. = 0.001	$F = 270.79$ Sig. = 0.002	$F = 206.07$ Sig. = 0.001	

Food samples having the same alphabet within the column are not significantly different from each other in attraction of the ants using ANOVA and Tukey's test at $p < 0.05$

N(R.A $\pm$ S.E (N) Number of insect attracted, R.A Relative abundance and S.E = Standard error). Total represents the numbers of *C. maculatus* that were attracted to the different food substances in each location



indoor which is more pronounced in the HDZ and MDZ locations that have reduced vegetation compared to LDZ which is surrounded by vegetation. Here, the ants relied on foraging outdoors than indoors. Again, comparing the field and laboratory, the attractiveness of *C. maculatus* to food samples decreases from sugar > honey > candy > cola drink > milk.

Foraging patterns and time are dependent on locations and food types

The ants forage during the day; however, the research revealed in the field unlike indoor, the peak of foraging activities were observed during the late evenings to the early morning before sunrise. This suggests a diurnal foraging pattern with increased activity during the twilight hours.

Based on the results presented in Table 2, sugar and candy demonstrated the quickest attraction time to *C. maculatus*, while honey, cola drink and milk displayed the longest attraction time. In most cases, milk has the longest attraction time. Similarly, in Table 2, sugar demonstrated the quickest attraction time across the three study locations as indicated with average attraction time of 33.33 ± 0.96 , 31.89 ± 2.56 , and 29.22 ± 0.95 minutes across LDZ (Ibule), MDZ (FUTA) and HDZ (Orita Obele, respectively). Notably, in the location 1 (LDZ), a significant difference was observed between the attraction time of sugar and milk (Table 2). Conversely, the attractiveness of honey, cola drink, and milk in HDZ (Orita Obele) was statistically similar to each other.

It becomes evident that milk has the highest attraction time, averaging times of 45.56 ± 3.09 , 39.45 ± 3.47 , and 33.11 ± 0.97 minutes across the three study locations of LDZ (Ibule), MDZ (FUTA) and HDZ (Orita Obele) respectively.

The environmental factors of temperature and relative humidity recorded in the study locations did not significantly affect the ants' attraction to the food items because the areas shared almost similar temperature and relative humidity. Orita Obele and FUTA recorded average environmental temperature of 76°F (24.44°C) and relative humidity of 85% while Ibule recorded average environmental temperature of 76.37°F (24.66°C) and relative humidity of 89%. Similarly,

the laboratory conditions of temperature and relative humidity recorded 75.2°F (24°C) and 83% respectively. This did not vary too much from the average temperature and relative humidity of the environment and it showed little or no influence on the ants' attraction to the food items by comparison.

Ant abundance and rate of attraction to food is dependent on conditions of the food (moist or dry)

C. maculatus showed preference towards moist sugary food in all the locations (Table 3), the ants were more abundant after the 1st hour (Table 4). In Orita Obele (HDZ), the abundance of *C. maculatus* after hours rose to 210.33 ± 7.22 , from the initial 59.67 ± 2.73 (Table 4). Although dry sugar exhibited some level of attraction to ant in high abundance, however, moist sugar showed stronger attraction levels. The results were similar in the other two locations FUTA (MDZ), Ibule (LDZ). Moist sugar had an abundance of 51.00 ± 3.46 in the 1st hour, thereafter, 192.67 ± 9.03 after the 2nd hour and 190.67 ± 3.84 in the 3rd hour. However, dry sugar demonstrated significantly less attraction, with the 1st, 2nd and 3rd hour having an abundance of 13.67 ± 1.45 , 11.00 ± 2.08 , and 14.67 ± 1.45 respectively (Table 4). Ibule had *C. maculatus* population of 48.67 ± 1.20 on moist sugar after one hour, this abundance increased to 205.00 ± 6.35 and 208.33 ± 6.98 after two and three hours respectively. Dry sugar demonstrated a similarly low level of ant abundance in Ibule compared to the other two locations; with an abundance of 9.67 ± 1.67 , 9.67 ± 1.53 , and 15.67 ± 1.20 respectively in the 1st, 2nd and 3rd hour respectively (Table 4).

Table 3 Average abundance of *C. maculatus* attracted to moist and dry sugar food in different locations in 4 h

Locations	Moist sugar	Dry sugar	P-value
HDZ (Orita Obele/ City centre)	464.00 ± 0.58	41.00 ± 2.52	< 0.01
MDZ (FUTA)	434.33 ± 9.91	39.33 ± 4.91	< 0.01
LDZ (Ibule)	462.00 ± 2.89	35.00 ± 3.51	< 0.01

Table 2 Mean attractiveness of Food Items in different locations

Food items	Ibule (LDZ)	FUTA (MDZ)	Orita Obele (HDZ)	Laboratory
Sugar	33.33 ± 0.96^a	31.89 ± 2.56^a	29.22 ± 0.95^a	30.41 ± 0.76^a
Honey	39.56 ± 0.44^{ab}	37.56 ± 2.63^a	31.44 ± 0.89^b	32.13 ± 0.51^b
cola drink	42.33 ± 2.91^{ab}	38.44 ± 4.07^a	33.56 ± 0.11^b	35.04 ± 1.27^b
Milk	45.56 ± 3.09^a	39.45 ± 3.47^a	33.11 ± 0.97^b	34.44 ± 1.17^b
Candy	40.89 ± 2.15^{ab}	35.33 ± 3.59^a	31.89 ± 0.56^c	36.11 ± 0.77^a

Means having the same alphabet within the column are not significantly different from each other using ANOVA and Tukey's test at $p < 0.05$

Table 4 Rate of attraction of *C. maculatus* to moist and dry sugar food conditions at various time intervals

Time	Sugar	Average abundance of <i>C. maculatus</i> (Mean $\pm$ SEM)				Significant test between dry/moist sugar
		Orita Obele (HDZ)	FUTA (MDZ)	Ibule (LDZ)	Laboratory	
After 1 h	Moist	59.67 $\pm$ 2.73 ^a	51.00 $\pm$ 3.46 ^b	48.67 $\pm$ 1.20 ^b	44.8 $\pm$ 7.84 ^a	F = 20.823
	Dry	9.00 $\pm$ 0.58 ^a	13.67 $\pm$ 1.45 ^b	9.67 $\pm$ 1.67 ^a	8.9 $\pm$ 1.68 ^a	Sig. = 0.001
After 2 h	Moist	194.0 $\pm$ 5.13 ^a	192.67 $\pm$ 9.03 ^a	205.00 $\pm$ 6.35 ^b	166.9 $\pm$ 8.3 ^c	F = 97.288
	Dry	15.33 $\pm$ 0.88 ^a	11.00 $\pm$ 2.08 ^b	9.67 $\pm$ 1.53 ^c	13.1 $\pm$ 1.7 ^d	Sig. = 0.002
After 3 h	Moist	210.33 $\pm$ 7.22 ^a	190.67 $\pm$ 3.84 ^b	208.33 $\pm$ 6.98 ^a	191.9 $\pm$ 17.8 ^b	F = 67.30
	Dry	16.67 $\pm$ 1.20 ^a	14.67 $\pm$ 1.45 ^a	15.67 $\pm$ 1.20 ^a	14.9 $\pm$ 3.68 ^a	Sig. = 0.001

Analyses of *P* values in all cases indicates that there is a significant difference in the attraction of ants to moist sugar compared to dry sugar in all the three locations examined

Means having the same alphabet within the column are not significantly different from each other using ANOVA and Tukey's test at *p* < 0.05

There is a significant difference (*P* < 0.05) between the rate of attraction of the ants to moist food compared to dry food in all locations and in the laboratory (Table 4). Large number of ants were observed foraging on the moist sugar while only a few ants were attracted to the dry sugar (Table 3). This finding confirmed that in addition to the ants preference for certain foods, higher moisture content of the food facilitates attraction. The environmental temperature and relative humidity in the three locations and in the laboratory are slightly different.

Assessment of the Microbial Pathogen Load in *C. maculatus* in the designated study locations

The microbial pathogens cultured from *C. maculatus* ants on the selective media, showed no growth after 24 h of incubation, except for the pathogens cultured on Nutrient agar (NA) in each of the three locations which displayed visible bacterial colonies of *Bacillus* spp (Table 5). Similarly, for Potato Dextrose Agar (PDA), the growth of *Aspergillus* spp. was also observed. For Orita Obele, *Bacillus* spp.

displayed the highest bacterial count (89 $\pm$ 2.65 CFU/ml) in site 1. *Aspergillus* spp. also exhibited significant colony formation with counts of 13.67 $\pm$ 1.45, 85.33 $\pm$ 2.03, and 72.00 $\pm$ 2.65 SFU/ml respectively in sites 1, 2 and 3 respectively in Orita Obele (HDZ) (Table 5). Findings from MDZ (FUTA) revealed that *Bacillus* spp. exhibited more colony growth compared to *Aspergillus* spp. (Table 5). No growths of coliforms, *Staphylococcus* spp., *Salmonella*, and *Shigella* were observed on the plates after 24 h. The highest colony count of 18.00 $\pm$ 2.08 CFU/ml was recorded for *Bacillus* spp in MDZ (FUTA).

Furthermore, our results indicate, in Ibule (LDZ) like in other 2 locations, only *Bacillus* spp. and *Aspergillus* spp. growth were observed on the plates. No visible growths of other pathogens were noted. *Bacillus* spp. displayed distinct colony growth with pathogen load counts of 33.00 $\pm$ 2.65, 22.00 $\pm$ 2.89, and 22.00 $\pm$ 1.53 CFU/ml in sites 1, 2 and 3 in Ibule (LDZ) respectively and *Aspergillus* spp. showed colony variations with counts of 18.00 $\pm$ 0.58, 20.33 $\pm$ 1.76 and 14.67 $\pm$ 1.86 SFU/ml in sites 1, 2 and 3 in Ibule (LDZ) respectively (Table 5).

Table 5 Microbial Pathogen Load of *C. maculatus* in Selected Sites in different locations

Isolates	Sites	Location counts		
		Bacterial colony counts ($\times 10^9$ CFU/ml)		
		Ibule (LDZ)	FUTA (MDZ)	Orita Obele (HDZ)
<i>Bacillus</i> spp	1	33.00 $\pm$ 2.65 ^b	18.00 $\pm$ 2.08 ^b	78.00 $\pm$ 3.46 ^a
	2	22.00 $\pm$ 2.89 ^a	8.67 $\pm$ 0.88 ^a	80.00 $\pm$ 0.58 ^a
	3	22.00 $\pm$ 1.53 ^a	13.67 $\pm$ 1.20 ^{ab}	89.00 $\pm$ 2.65 ^a
<i>Aspergillus</i> spp.	1	18.00 $\pm$ 0.58 ^a	13.67 $\pm$ 1.45 ^a	13.67 $\pm$ 1.45 ^a
	2	20.33 $\pm$ 1.76 ^a	8.67 $\pm$ 0.33 ^a	85.33 $\pm$ 2.03 ^c
	3	14.67 $\pm$ 1.86 ^a	9.33 $\pm$ 1.45 ^a	72.00 $\pm$ 2.65 ^b

Coliforms, *S. aureus*, *Salmonella* spp. and *Shigella* spp. recorded 0.00 $\pm$ 0.00. Means having the same alphabet within the column are not significantly different from each other using ANOVA and Tukey's test at *p* < 0.05



Assessment of the Microbial Pathogen Load in the spoilt food samples

Upon subjecting the spoilt food samples to a dilution factor of 10^9 , noticeable and significant colony growth was observed in the microbial analysis. Notably, *Bacillus* spp. exhibited the highest colony growth on Nutrient agar, recorded at 133.44 ± 11.12 CFU/ml (Table 6). In contrast, *S. aureus* displayed the lowest colony growth at 9.33 ± 2.42 CFU/ml among the cultured samples (Table 6). *Escherichia coli* presented a substantial level of contamination with a colony growth of 59.78 ± 9.07 CFU/ml, although it did not reach the levels observed for *Bacillus* spp. (133.44 ± 11.12 CFU/ml). This suggests a significant presence of *E. coli* in the spoilt food samples (Table 6). While *S. aureus* showed a comparatively lower contamination level (9.33 ± 2.42) than *Bacillus* spp. (133.44 ± 11.12 CFU/ml) and *E. coli* (59.78 ± 9.07 CFU/ml), it remained statistically significant, indicating its presence in the spoilt food. Moderate levels of *Salmonella* and *Shigella* spp. contamination, with a colony growth of 28.44 ± 0.60 CFU/ml (Table 6) suggested the presence of *Salmonella* and *Shigella* spp. in the spoilt food samples. Similarly, Potato Dextrose Agar containing *Aspergillus* spp. displayed a moderate contamination level of 42.00 ± 7.64 SFU/ml (Table 6), indicating the presence of *Aspergillus* spp. in the spoilt food samples.

Microbial evaluation of the fresh food exposed to infected ants from the contaminated food

In Orita Obele, *Bacillus* spp. had the highest mean count of 20.22 ± 0.89 CFU/ml with *Aspergillus* spp. recording the lowest (6.67 ± 0.62 SFU/ml). Significant microbial loads containing *E. coli* (11.33 ± 0.76 CFU/ml), *S. aureus* (13.78 ± 1.53 CFU/ml), and *Salmonella/Shigella* spp. (9.00 ± 0.82 CFU/ml) were observed (Table 6). Variations in microbial load ($p < 0.05$) showed that the SFU/ml of *Aspergillus* spp. and CFU/ml of *Salmonella/Shigella* were

not significantly different ($p > 0.05$). Meanwhile, *Bacillus* spp. showed a significant difference compared to *S. aureus* and *E. coli*.

The *C. maculatus* ants in MDZ (FUTA) showed significant variations in microbial pathogen population after interacting with healthy food, sequenced to foraging on spoilt food samples. There was a significant difference ($p < 0.05$) among some CFU units of pathogens observed from this location, while some showed no significant differences ($p > 0.05$). For example, results obtained from *E. coli* (6.89 ± 0.56 CFU/ml) showed no significant difference compared to that of *Aspergillus* spp. (10.33 ± 0.62 SFU/ml). *Bacillus* spp. showed significant variation (17.89 ± 0.92 CFU/ml) in microbial load compared to that of *Salmonella/Shigella* and *S. aureus*, *E. coli* and spore forming unit of *Aspergillus* spp.

Results obtained after *C. maculatus* interacted with healthy food in Ibule showed there was no significant difference ($p > 0.05$) between the microbial colony count for *E. coli* (16.33 ± 0.87 CFU/ml) and *Salmonella/Shigella* spp. (14.22 ± 1.18 CFU/ml) (Table 6). Similarly, *S. aureus* colony count (11.11 ± 0.72 CFU/ml) showed no significant difference compared to that of *Aspergillus* spp. (11.22 ± 0.66 SFU/ml). Also, the microbial colony counted for *Aspergillus* spp. (11.22 ± 0.66 SFU/ml) and *S. aureus* (11.11 ± 0.72 CFU/ml) were significantly different ($p < 0.05$) from that of *Bacillus* spp. (20.78 ± 0.85 CFU/ml), *E. coli* (16.33 ± 0.87 CFU/ml), and *Salmonella/Shigella* spp. (19.11 ± 1.06 CFU/ml).

Discussion

The research conducted in the study areas revealed differences in relative abundance of *C. maculatus*. High relative abundance of *C. maculatus* foraging in the high density and anthropized places inferred that the primary factors driving foraging behaviour in ants indoor is the clearing of their habitats and food resources in the natural environment (Akinwande et al. 2024). The choice of foraging food attractants

Table 6 Microbial count of the spoilt food, ants that interacted with the fresh and spoilt food samples

Isolates	Spoilt food Samples	Microbial Count on the Ants after Interaction with the Spoilt Food Samples			Microbial Pathogen Load vectored to Fresh food Samples by the ants		
		Orita Obele (HDZ)	FUTA (MDZ)	Ibule (LDZ)	Orita Obele (HDZ)	FUTA (MDZ)	Ibule (LDZ)
<i>Bacillus</i> spp.	133.44 ± 11.12^d	26.11 ± 1.12^b	29.22 ± 2.59^c	26.67 ± 2.13^c	20.22 ± 0.89^d	17.89 ± 1.53^c	20.78 ± 0.85^c
<i>Escherichia coli</i>	59.78 ± 9.07^c	19.44 ± 1.86^{ab}	14.00 ± 1.20^b	17.00 ± 1.07^b	11.33 ± 0.76^{bc}	6.89 ± 0.56^a	16.33 ± 0.87^b
<i>S. aureus</i>	9.33 ± 2.42^a	17.78 ± 4.19^a	15.33 ± 1.26^b	24.11 ± 1.98^c	13.78 ± 1.53^c	11.11 ± 0.79^{bc}	11.11 ± 0.72^a
<i>Salmonella/Shigella</i> sp	28.44 ± 0.60^{ab}	21.56 ± 1.72^b	6.22 ± 0.78^a	19.11 ± 1.06^{bc}	9.00 ± 0.82^{ab}	14.22 ± 1.22^c	14.22 ± 1.18^a
<i>Aspergillus</i> spp. ($\times 10^9$ SFU/ml)	42.00 ± 7.64^{bc}	77.22 ± 6.92^c	14.56 ± 2.68^b	23.78 ± 1.76^c	6.67 ± 0.62^a	10.33 ± 0.62^{ab}	11.22 ± 0.66^a

Means having the same alphabet within the column are not significantly different from each other using ANOVA and Tukey's test at $p < 0.05$

for *C. maculatus* may inadvertently impact the abundance of the ants in the environment (Bambang et al. 2023). Furthermore, the variation in the distribution and presence indoor is dependent on the geographically delineation of the study areas using vegetation and human population density, this opinion aligned with Sharaf et al. (2017) and Akinwande et al. (2024). Site variations in the ants' presence and abundance suggest that ecological conditions at each location probably influence the ant distribution (Sharaf et al. 2017). Habitat characteristics, resource availability and interspecific competition are other known factors that might also influence the ant community structures (Andersen and Sparling 1997).

Again, in the study, the foraging behaviour of ants is influenced by the needs of the colony as a whole (Ganesh and Avalokiteswar 2018), Ants are highly adaptive, the insect can adjust their foraging behaviour based on the availability, abundance and distribution of food resources (Nyamukondiwa and Addison 2014). Ant colonies operate as superorganisms, with individual ants working together or cooperatively (Wilson 2003) to meet the needs of the colony. In this regards, colony size, reproductive status, and nutritional requirements influence the intensity and direction of foraging efforts. Observations showed the food attractiveness, the food quality and location drive the ants discovery of food for forage, recruitment of foraging members and foraging process. Although, we do not investigate the chemical driver of the foraging behaviour of the ants, but the ants were known to use pheromones to recruit nest mates to food discoveries, establish efficient foraging trails, and navigate complex environments (Dussutour et al. 2009).

Foraging patterns of *C. maculatus*, observed to be diurnal with peak activity during late evenings to early morning align with general ant foraging behaviour (Hölldobler and Wilson 1990). The preferences for group foraging and reliance on pheromone trails emphasize the cooperative nature of *C. maculatus* colonies, supporting existing knowledge of the ant foraging behaviour (Wilson 2003). The capability of *C. maculatus* to cover substantial distances in search of food stresses broader understanding of ant foraging behaviour, crucial for their survival and efficient resource exploitation (Hölldobler and Wilson 1990; Wilson 2003). For survival and efficient resource exploitation, the worker ants display a swift and coordinated response to the discovery of food (Hölldobler and Wilson 1990; Gordon 2010) and the behaviour of diligent food storage by *C. maculatus* ants contributes to the overall resilience of the colony foraging in the presence of continuous supply of food resources over time (Judd 2011).

The study established that *C. maculatus* ants have preference for sugary food sources and food with high moisture contents. The ant exhibition for a strong preference for sugar is not only because of taste but also for the moisture

contents which add a nuanced layer to their foraging behaviour (Nyamukondiwa and Addison 2014). The question has been why sugary food? Perhaps due to its abundance, ease of storage, high energy levels, and straightforward digestibility (Barbani 2003; Buczkowski and Bennett 2006; Went et al. 1972). The liquid nature of sugar makes it an optimal choice for ants, which lack the ability to digest solid food particles and rely on semi-liquid substances (Ricks and Vinson 2004). The moisture creates a solution that disperses the sugar molecules, making it more detectable through chemical cues such as scent trails (Lanza et al. 1993). Moistened sugar contain trace amounts of other substances such as minerals, that are important for ant nutrition (Blüthgen and Fiedler 2004). This nutritional factors influence the ants' attraction to moistened sugar in the study.

The research findings using selective media analysis revealed the absence of certain pathogens in *C. maculatus*, suggesting lack of association. However, the presence of *Bacillus* spp. and *Aspergillus* spp. indicates the natural microbiota harboured by these ants. This is in agreement with studies by Máximo et al. (2014); Simothy and Mahmoodally (2018) and Alharbi et al. (2019). These researchers have explored the microbial composition in ants, emphasizing the prevalence of bacteria species and highlighting the selective nature of different growth media. Variation in microbial counts and the presence of diverse fungi across study locations indicates the influence of environment. Therefore, foraging environmental factor affects microbial diversity vectored by ants (Zientz 2005; Simothy and Mahmoodally 2018) and the presence and growth of diverse fungal species in ant-associated environments (Zientz 2005; Simothy and Mahmoodally 2018). In this study, we do not investigate the mechanism by which the ants dispersed the pathogens on the foraging food substances, However, Paul and Roces (2019) investigated the mouth part morphology of ants and its implications for pathogen transmission, certain ant species has a specialized mandibles and mouth parts which are more efficient at piercing plant tissues and insect cuticles, potentially facilitating the transmission of pathogens. The dispersal of pathogens by ants and their potential impact on environmental contamination consented with research by Alharbi et al. (2019), in which ants were discovered to transport and disseminate pathogens across landscapes, particularly in agricultural and urban settings where ants interact with human activities and structures.

Spoilt food samples showed significant colony growth of *Bacillus* spp. and *E. coli* supporting findings by Zarzuela et al. (2005) regarding potential health risks associated with food-borne pathogens in spoilt foods foraged by ants. The ants demonstrated the potential to pick up and carry these food-borne pathogens from contaminated food sources to healthy food with variations across locations suggesting influence of environmental factor. In one of



the study areas here, Orita-Obele, *C. maculatus* exhibited the potential to carry and disseminate diverse pathogens. Similar observations were made by Zarzuela et al. (2005) and Ogba et al. (2017) with research study highlighting ants as vectors for various microorganisms in some studied environment. The significant variations in microbial pathogen populations from ants in FUTA highlight the dynamic nature of ant-mediated transmission, this poses a great challenge for food safety protocols according to Konrad et al. (2015) and Ramalho and Moreau (2023). In Ibule, similar transmission dynamics were observed for Coliforms, *Salmonella* spp and *Shigella* spp., suggesting shared transmission patterns within the ant population. Segers and Kaltenpoth (2019) suggests that certain bacterial species may have comparable transmission patterns within ant colonies. Therefore, the significant differences in microbial colony counts suggests a selective transmission pattern by *C. maculatus* with variations across different environmental locations.

Conclusion

C. maculatus are attracted to food by preference and the nature of the food. The number of ants attracted to the food is dependent on the food attractiveness and the location as factored by the environmental conditions that drive the ants indoor. The environment and the evolutionary relationship or interactions with some pathogens influence the potential to carry some of the pathogens from contaminated food sources to stored, freshly cooked or ready-to-eat food. Our study emphasizes the need for awareness regarding the presence of ants in indoor environment and on food. The variations in microbial colony counts in *C. maculatus* across the different locations show the pathogen transmission pattern is driven by the environment and the food sources. Hence, it is imperative to control ants particularly *C. maculatus* spp invading indoor.

Acknowledgements We are grateful to Mr Adewale Praiseworth, Department of Biology, Federal University of Technology, Akure, for his contributions.

Author contribution KLA Conceived the experiments and supervised it. OOO carried out the experiments together with KLA, BA compiled the data and KLA analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

Funding The research did not receive any grant from funding agencies in the public, commercial or not for profit sectors.

Availability of data and material All data used in this study are available from the corresponding author upon reasonable request.

Declarations

Competing interest There is no conflict of interest exists for all participating authors.

Ethical approval Not applicable.

Consent to participate The entire areas of study are within the areas the university researchers use.

Consent for publication The two authors have agreed to publish the findings from the research.

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