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Lipid Food Preference and Transportation Using Tools by an Indian Ant Species *Monomorium pharaonis* (Hymenoptera: Formicidae): A Field Study

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Abstract

Ants have dietary requirements with different macromolecular compositions and the foraging activity is considered to be based on maximum energy acquisition. Besides proteins and carbohydrates, the Pharaoh ant (Monomorium pharaonis) has a preference for lipid foods especially when lipid becomes limiting in the colony. The personal observation indicated that the ant colony under investigation had preferred oil (lipidrich foods) thus we wanted to follow up by using different oils as foods in our next study. We used four different food-grade lipids such as mustard oil, sunflower oil, coconut oil and butter for seven consecutive days for a specified time. We observed that approximately 90 mins after the start of the experiment the highest number of ants accumulated in all four food baits. Sunflower oil was the most preferred food as far as the accumulation of ants was concerned and mustard oil showed the lowest number of ants. The differential number of ants in lipid food baits under observation can be attributed to the physical features of lipid like density, and smell along with the nutrient content. We recorded the specific time zones for all four lipid foods at which, the highest number of foragers gathered. We also noted an interesting feature that the major role of gathering and transporting food was performed primarily by two castes, intermediate and large, among the four distinct foraging castes found during the study. Further, we noted that ants used small absorbent earthen clods and sand particles (tools) to soak the oil and some of the tools were carried back to the nest. The most used tools in the present investigation were <0.5mm and naturally available sand particles. M. pharaonis transported the butter-soaked smallest tools only back to the nest and it is revealed that the butter was the most important lipid food for the particular ant colony of *M. pharaonis* under study.

Introduction

Most ants including *Monomorium pharaonis* have been found to generally prefer sugar solutions over honey, and other food items (Nyamukondiwa & Addison, 2014). Different ant species have different dietary requirements with macromolecular proportions (carbohydrate, protein, and lipid) required by them may also vary (Rust et al., 2000). Forager ants for the collection of essential nutrients in the wild are always at risk of being preyed on (Goteceitas, 1990). The foraging activity of many animals is assumed to be based on maximum energy intake per unit of time (Cuthill & Houston, 1997) to minimize the chance to be preyed upon. When foraging insects come across food resources, they immediately stop their movement and decide whether the food is acceptable or not because they have a systematic feeding sequence which helps detect, identification and transport food substances (Elizabeth, 1977; Joseph & Carlson, 2015; Thoma et al., 2017). Forager ants primarily collect large or small dead animals (Vinson & Sorensen, 1986) and they also collect foods rich



in micronutrients and microelements (Sengupta et al., 2010; Simpson & Raubenheimer, 2012; Sperfeld et al., 2017). The quickly growing colony with a large number of larvae requires a constant supply of protein food sources (Dadd, 1985). The larva is the only life stage in insects in which they eat voraciously to gain maximum energy. The energy intake helps them quickly build up the structure that eventually triggers the necessary function of the body to withstand the next non-feeding life stage, the pupa. All the important metabolic processes come into play during the pupal stage to become an active adult from the non-active predecessor. In this game, the protein plays a pivotal role. Likewise, carbohydrates and lipids also provide energy and lipid is the most efficient space-saving, and richest energy source while the energy in carbohydrates is more readily available. Lipid is a vital micronutrient for most organisms as it is the parent compound of many signal molecules and it chiefly contributes to the cell membrane structure (Bayes et al., 2014). In insects, lipids can be used as waxes, pheromones, defensive secretion, etc. (Stanley-Samuelson et al., 1988). The cuticular hydrocarbons are the important source for communication in ants and these are primarily made up of lipids (Barbero, 2016). Lipid is an important liquid food component and among the different food resources, most ants prefer liquid food to other food types (Qin et al., 2019; Wang et al., 2018). Lipids are essential for larval growth, egg production, ovary development, etc. (Blüthgen & Feldhaar, 2010). The foraging activity of ants depends upon the nutritional status of the colony however, the lipid nutrient sometimes becomes limiting. Lipid becomes difficult to acquire in nature due to its physical properties. During the procurement of liquid lipid food, the ants get stuck to this food and die. In such a scenario ants develop alternative strategies for lipid food procurement. In our preliminary observation, we found M. pharaonis had a preference for lipid food in addition to carbohydrates and proteins. Keeping this fact in mind, in the present experiment we used different types of lipids as food baits to address the preference of *M. pharaonis* to a specific lipid food type.

Animals are known to use tools for their survival, and these are primarily used for gathering food, defence, construction, etc. (Oakley, 1951; Shumaker et al., 2011; St. Amant & Horton 2008; Bentley-Condit & Smith, 2010; Hunt et al., 2013). For ants, few species have been found to use tools, and species like Oecophylla, and Polyrhachis were documented to use tools for nest building. They use silk material (from their larvae), earthen clods and other debris to construct the nest (Wheeler, 1910; Hölldobler & Wilson, 1990). They sometimes use dry plant twigs and sand particles while foraging. Tool use is a well-known feature in ants but as of now very little is known regarding how, when, and in what situation ants use tools (Maák et al., 2017; Lőrinczi et al., 2018; Richards, 2022). Until now, only eight genera of ants have been recorded to use tools, which are mainly associated with foraging activity and the first documentation of tools used by ants was made by Fellers and Fellers (1976) in Aphaenogaster, in which the ant was found to drop debris like particles of soil, bits of leaf etc. as tools into the liquid food to transport the food-soaked tools back to the colony. Since then, not much research was carried out during the last forty-odd years on the issue of transportation of liquid lipid food back to the colony using various tools. This is a very important issue because the transportation of lipid-like liquid food to the colony is a great challenge to the ants. Considering the aforesaid facts, we designed the present investigation to find out among four distinct foraging castes of our ants, which was taking the primary responsibility in the transportation of liquid lipid food into the nest and how many and, what types of tools they were using during the process of transportation. In the present study, we also investigated, which type of liquid lipid food they liked most.

We predicted that the present ants would have differential choices for the different liquid lipid food and due to the nature and the texture of the liquid lipid food the present ants would use tools to collect and carry it back to the nest. In the present study, we used *M. pharaonis* from a natural colony for our experiment and we allowed the ants to freely access the lipid food baits.

Objectives of the present investigation

(1) What type of lipid food bait was liked most by our forager ants?

(2) What caste of forager ants accumulated more at the lipid food site?

(3) When did the foragers start using tools for the lipid food bait?

(4) How many types and the number of tools were used?

Materials and Methods

Study species

The tropical pest ant, Monomorium pharaonis (Linnaeus, 1758), the Pharaoh ant is the most ubiquitous house ant in the world (Erdos & Koncz, 1977; Wilson & Booth, 1981) and this ant has been studied extensively due to their wellestablished caste-hierarchy system (Boonen & Billen, 2017). M. pharaonis is one of the smallest ants with the length of foragers ranging from 2.00 - 3.00 mm. The distribution of this ant is limited by cool climates and relies primarily upon humans for a suitable home (Klots & Klots, 1959). These ants are commonly seen in the backyard, garden, park, and even inside human habitation. M. pharaonis is native to Africa but it can quickly expand into new territories and propagate rapidly as an invasive species and it is predominant in tropical regions across the globe. They can be seen to take all types of foods. Their nests are made in every possible habitation such as in soil, under leaf litter, in cracks in buildings, etc. (Narendra & Sunil Kumar, 2006).

The present ant species (*M. pharaonis*) was selected from the natural colony in the field. For our experimental design to test lipid procurement activity by our ants, we used four types of lipid food baits. The foods were provided to them in white food plates which were made up of non-soaked photo paper. The ants of the present investigation were allowed to access easily the all food baits ad libitum provided to them for the experiment. There was an approximate distance between the food plates and the ant trail of 15.0 cm. Different types of lipid food baits such as mustard oil (MO), coconut oil (CO) sunflower oil (SO), and butter (BU) were provided together to them on four separate white photographic square papers $(5.0 \times 5.0 \text{ cm}^2)$ as food plates. Different lipids used in the investigation were of the highest grade, for human use, and from well-known Indian branded companies. We used one drop of liquid lipid bait in three cases of mustard oil, coconut oil and sunflower oil and in the case of butter we used an almost similar amount considering the periphery of the other liquid food drops initially applied. The four food bait plates were arranged 2.0 cm apart from each other in a straight line (Fig 1). The experiment was conducted in July from 10:30 h to 14:30 h for seven consecutive days with an ambient temperature of 34.2 ± 1.1 °C. The seven experimental days included sunny and cloudy days with occasional rains. We did not provide any tools to our ants but we observed diverse sizes and kinds of tools in the food baits and we measured the size of tools present on baits. We measured the diameters of those roughly circled tools and categorized them as <0.5mm, 0.5-1.0mm, and >1.0mm and subsequently counted the number of tools at all time points of our observations. We distinctly observed four different types of foragers in the food bait plates and we categorized them into small (S), intermediate (I), large (L), and huge (H) foragers based on their size. We recorded the number of them in the food baits at different time points starting at 11:15 h and ending at 14:15 h with 10 minutes intervals. The same experiment was repeated for seven consecutive days (N = 7).

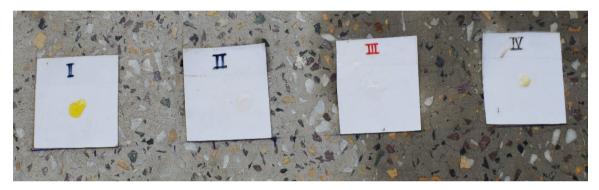


Fig 1. The arrangement of lipid food baits. Mustard oil, Coconut oil, Sunflower oil, and Butter are on food plates I, II, III, and IV respectively.

Statistical analyses

For the one-way ANOVA test, we have used different lipid foods as independent variables. The number of ants and the number of tools are the dependent variables and the level of significance considered is p<0.05. When we have found any significant F value from our data calculation, we have tested the data using Schefee's multiple comparison test as a post hoc test to work out which two groups are significantly different from one another (p<0.05) both in case of the number of ants and the number of tools as well (Das & Das, 2003).

Results

The ant aggregation at lipid food baits

Overall, significantly, more and more numbers of ants started accumulating in the food bait plates with the increment of time. Among all four food baits, sunflower oil was found to be the most preferred concerning the accumulation of different-sized ants under observation. Almost at all time points, the highest number of ants accumulated was in the sunflower food bait. The peak number of ants found was always higher in this particular food bait in all seven days of observations except on the 7th day (Fig 9). The preferred food bait next to sunflower oil was butter. Conversely, the mustard oil was found to be the least-liked food bait by this particular ant species and the coconut oil remained in the third position. There was a time zone on all experimental days (12:05 h to 13:05 h), at which more ants gathered at all four food bait plates under observation, with varying numbers. Before and after the specified time zone, the ant number was getting decreased on either side of the time zone (Fig 3-9). This observation indicates the fact that there was a lag phase for the ants in finding out the lipid food source for the first time on each experimental day and it was about 10 - 40 mins for different food baits. After having reached the highest number, they started disintegrating. Such a phenomenon was noted on all seven days of the experiment. One-way ANOVA has been performed to find out whether or not there is any significant difference in the total number of ants in all four food baits under study and the calculated F value (32.76) indicates the significant difference among the ant numbers (p < 0.05). Further analysis of these data with the post hoc test mentions which two food baits are significantly different from each other (Table 1). The four groups of food baits

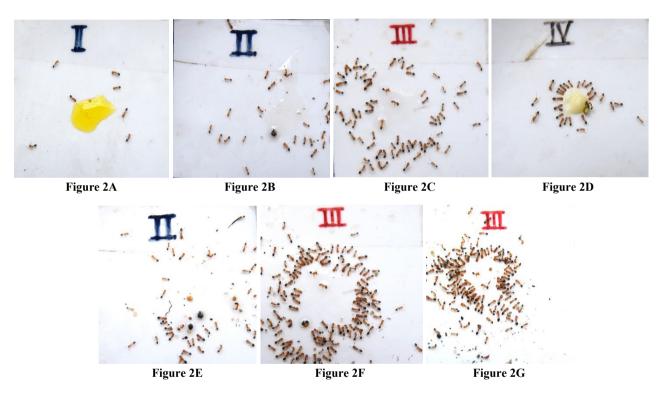
produce six combinations of two each and these are mustard oil vs coconut oil; mustard oil vs sunflower oil; mustard oil vs butter; coconut oil vs sunflower oil; coconut oil vs butter and sunflower oil vs butter. The calculated *t* values obtained using the post hoc test are significantly different between all two groups of six such combinations.

When we plot the data to prepare a 100% stacked column using the values of different castes gathered in food baits, we find that among all four ants' forager castes, the values of intermediate and large castes are always more in all four food plates (Fig 10). The total number of ants (all types) is found to be significantly highest in sunflower oil (587.43 \pm 148.40) and significantly lowest in mustard oil (70.14 \pm 12.88) with intermediate values of 489.57 \pm 128.11 and 333.29 \pm 70.30 in butter and coconut oil respectively.

The total number (Mean \pm SD) (Fig 11) has also been noted to show a similar pattern to what has been seen in the 100% stacked column (Fig 10).

Table 1 - Post hoc test (Schefee's Multiple Comparison Test)

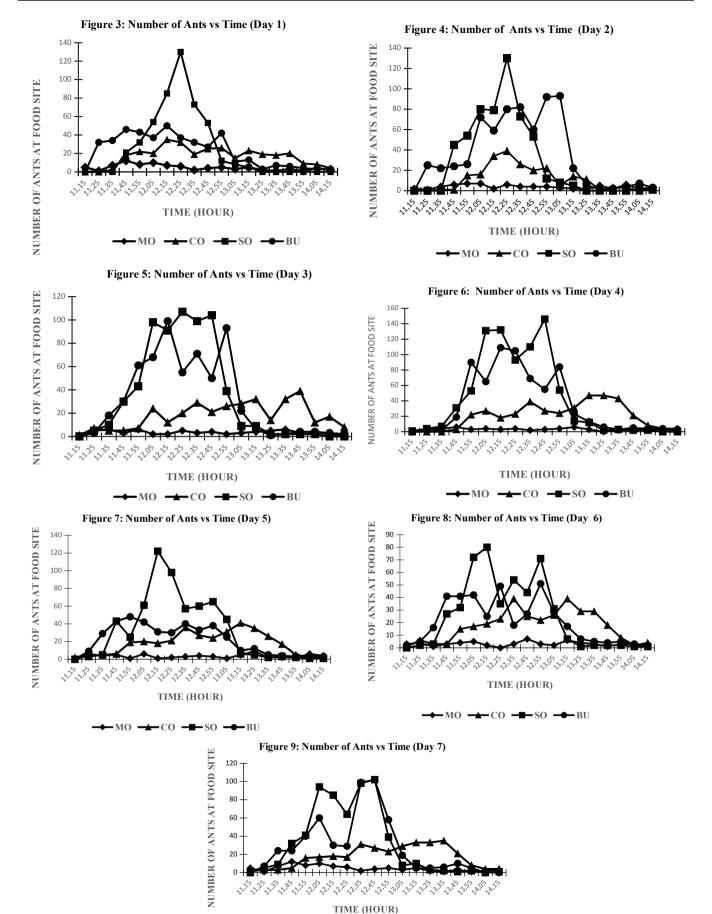
| Comparing Group | t Value | P<0.05 |
|------------------------------|---------|-------------|
| Mustard Oil vs Coconut Oil | 4.719 | Significant |
| Mustard Oil vs Sunflower Oil | 4.558 | Significant |
| Mustard Oil vs Butter | 1.754 | Significant |
| Coconut Oil vs Sunflower Oil | 9.276 | Significant |
| Coconut Oil vs Butter | 7.522 | Significant |
| Sunflower Oil vs Butter | 2.802 | Significant |



Figs 2A-G. A: Ants in the mustard oil food bait during the early phase of observation. **B:** Ants are in the coconut oil. Different-sized tools are seen. **C:** Ants in the sunflower oil bait during the same time as observed in mustard and coconut oils. No tool is seen. **D:** Ants in the butter food bait. Few tools are observed with one unusually large tool. **E:** Ants in the coconut oil food bait. Ants have collected more tools with time. **F:** Ants in the sunflower oil food bait with different-sized tools. **G.** Ants in the sunflower oil food bait. More tools are seen as compared with the previous figure (Fig 2F).

The dropping and retrieval of tools

We recorded the dropping of different tools in the lipid food baits (Table 2A-G). Mostly the sand particles and earthen clods were dropped into the baits along with dry and dead plant debris in a few cases. The tool dropping was started at 11:25 h (butter), 11.35 h (mustard oil), and 11:55 h (sunflower oil) but the same was started further late in the case of coconut oil food bait. For tool-dropping timing, we found a lag phase of 10, 20, and 40 mins for butter, mustard oil and sunflower oil food baits respectively from the start of the experiment but the event was started 100 min later (at 13:05 h) in coconut oil food bait (Fig 12). Though the tool-dropping started late in coconut oil, the number of tools was increasing steadily with the increased time points. On the contrary, the number of tools was found either to be decreased or remained the same in the other three food baits. We noted the tools of different sizes in the food baits and in a few rare cases, the size of the tool went up to 10 times that of the body size of the foragers, if not less. No further increase in the number of tools after a specified time (12:45 h) was recorded both in mustard oil and sunflower



→ MO → CO → SO → BU

Figs 3-9. The trend of ant accumulation at different lipid food sites was observed and recorded for seven consecutive days.

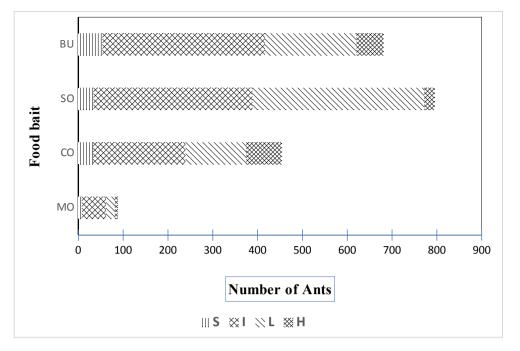


Fig 10. Average number of ants at peak accumulation along with their caste distribution.

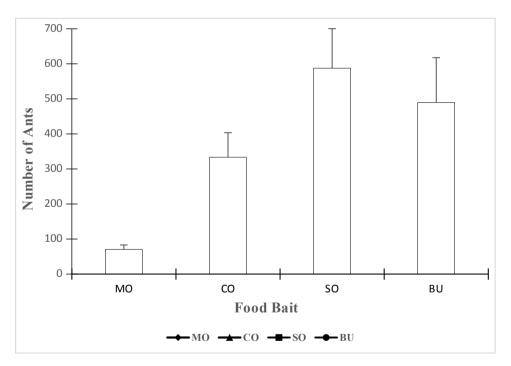


Fig 11. Total number of ants (Mean \pm SD) accumulated at different food baits irrespective of any time point and caste variation.

oil but an opposite relation was noted between the number of tools and the time points in coconut oil and butter; while the number of tools decreased in butter, it increased in coconut oil with time. Table 2A shows the individual tool types used by the *M. pharaonis*. The tools in >1.0mm category were huge and most of the tools observed in all food baits were of <0.5mm category and were sand particles. During the entire experiment, we did not provide our ants with any kind of tool for the experimental purpose but they collected the same from the surroundings and put those into the food baits.

A one-way ANOVA test among the types of lipid food baits and the number of tools has been performed and the F value (9.62) shows the significant (P<0.05) difference in numbers in all four food baits. The post hoc test (Table 2B) indicates the number of tools are not significantly different between mustard oil vs sunflower oil; mustard oil vs butter and sunflower oil vs butter but the rest three combinations such as coconut oil vs mustard oil; coconut oil vs sunflower oil and coconut oil vs butter are showing significant differences.

Table 2A - Individual and average number of tools accumulated at four food baits along with different time points.

| | | | | | | | | | | | | | | | | | ř I |
|----|-------|--------|---------|-------|-------|--------|---------|------|-------|--------|---------|-------|-------|--------|---------|------|-------|
| | TIME | МО | | Total | СО | | Total | SO | | | Total | BU | | | Total | | |
| | | <0.5mm | 0.5-1mm | >1mm | TOUAL | <0.5mm | 0.5-1mm | >1mm | Total | <0.5mm | 0.5-1mm | >1mm | TOTAL | <0.5mm | 0.5-1mm | >1mm | Total |
| 1 | 11.15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 11.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 1 | 5 |
| 3 | 11.35 | 5 | 3 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 3 | 1 | 8 |
| 4 | 11.45 | 4 | 2 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 3 | 1 | 8 |
| 5 | 11.55 | 8 | 2 | 0 | 10 | 0 | 0 | 0 | 0 | 4 | 0 | 1 | 5 | 4 | 4 | 4 | 12 |
| 6 | 12.05 | 11 | 3 | 1+H | 16 | 0 | 0 | 0 | 0 | 9 | 2 | 1 | 12 | 8 | 6 | 4 | 18 |
| 7 | 12.15 | 9 | 3 | 1+H | 14 | 0 | 0 | 0 | 0 | 11 | 3 | 1 | 15 | 6 | 4 | 2 | 12 |
| 8 | 12.25 | 10 | 3 | 1+H | 15 | 0 | 0 | 0 | 0 | 11 | 3 | 1 | 15 | 7 | 4 | 2 | 13 |
| 9 | 12.35 | 14 | 2 | 1+H | 18 | 0 | 0 | 0 | 0 | 11 | 3 | 1+H | 16 | 7 | 4 | 2 | 13 |
| 10 | 12.45 | 15 | 2 | 1+H | 19 | 0 | 0 | 0 | 0 | 12 | 3 | 1+H+W | 18 | 8 | 4 | 2 | 14 |
| 11 | 12.55 | 15 | 2 | 1+H | 19 | 0 | 0 | 0* | 0 | 12 | 3 | 1+H+W | 18 | 8 | 4 | 2 | 14 |
| 12 | 13.05 | 15 | 2 | 1+H | 19 | 4 | 0 | 0 | 4 | 12 | 3 | 1+H+W | 18 | 10 | 6 | 2 | 18 |
| 13 | 13.15 | 15 | 2 | 1+H | 19 | 4 | 0 | 0 | 4 | 12 | 3 | 1+H+W | 18 | 11 | 8 | 4 | 23 |
| 14 | 13.25 | 15 | 2 | 1+H | 19 | 4 | 0 | 0 | 4 | 12 | 3 | 1+H+W | 18 | 8 | б | 3 | 17 |
| 15 | 13.35 | 15 | 2 | 1+H | 19 | 4 | 2 | 0 | 6 | 12 | 3 | 1+H+W | 18 | 7 | 5 | 4 | 18 |
| 16 | 13.45 | 15 | 2 | 1+H | 19 | 4 | 4 | 0 | 8 | 12 | 3 | 1+H+W | 18 | 8 | 6 | 5 | 19 |
| 17 | 13.55 | 15 | 2 | 1+H | 19 | 6 | 4 | 3 | 10 | 12 | 3 | 1+H+W | 18 | 6 | 4 | 5 | 15 |
| 18 | 14.05 | 15 | 2 | 1+H | 19 | 8 | 10 | 11 | 29 | 12 | 3 | 1+H+W | 18 | 4 | 3 | 3 | 10 |

* H = Huge sized tool; W = wooden structure. MO = Mustard Oil; CO = Coconut Oil; SO = Sunflower Oil; BU = Butter.

Table 2B - Post hoc Test (Schefee's Multiple Comparison Test).

| Comparing Group | t Value | P<0.05 |
|------------------------------|---------|-----------------|
| Mustard Oil vs Coconut Oil | 4.765 | Significant |
| Mustard Oil vs Sunflower Oil | 0.814 | Not Significant |
| Mustard Oil vs Butter | 0.518 | Not Significant |
| Coconut Oil vs Sunflower Oil | 3.954 | Significant |
| Coconut Oil vs Butter | 4.245 | Significant |
| Sunflower Oil vs Butter | 0.296 | Not Significant |

Discussion

Experiment I: Lipid food preference

The results of our study demonstrate that M. pharaonis exhibits a considerable amount of flexibility in choosing different kinds of lipid foods. Two among four castes of the ants, intermediate and large, take the primary responsibility in the transportation of food substances. They also show a differential response towards the four different lipid food baits. The differential choice among lipid food baits can be assumed that some factors of the food baits may play a role in addition to the nutrient contents of the food. The lipid food baits used in the present experiment were of food-grade quality and the M. pharaonis showed a differential response towards those lipid baits. Lipid is a major food source required by the workers of M. orientale (Loke & Lee, 2006) and the ants of urbanized areas have a natural choice for lipid because lipid may be the limiting resource in such areas (Gozman-Hernandez, 2019). The massive urbanization generates the heat-island effect, which subsequently develops microclimates (Oliveira et al., 2011; Aminipouri et al., 2019). Such microclimates have a great effect on ants' life including their food choice. The different number of ants in different lipid food baits as found in the present investigation could mean that there are other factors besides the nutrient value affecting their lipid preference and the differential choice could also be attributed to the physical nature of the lipid food bait provided. The less-dense lipid food bait (sunflower oil) was liked more than the high-density lipid food. Factors like smell and density of lipid food bait as observed in mustard oil, may hinder the choice of it. As was seen, the density of lipid food bait could be associated with the risk of fatality of foraging ants, and the more the dense lipid food bait more the chance of casualty. In addition, there may be nutritional differences in the four different lipid food baits. The omnivorous ants can obtain lipids through a diet of arthropods, seeds, and plant exudates (Rosumek et al., 2017) and human foods, to a greater extent helping them meet up with oil sources (Penick et al., 2015). Prey like arthropods, as lipid sources, cannot provide all the necessary nutrients that ants need, which may make them look for other food resources (oil). Judd (2006) suggested that in seed-consuming ants, foraging can occur when essential nutrients are not sufficiently provided by seeds. However, it is important to note that for ants to have lipids it is not necessary to consume just oils because neutral lipid fatty acids (NLFAs) can be synthesized from sugars also (Rosumek et al., 2017). From our observation, it could be said that the ant colony under observation was the lipid-deprived colony because the lack of both lipids and sugar sources in the microenvironment caused the colony to accumulate a high amount of lipid sources, and that could be obtained from lipid food baits supplied to them.

On all experimental days, the food baits were provided at the same time and at the same site, and we noted that within 10 mins the first batch of foragers gathered at the food site, especially in the sunflower oil food bait. Ants can acquire spatiotemporal learning (Cammaerts, 2013a) though the young members do not exhibit this ability (Cammaerts, 2013b). After having experienced feeding times ants of *Myrmica* species

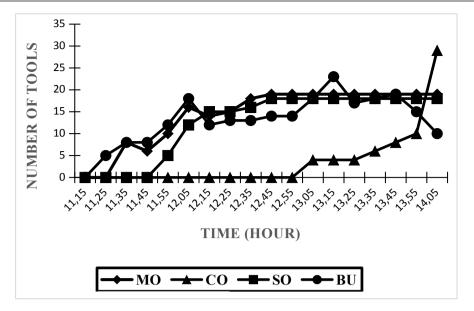


Fig 12. Accumulation of different-sized tools at food baits along different time points.

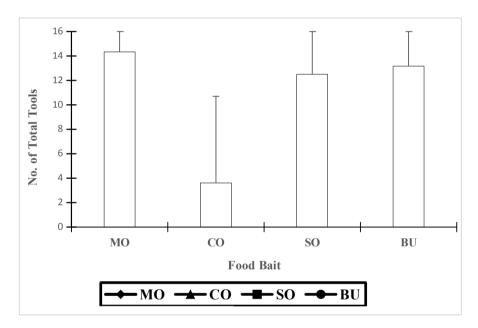


Fig 13. Accumulation of different-sized tools (Mean \pm SD) at four different food baits irrespective of time points.

could adapt themselves to the situation and this expectative behaviour of ants is an advantageous ethological trait (Cammaerts & Cammaerts, 2015; Cammaerts & Cammaerts, 2016). The *M. pharaonis*, in our pilot study, took about 20 min to find out the food source for the first time (irrespective of food bait) but after having gathered experience, the average time decreased to about 10 min almost on all days of experiments. On the contrary, the least favoured food baits took more time as compared with sunflower oil. The ants can memorize the favoured food bait location and they have memories, and our study can be corroborated with the earlier similar study (Heyman et al., 2019).

Another interesting feature was observed in our investigation, in which two castes of *M. pharaonis* small and

intermediate were seen to take a major responsibility in lipid food collection and transportation as well. This behaviour was observed in all four lipid food bait types *i.e.* in the case of both least-liked and most favoured food baits including the moderately preferred food baits. To the best of our knowledge, this type of behaviour of this species of ant has not been reported in any earlier observations. In one of our studies with *Camponotus compressus*, the major caste was found never took the responsibility to collect and transport any kind of food resource (Banik et al., 2011). Like *C. compressus*, the *M. pharaonis* has a clearcut caste division and among four worker castes (as seen outside the colony), all four castes took part in food gathering and transportation but the major role was displayed by two specific castes as mentioned earlier.

Experiment II: Tool using behaviour

M. pharaonis here also shows a certain amount of flexibility towards the tool-using behaviour. Beyond the tool characteristics, a few other factors influenced the decision of ants as to which tools to use. Although our ants exhibited a preference for small sand particles, the tool selection was found to be varied. It can be assumed that the aim of tool dropping was simply to soak the liquid lipid food, thereby protecting nestmates from drowning or becoming entangled in the dense oil. We observed initially, that our *M. pharaonis* tried to collect lipid food baits directly without using any tools but at later time points and later days, the tool-dropping activity became evident. This kind of protective behaviour, which is not uncommon in ants (Wheeler, 1910), is of great interest from the evolutionary point of view because it can be considered a pre-existing behaviour (Fellers & Fellers, 1976; McDonald, 1984). In the present study, M. pharaonis preferred dropping small sand particles into the food baits because those small-sized tools can be carried easily to the food bait site and can also be retrieved equally easily and transported back to the nest. With the advancement of time points, the number of tools in the food baits increased except in butter, where decreasing value was recorded after the number reached its peak at 13:15 h. For the butter, M. pharaonis transported the food baits using butter-soaked tools back to the nest. The butter is thus found to be the most important lipid food source at least for the present ant colony as compared with other lipid food under investigation.

The size of mostly used small sand particles as tools was well in the range of the maximum mandible opening of the ant under study, which was about 0.5 mm or less (Oliveras et al., 2005), allowing the ants to manipulate those objects easily and efficiently. A. famelica, showed a similar result, in which the tool use frequency became highest when the size of tools was less than 2.0 mm (Tanaka & Ono, 1978) and the most frequently dropped tool items were the sand grains and conversely, the pieces of leaves were used least. The underuse of leaves and other large wooden debris by M. pharaonis in the present investigation can be attributed to the difficulty of handling and orienting such tools due to their large diameter and surface area. Ants may have preferred retrieving small sand grains because these, due to their relatively low weight and irregular texture, can be retrieved more easily and with less tensile force from a highly viscous liquid (Maák et al., 2017).

It can be concluded from our observation of lipid food preference and tool use strategy that the ant, *M. pharaonis* has a selective preference for lipid food depending on the demand of the colony as a limiting resource and also the physical nature of the resource. As far as the tools are concerned, small size and easy transportability are the main determinants for selecting the appropriate tool items (Rudolph & Loudon, 1986; Lighton et al., 1993; Torres-Contreras & Vasquez, 2004). In addition, our findings regarding differential lipid food choice and the major role of lipid transportation by the two leading castes (intermediate and large), require further research. While there was no concrete evidence found to support our hypothesis based on our available data set, more research can be made on this particular issue. Further research could look at if lipids are used in different seasons of the year. This, we think, would help demonstrate which type of lipid they require all through the year and what functions of fatty acids are used to help adapt to urban environments. Additional research could look at the role of the kinds and sizes of tools with ants' oil preferences. This would allow us to see if an increased number of tools expedite lipid food transportation during foraging for oil. Another interesting point would be to look into if there are any differences in oil preference between

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ants in urban habitats and other habitats because there is

ample scope for gathering knowledge on this particular issue.

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Authors' Contribution

RS: Investigation.

SB: Formal analysis, writing: original-draft.

RK: Methodology, investigation, writing: original-draft, writing: review & editing.

Competing interests

All the contributing authors declare that they have no conflict of interest.

Availability of data and materials

The raw data are available with the corresponding author. It is available upon request.

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All authors have consented to the publication of this article

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