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Competition Between Mealybug Attendant Ant Species for the Honeydew of Mealybugs in the Same Niche

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ABSTRACT

Considering limited studies on the competitive behaviours in ants in similar ecological niches, this study investigated such behaviours between three ant species. Ant species, *Tapinoma melanocephalum*, *Technomyrmex albipes* and *Camponotus variegates* were attracted to a honeydew food source of mealybug *Planococcus citri* infested pumpkins in field trials and competitive behaviour in terms of the presence of these ant species over a different time frame (every 2hrs for a total of 24hrs) was studied. Endpoints assessed were in terms of number and the ant species that were present during diurnal and nocturnal competitive foraging sprees. Significance was drawn in terms of overlap in terms of time and the population of ants. The results showed that between diurnal ant colonies i.e., *T. albipes* and *T. melanocephalum*, later was more dominant. During nocturnal foraging at the overlapping time (6 am and 6 pm) less number of *C. variegates* ants tended to the pumpkin. Similarly, between *T. melanocephalum* and *C. variegates* for nocturnal foraging from 6 pm to 6 am *C. variegates* was more during the night but around 6 am, *T. melanocephalum population* increased. Holistically, *T. melanocephalum emerged* as the most dominant species in the field.

Key words: *Planococcus citri, Tapinoma melanocephalum, Technomyrmex albipes, Camponotus variegates*

Ecological interactions in the ant community depend to a greater extent on the balance of nutritional supply. The ant ecological stoichiometry revolves around the availability of food resources and the general display of dominant behaviour for its protection [1]. Macronutrient availability plays a constructive role in shaping the structure of ant dominance. Of the many mutualistic interactions that facilitate the supply of food resources is the ant mealybug interaction that is highly commendable in shaping the food webs and social interactions between Hymenoptera and Hemiptera. Considerable data highlights that mealybugs supply honeydew to the ants that protect them from their natural enemies and thus help in sustaining the mutualistic interaction.

Ants display various behaviours to protect themselves from threats both within and from outside. Territorial competitiveness in ants is marked by aggressive physiological regulation of dominant behaviours such as the use of chemical/pheromonal defenses or signaling through raiding of others' nests and display of general aggression. Major conflicts of food dominance are modulated through chemical foot printing, and pheromone or chemical training. Dominant behaviour is said to correlate positively with the availability of food resources and it is said that such ant species also display more aggressive behaviour as compared to other species. Similarly, Davidson *et al.* [2] studied the correlation between carbohydrate availability and behavioural dominance in ant species. Likewise, Yanoviak and Kaspari [3] studied the correlation of ant aggression and dominance to the availability of carbohydrate resources in forest lands.

Dominant behaviour in ants also correlates with the colony strength and the size of the nest which could be built at various locations such as in the soil, under rock beds, in tree canopies, decaying trunks, crevices, roots and in litters made by fallen lives. Nesting locations both soil and arboreal, reflect the strength and dominance of the ant species. Foraging for food resources categorizes the ant community as either diurnal (day), nocturnal (night) or both. Diurnal or nocturnal foraging of food is dependent on many factors such as temperature tolerance, ability to function at different light intensities, and visual capacities. Nocturnal ants usually have better vision with complementary compound visions to facilitate their food hunts [4]. Comparative studies amongst various ant species have shown that white foot ants (WFA) *Technomyrmex albipes* (Fr. Smith) (Dolichoderinae) are diurnal in habit and usually canopy dwellings but can also be sighted under debris and in leaf-litters. Food foraging of this species is usually on dead insects and honeydew [5]. Female workers are predominant and participate in colony maintenance and foraging and adopt aggressive behaviour if disturbed during foraging activities [6].

Likewise, the ghost ant *Tapinoma melanocephalum* (Fabricius) is also diurnal in habit and found below grass or

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fallen leaves or in-wall crevices or plant pots. They are polygynous in colony organization [7]. Members of the colony do not display aggressive behaviour both conspecific or from outside communities [8]. The species forage for honey-dew and debris of dead insects. They usually disperse haphazardly when alarmed. On the contrary, the Hawaiian ant/carpenter ant *Camponotus variegates*, due to their habituated dwelling in the wood carvings by termites, display aggressive behaviour. *Camponotus variegates* are sensitive to humid temperatures and thus prefer nocturnal foraging. They have a distinct division of labour and can be found either in groups or individually. They are omnivorous feeding on dead insects as well as plant nectar or honeydew. Carpenter ants can dwell both indoors and outdoors. They can be found in carvings of logs or the crevices of wood furniture.

Although there is considerable literature available on these ant species, not much is known about their interactions when they are in the same niche. Therefore, in this paper, we studied the competitive behaviour amongst three ant species namely *T. albipes*, *T. melanocephalum* and *C. variegates*, when they are attracted to a honeydew food source in the same niche in a field trial. Competitive behaviour was studied in terms of the presence of various ant species over a different time frame (every 2hrs for a total of 24hrs). Endpoints that were assessed were in terms of number and the type of ant species that were present during diurnal and nocturnal competitive foraging sprees. Significance was drawn in terms of overlap found between the species of ants both in terms of time (in hrs) and the population of ants (type and number). The significance of the work lies in understanding the ecology of ant species in commonly invaded areas of food foraging and survivability in terms of competition and dominance.

MATERIALS AND METHODS

Laboratory rearing of mealybugs

Pumpkin selection was done based on earlier published literature. Briefly based on criteria of body surface (green colour), less damaged body and the number of ridges pumpkins were selected. They were washed with water and then treated with 1% fungicide to reduce the fungal contamination. Pumpkins were infested with *Planococcus citri* egg masses and the culture was maintained in the laboratory [9]. Mealybug infested pumpkins attract different ant species that were drawn to the honeydew exuded by the mealybugs.

Experiment 1- Ant population that attended on mealybug infested pumpkin at site A (T. albipes vs C. variegates)

The experimental setup entailed placing a mealybug infested pumpkin at site A that corresponded to the availability of specific ant species in those areas. The major objective of this experiment was to compare the competition between the different ant species for the food source in the same niche at site A. Site A was predominantly attended by *T. albipes* ant species during the day and by *C. variegates* ant species during the night times. Observations were drawn in terms of competitive behaviour between *T. albipes* and *C. variegates* ant species over a time frame of every 2hrs for a total of 24hrs at 6, 8, 10, 12, 2, 4 and 6 pm). Endpoints assessed were in terms of number and the type of ant species that attended the mealybugs during diurnal and nocturnal foraging and the population of the two species during overlapping times.

Experiment 2- Ant population that attended on mealybug infested pumpkin at site B (T. melanocephalum vs C. variegates)

The mealybug infested pumpkin placed at site B, which was predominantly attended by *T. melanocephalum* ant species during the day and by *C. variegates* ant species during night times. Observations were drawn in terms of competitive behaviour between *T. melanocephalum* and *C. variegates* ant species over a time frame (every 2hrs for a total of 24hrs). Observations were made as explained for site A. Experiments at sites A and B were replicated 10 times.

Statistics

Statistics were performed using one-way ANOVA with post-hoc analysis by Kruskal Wallis test or t-test with post-hoc analysis using Dunn's multiple comparisons test using graph pad prism version 6.0 software.

RESULTS AND DISCUSSION

Ant population (T. albipes vs C. variegates) that attended on mealybug infested pumpkin at site A

Ant population at site A at different time intervals

The two ant colonies *T. albipes* and *C. variegates* showed variation in the number of ants that attended the mealybug infested pumpkins during diurnal and nocturnal foraging hours.

T. albipes attended mealybugs for honeydew during the day and observations were made every 2hrs for a total period of 24hrs. The results of the one-way non-parametric ANOVA using the Kruskal Wallis test showed that there was a significant difference (P-value of 0.0032 and $P < 0.05$) in the number of ants that attended the pumpkin during the study period. Further, posthoc analysis using Dunn's multiple comparisons between the groups revealed that at 6 am vs 2 pm time points (P-value of 0.0080) there were significant differences in the number of ants that attended the mealybug infested pumpkin (Fig 1.1A, Fig. 1.2).

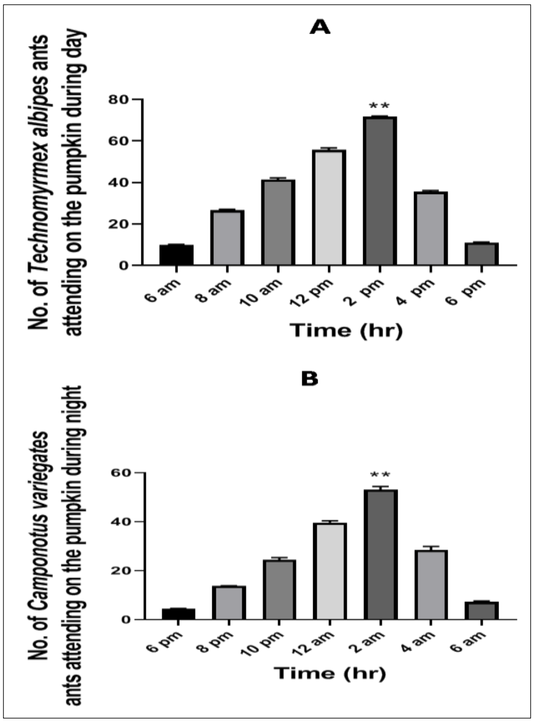

Fig 1.1 Mean (± S.E) *Technomyrmex albipes and Camponotus variegates* ant species that attended on mealybug infested pumpkin at site A at different time intervals. (A) *Technomyrme albipes* and (B) *Camponotus variegates.* (one-way non-parametric ANOVA using the Kruskal Wallis test-posthoc Dunn's multiple comparisons). (*P < 0.05, ** P < 0.01; *** P < 0.001)

Ant population (C. variegates) that attended mealybug infested pumpkin in a field trial at different time intervals

Similarly, after the 6 pm (nocturnal) site A pumpkin was attended by *C. variegates* ant species it replaced *T. albipes* that were present during the day. The results of the one-way non-parametric ANOVA using the Kruskal Wallis test showed a significant difference (P-value of 0.0033 and P < 0.05) in the number of ants that attended the pumpkin during the study period. Further, posthoc analysis using Dunn's multiple comparisons between the groups revealed that at 6 pm vs 2 am time points (P-value of 0.0079) there was a significant difference in the number of ants that attended the mealybug infested pumpkin (Fig 1.1B, Fig 1.3).

Fig 1.2 *Technomyrmex albipes* ants that attended on mealybug infested pumpkin at site A at different time intervals

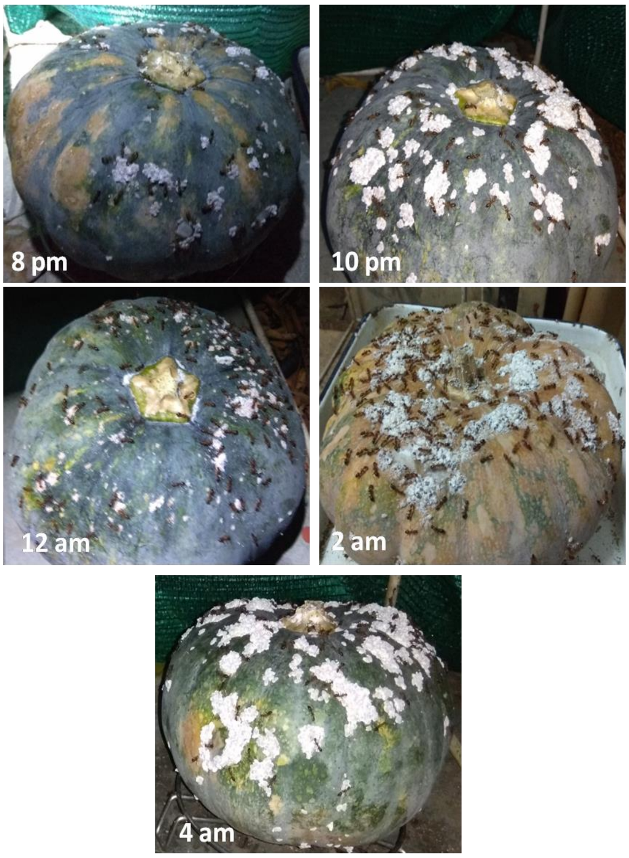

Fig 1.3 *Camponotus variegates* ants that attended on mealybug infested pumpkin in a field trial at site A at different time interval

Comparative study between the ant populations (T. albipes vs C. variegates) that were displaced at site A at overlapping times i.e., 6 am and 6 pm

The results of our study highlight that during the transition from dawn to dusk, between the two ant colonies *T. albipes* was found more dominant in terms of ant strength as compared to *C. variegates* for foraging. We found that at two incidences i.e., 6 am and 6 pm there was an overlap between *T. albipes* and *C. variegates* for foraging. The results of our study showed that as compared to *C. variegates, T. albipes* showed significant differences (P-value of 0.0001 and P < 0.05) in the number of ants that attended the pumpkin at 6 pm through one-way non-parametric ANOVA using Kruskal Wallis test. Further, posthoc analysis was done using Dunn's multiple comparisons between the groups for 6 pm time points (P-value of 0.0132) (Fig 1.4).

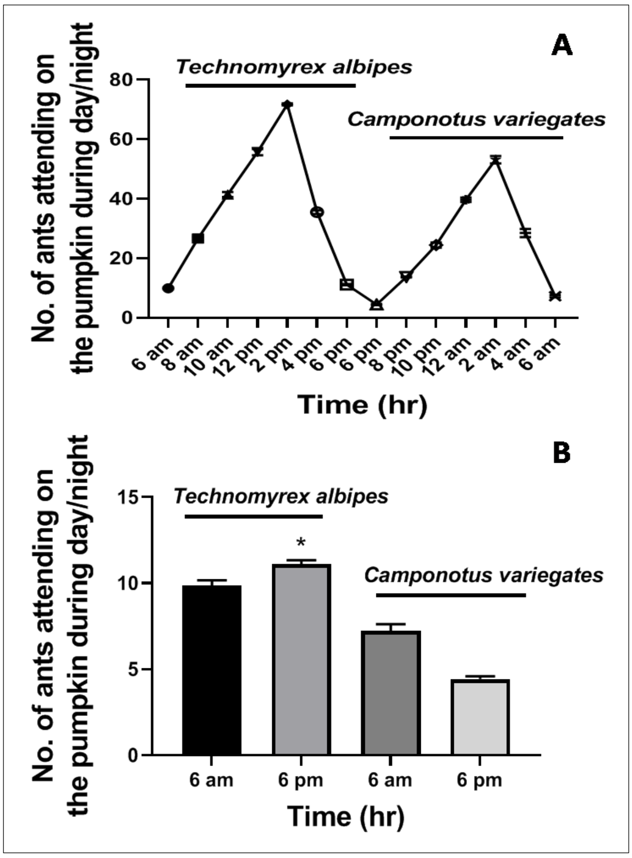

Fig 1.4 Mean (± SE) Comparative study between the ant populations (one-way non-parametric ANOVA using the Kruskal Wallis test -posthoc Dunn's multiple comparisons) (*P < 0.05, ** P < 0.01; *** P < 0.001)

Ant population (T. melanocephalum vs C. variegates) that attended on mealybug infested pumpkin at site B

Ant population (T. melanocephalum) that attended on mealybug infested pumpkin at site B at different time intervals

The results of our study highlighted that the two ant colonies *T. melanocephalum* and *C. variegates* showed variation in the number of ants that attended the mealybug infested pumpkin both during diurnal and nocturnal foraging. Mealybug infested pumpkin at site B was attended by *T. melanocephalum* ants during the day and observations were drawn for 24hrs at every 2hrs (6 am, 8 am, 10 am, 12 pm, 2 pm, 4 pm and 6 pm). The results of the one-way non-parametric ANOVA using the Kruskal Wallis test showed that there was a significant difference (P-value of 0.0032 and P < 0.05) in the number of ants that attended the pumpkin during the study period. Further post-hoc analysis using Dunn's multiple comparisons between the groups revealed that at 2 pm vs 6 pm time points (P-value of 0.0080) and there was a significant difference in the number of ants that attended the mealybug infested pumpkin as illustrated in (Fig 1.5A, Fig 1.6).

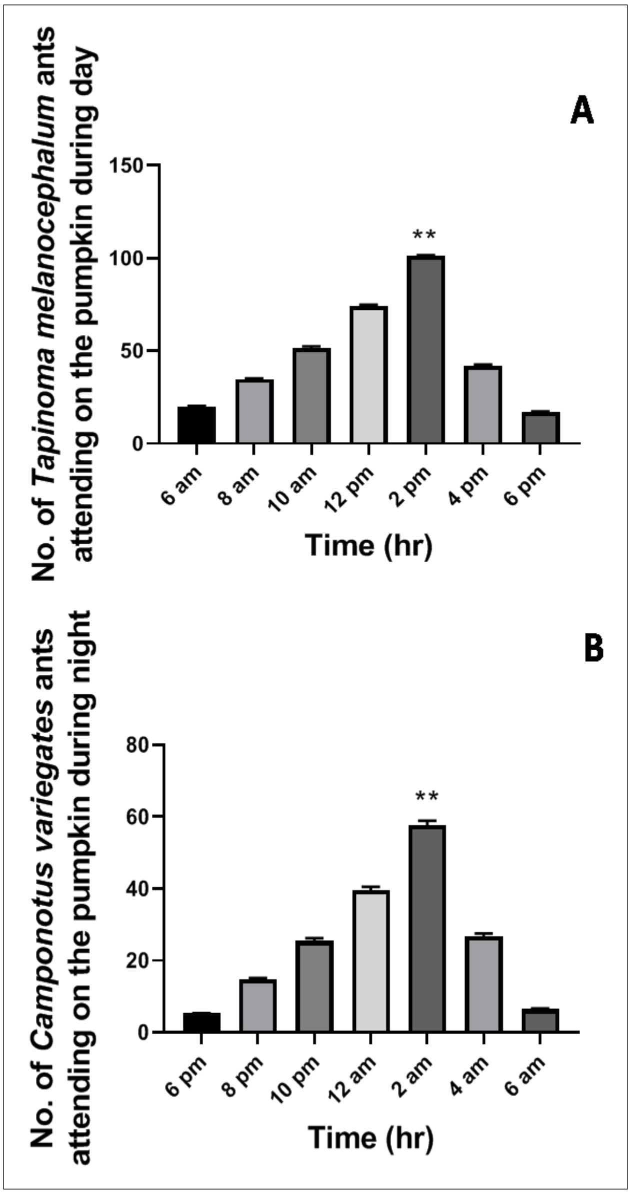

Fig 1.5 Mean (± S.E) *Tapinoma melanocephalum* and *Camponotus variegates* ants that attended on mealybug infested pumpkin at site B at different time intervals. (A) *Tapinoma melanocephalum*. and (B) *Camponotus variegates.* (one-way non-parametric ANOVA using the Kruskal Wallis test -posthoc Dunn's multiple comparisons). (*P < 0.05, ** P < 0.01; *** P < 0.001)

Ant population (C. variegates) that attended on mealybug infested pumpkin at site B at different time intervals

Similarly, after 6 pm (nocturnal) the pumpkin infested with mealybugs was attended by *C. variegates* ant species. They replaced the *T. melanocephalum*. ants that were present in the day. For *C. variegates* ant species the observation period was 24hrs at every 2hrs (6 pm, 8 pm, 10 pm, 12 am, 2 am, 4 am and 6 am). The results of the one-way non-parametric ANOVA using the Kruskal Wallis test showed significant differences (P-value of 0.0033 and P < 0.05) in the number of ants that attended the pumpkin during the study period. Further posthoc analysis using Dunn's multiple comparisons between the groups revealed that at 6 pm vs 2 am time points (P-value of 0.0080) there was a significant difference in the number of ants that attended as illustrated in (Fig 1.5 B).

Comparative study between the ant populations (T. melanocephalum vs C. variegates) that were displaced in field trial at overlapping time of 6 am and 6 pm

The results showed that *T. melanocephalum* was found to be more dominant in terms of ant strength as compared to *C. variegates*. At two time intervals, we found an overlap between the time interval for *T. melanocephalum* and *C. variegates* ants. As compared to *C. variegates, T. melanocephalum* ants showed a significant difference (P-value of 0.0001 and P < 0.05) in the number of ants that attended the pumpkin at 6 pm through one-way non-parametric ANOVA using Kruskal Wallis test. Further post-hoc analysis using Dunn's multiple comparisons between the groups revealed significant differences at 6 pm time points (P-value of 0.0134) illustrated in (Fig 1.7).

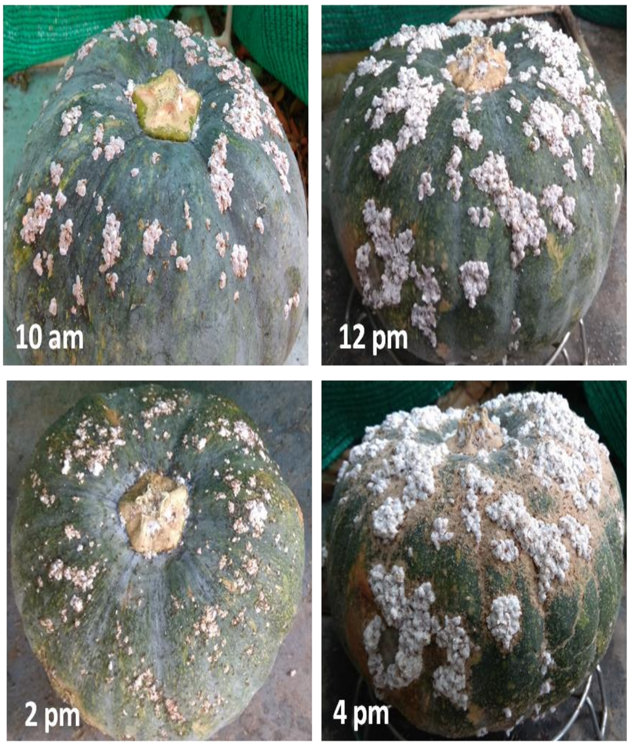

Fig 1.6 *Tapinoma melanocephalum* ants that attended on mealybug infested pumpkin at site B at different time intervals

The current paper focuses on highlighting the competitive behavior of three ant species namely *T albipes*, *T. melanocephalum* and *C. variegates* that were introduced to a food source in the same niche at two different sites (A & B) in a field trial. Competitive behaviors were studied in terms of the number and the type of ant species that predominated in the diurnal and nocturnal competitive foraging.

Ant-hemipteran mutualism and the ant-ant dominance or co-existence are subject to much scrutiny. The ant-hemipteran mutualism is the aftermath of the benefit that this association draws for both ants and the Hemiptera family. Ant-tending behaviors protect the hemipteran against their enemies, and also sanitize them of extra wax, soot or honeydews. This is important to keep them free of fungal contaminations. In exchange, the hemipterans provide food in the form of honeydew to the ant, which shapes the ant community and their survival. Likewise, the ant-ant interactions are subject to many factors namely their requirement for food, the size of their colony, their fear of predation and the time requirement for their searches. Ants would behave aggressive or dominant either when they run short of food reserves, or the workers designated as foragers have to fend for a large colony structure or they are subject to climatic conditions of restrains in temperature or the effect of light sources. That is to say that the ant-ant interactions are dependent on many co-variables that shape their community and social structures.

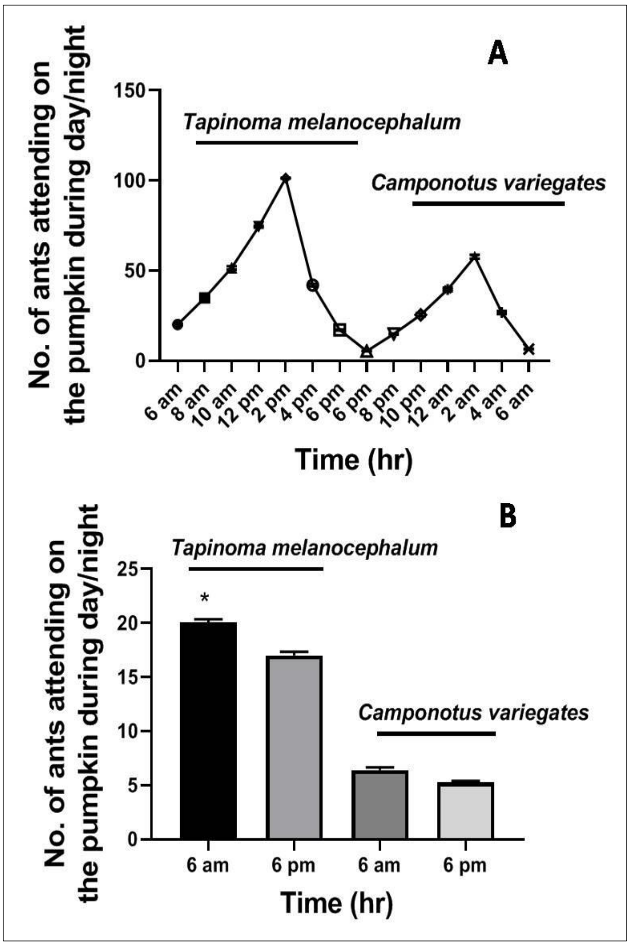

Fig 1.7 Mean (± S.E) Comparative study between the ant populations (one-way non-parametric ANOVA using the Kruskal Wallis test -posthoc Dunn's multiple comparisons) (*P < 0.05, ** P < 0.01; *** P < 0.001)

Ant community modulates its competitive foraging through various strategies of territorial dominance namely pheromone defences or chemical trailing behaviors. This behavior compartmentalizes them as either diurnal (day), nocturnal (night) or both types of hunters. Diurnal or nocturnal foraging of food is dependent on many factors such as temperature tolerance, ability to function at different light intensities, and visual capacities of the ant species.

The results of our study highlight that between the two diurnal ant colonies that were studied i.e., *T. albipes* and *T. melanocephalum*, *T. melanocephalum* appeared more dominant in terms of the number of ants that attended the mealybug infested pumpkins in the field trial (30% more ants than *T. albipes*). Certain explanations could be extended in this regard. Firstly, between the two diurnals, *T. albipes* is larger and therefore as compared to *T. melanocephalum,* requires a fewer number of workers to carry food resources to its colony. Secondly, it could be argued that although *T. albipes* is more aggressive, in this case, the ant-hemipteran tending was fewer favourable as compared to *Tapinoma sp* for the same food niche. Similar studies were carried out by Le Brun *et al*. [10] who studied intraspecific aggressive behaviour in ants.

Likewise, we also compared the number of ants tending on the mealybug infested pumpkins during nocturnal foraging. The results of our study using *T. albipes* and *C. variegates* showed that between the overlapping time (6 am and 6 pm) when *C. variegates* species overtakes the *T. albipes* on the pumpkin, less number of *C. variegates* ants tended the infested pumpkin (60% less). This could again be due to the larger size of *C. variegates* as compared to *T. albipes.* Similarly, between *T. melanocephalum* and *C. variegates* nocturnal foraging for 24hrs (6 pm, 8 pm, 10 pm, 12 am, 2 am, 4 am and 6 am) our results showed that *C. variegates* was more during the night but around 6 am, *T. melanocephalum* increased by (75%). Chen *et al.* [11], similarly studied intraspecific aggression and the colony structure of the invasive ant *Myrmicarubra.*

The ant community is highly competitive in choosing its food resources. Many investigators have reported that as compared to extra plant nectarines (that usually contain constituents of plant sap and glucose) honeydew produced by hemipterans (containing more sugars, amino acids and nitrogen bases) is usually preferred mostly based on the superiority of the nutritious content of honeydew [12-13]. Honeydew as a food source is usually in scarce supply thus leading to display of thick competition and aggression between ants. It is reported that between ground and arboreal ant dwellers, arboreal ants show higher level of competition for honeydew as their food source [14]. Luo *et al*. [15] and Luo *et al.* [16] in their independent studies looked into the pollination potential of *Jatropha curcas* in China region in reference to the nocturnal-diurnal insects. Their studies lend credence to the impact of circadian rhythm of insects with their capacity to act as potential pollinators. Similarly, Ness and Bronstei [17] further studied interaction of ant invasiveness during mutualistic interactions and competition for food sources in the same niche. Holistically ant aggression or dominance is subject to supply and type of food reserves, time required to research the source, visual acumen and tendency for diurnal or nocturnal hunting habits.

CONCLUSION

Conclusively this paper addresses the competitive behavior amongst three ant species and establishes that amongst the three species of ants that were studied *T. melanocephalum* was the most dominant species in comparison to *T. albipes*, and *C. variegates,* when introduced to a food source in the same niche in a field trial study. This study lends credence to understanding physiological behaviours in ant ecology that could be used in ant management programs. Also, this study helps us in understanding the behavioural underpinning of the ant community.

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