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Assessment of Ant-Mealybug Mutualism on Pumpkin and their Impact on Shelf-life of Pumpkin

G. P. Prashanth, Melally G. Venkatesha

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ABSTRACT

Mealybugs are sap-sucking pests that mainly damage crops and horticultural plants. Mealybugs exhibit mutualism with ants, feed on honeydew secreted by mealybugs, which provide a vital carbohydrate source to ants. In turn, ants protect mealybugs from their natural enemies. Studies regarding the ant-mealybug mutualism on pumpkin *Cucurbeta maxima* as a host are minimal. An investigation was carried out on mealybug *Planococcus citri* infested *Cucurbeta maxima* to analyze the ant-mealybug interactions in the presence of two ant species, namely *Tapinoma melanocephalum* and *Technomyrmex albipes* and observed for the possibilities of maximum ant population attending the mealybug infested pumpkin and the full spread of mealybugs on the pumpkin in the presence and absence of ants, along with the impact of ant-mealybug mutualism on the self-life of host pumpkin. It was observed that the ant population of *T. melanocephalum* on mealybug infested pumpkin was 30% higher as against the *T. albipes* population. The maximum mealybug spread was observed in the presence of ants than in their absence, where *T. albipes* exhibited a faster and more extensive spread of mealybugs than *T. melanocephalum*. Overall ant-mealybug interaction also leads to prolonged self-life of pumpkins by 20 days compared to the absence of ant species. This study revealed that their association positively impacts the existence of each other and aids to the self-life of host *Cucurbeta maxima*.

Keywords Ant-mealybug mutualism, Presence, Absence, Impact, Pumpkin.

G. P. Prashanth, Melally G. Venkatesha*
Insect Science Laboratory, Department of Zoology, Bangalore University, JB Campus, Bengaluru 560056, Karnataka, India
Prof. M. G. Venkatesha, Insect Science Laboratory, Department of Zoology, Bangalore University, JB Campus, Bengaluru 560 056, Karnataka, India
Email : venkatmelally@gmail.com
*Corresponding author

INTRODUCTION

Mealybugs (Homoptera : Pseudococcidae) are small, oval, slow-moving, soft-bodied insects with a waxy coating and generally measure up to 3–4 mm in length. They are usually found in moist, humid, tropical regions resembling a typical insect meal and get their name owing to the whitish cottony secretions covering them. Adult females measure 2.5– 4.0 mm, are wingless and lay 300- 800 eggs in ovisacs which hatch into nymphs/crawlers in 10 to 20 days. The females have three nymphal instar stages and attain total growth in 6-8 weeks. On the contrary, males have wings, no mouthparts and appear greyish. After hatching, the males go through 4 nymphal instar stages and in the last stage, they weave cocoons in 2-3 weeks (Correa *et al.* 2008).

Mealybugs suck on the sap from plants, leaves, stems, roots. Also, they feed on fruits, i.e. guava, papaya, grapes, banana, apple, mango, avocado, citrus, coffee, cocoa and ornamental plants like hibiscus and oleander (Ahmed and Abd-Rabou 2010). They cause discoloration and defoliation of leaves, along with the premature dropping of fruits. Mealybugs exude honeydew from their body surface, forming a black sooty mold on the leaves, blocking photosynthesis. The waxy coating of mealybugs makes insecticidal actions ineffective; hence, they are regarded as serious pests and a potential threat to crop production and cultivation (García Morales *et al.* 2016). Mealybug infestation in India is heaviest during April-May and lowest around August-October (Rao *et al.* 2006).

Propagation of mealybug infestations is either by crawling onto new plants or sometimes by hiding in the small crevices of the bark of plants. Among the important species of mealybugs, the major pests of economically important crops are the citrus mealybug *Plannococcus citri* (Risso) (Goldasteh *et al.* 2009, Karamaouna *et al.* 2010, Ahmed and Abd-Rabou 2010, El-Aw *et al.* 2016), cotton mealybug *Phenncoccus solenopsis* (Abbas *et al.* 2010) and the pink hibiscus mealybug *Mnconellicocctis hirsutus* (Green) (Mani *et al.* 2011). Mealybugs may act as vectors for various diseases and can be carried through wind or other transport services (Mansour *et al.* 2011). Nine species of mealybugs infest citrus, amongst them, the citrus mealybug *P. citri* is a cause of concern in Indian regions of Meghalaya, Rajasthan, Punjab, Maharashtra, Gujarat and Nagpur (Rao *et al.* 2016). *P. citri* is commonly called citrus mealybug, having a yellowish pink coloration with a medial strip present dorsally in adults, and the anal filament is comparatively tiny.

The ant population on the earth is tremendous and equals approximately half of the earth's biomass (Hölldobler and Wilson 1994). Of the many mutualistic relationships adapted by organisms for survival, the ant and mealybug tending are largely commendable (Stadler and Dixon 2005). Usually, they are present in the same areas or niches and ant-tending behaviors protect the mealybugs against their natural predators or parasitoids. In exchange, the hemipterans provide food in honeydew to ants, which is essential for ant survival. Ant community is highly competitive in generating resources for itself, which shapes its community and population dynamics to a more considerable extent (Holldobler and Wilson 1990).

Mutualistic interactions generally shape the dynamics of niche-related communities. The ant (Hymenoptera) mealybug (Homoptera : Pseudococcidae) mutualistic interactions exemplify strong trophobiotic relationships (Bastolla *et al*. 2009, Brightwell and Silverman 2010, Zhang *et al.* 2012). Ant-tending behaviors protect hemipterans against their natural predators or parasitoids in exchange for the rich source of honeydew excreted by hemipterans. Honeydew acts as a rich food source for ants and shapes their colony size (Freitas *and Rossi* 2015, Beltrà. *et al.* 2017, Clark and Singer 2018).

Ghost ant, *Tapinoma melanocephalum* (Fabricius), is diurnal and inhabit underneath grass tufts or dead leaves when outdoor or in various small crevices in the walls, doors or under plant pots indoor. Their colony structure can vary from small to big with the polygynous organization (Oster and Wilson 1978). Members of the colony do not display aggressive behaviors or indulge in inter or intraspecies fights (Smith and Whitman 1992). Foraging is both for honeydew and debris of dead insects. When alarmed, they usually disperse haphazardly.

Similarly, *Technomyrmex albipes* (Fr. Smith) or white-footed ants are wingless that are 3.5 to 4 mm in length. They belong to the subfamily *Dolichoderinae*, with five abdominal segments, 12-segments of antennae and no sting. Workers generally live for a year and participate in colony maintenance and food foraging (Manjushree and Chellappan 2019). Like *T. melanocephalum*, *T. albipes* also feed on honeydew producing insects (mealybugs, aphids, soft scales, whiteflies) for food besides feeding on dead insects. *T. albipes* nests are seen in trees, old leaf boots, under debris, leaf litter, wall voids and attics. They usually follow a heavy trailing pattern for locomotion (Lach *et al.* 2010).

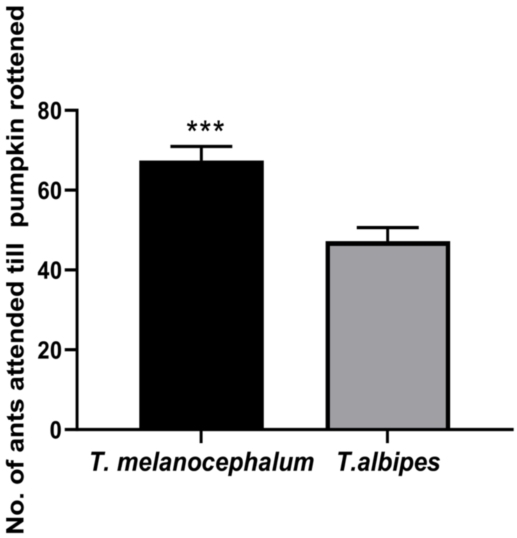

Fig. 1. Mean (± SE) Total ant population (*T. melanocephalum* and *T. albipes*) that attended on mealybug infested pumpkin. (t-test followed by the post-hoc analysis using Mann-Whitney) (*p < 0.05, ** p < 0.01, *** p < 0.001).

The ant-hemipteran interactions are essential as they help understand the food web in trophobiotic solid communities. In this study, we focused on studying the growth of mealybug *P. citri* in the presence and absence of mealybug attendant ants *T. melanocephalum* and *T. albipes* using pumpkin as a host. *P. citri* quickly proliferate on pumpkins providing an ideal surface to study the ant-hemipteran interactions. Ant behavior was assessed in terms of its ability to populate the pumpkin. Also, studies were carried out to ascertain the self-life of pumpkins infested with *P. citri* and in the presence and absence of ant species.

MATERIALS AND METHODS

Laboratory procedure and experimental setup for *P.citri* rearing

P. citri were reared in the laboratory on medium-sized (70×20 cm, diameter × height) fresh pumpkins (*Cucurbita maxima* Duchesne) following the technology for production of natural enemies (1995). Pumpkin selection was based on their similarity for size (cir-

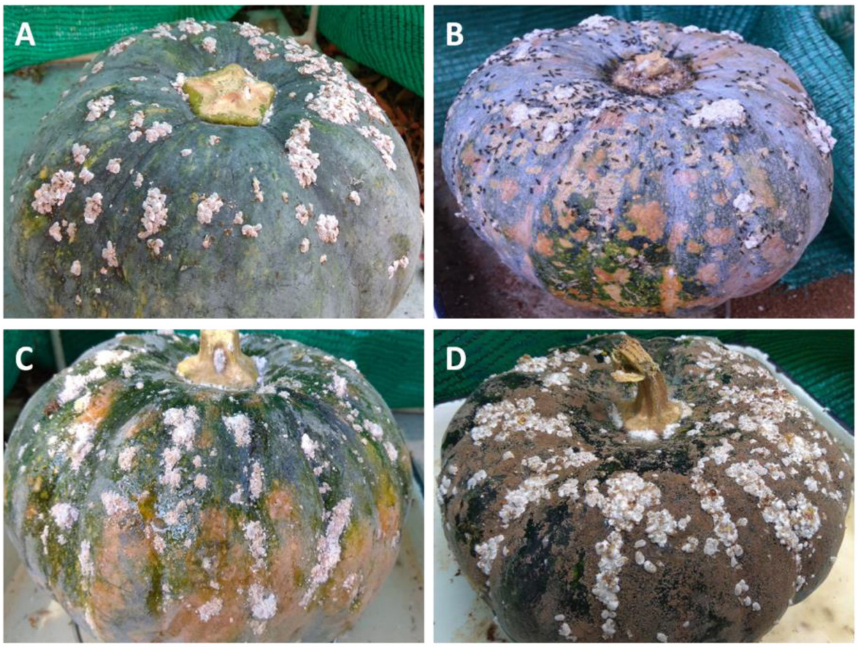

Fig. 2. Ant population attending on the pumpkin. (A) *T. melanocephalum* attending on mealybug infested pumpkin. (B) *T. albipes* attending on mealybug infested pumpkin. (C) Presence of honeydew in the absence of mealybug attendant ants. (D) In the absence of ants, honeydew acts as a good source of fungal contamination.

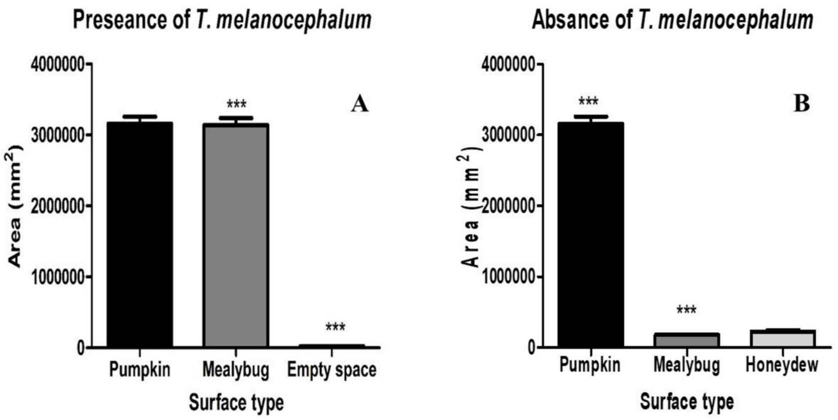

Fig. 3. Mean (± SE) The overall surface of pumpkin, spread of mealybugs on the pumpkin surface, empty space on pumpkin surface in presence of ants and honeydew on pumpkin surface where as in absence of ants (A) In the presence and absenc of ants. *T. melanocephalum,* (B) In the presence and absence of ants *T. melanocephalum.* (one-way non-parametric ANOVA using the Kruskal Wallis test-posthoc Dunn's multiple comparisons) (*$p < 0.05$, ** $p < 0.01$; *** $p < 0.001$).

cumference and number of ridges) and weight. They were prewashed /soaked in water and then exposed to 1% fungicide to reduce the fungal contamination and shade dried. For individual experimental setup, two pumpkins were used for the presence and absence of ants and five egg masses of *P.citri* were released on both the experimental setup. Both pumpkins were placed under the same natural environmental conditions (in a tray with 1/4th of water and the outer surface of the tray coated with grease) to avoid ants. The experimental setup/ trays were covered with 50% agriculture shade nets to protect the mealybugs from their natural enemies. Initially, the eggs and nymphs of *P. citri* were collected with the help of a camel hair brush from croton plants (*Codiaeum* spp.) in the field at Bangalore University campus, Bengaluru, India (latitude 12°58´N, longitude 77°35´E, elevation 921 m above sea level) and were transferred onto the

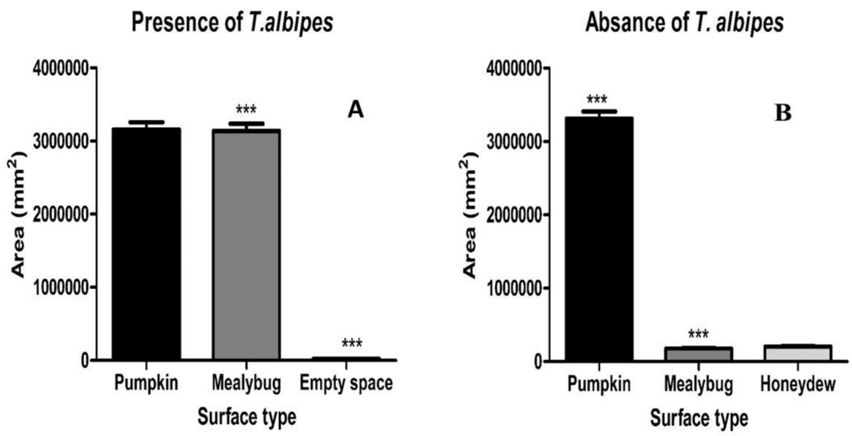

Fig. 4. Mean (± SE) The overall surface of pumpkin, spread of mealybugs on the pumpkin surface, empty space on pumpkin surface in presence of ants and honeydew on pumpkin surface where as in absence of ants (A) In the presence and absence of ants *T. albipes,* (B) In the presence and absence of ants *T. albipes.* (one-way non-parametric ANOVA using the Kruskal Wallis test-posthoc Dunn's multiple comparisons) (*$p < 0.05$, ** $p < 0.01$; *** $p < 0.001$).

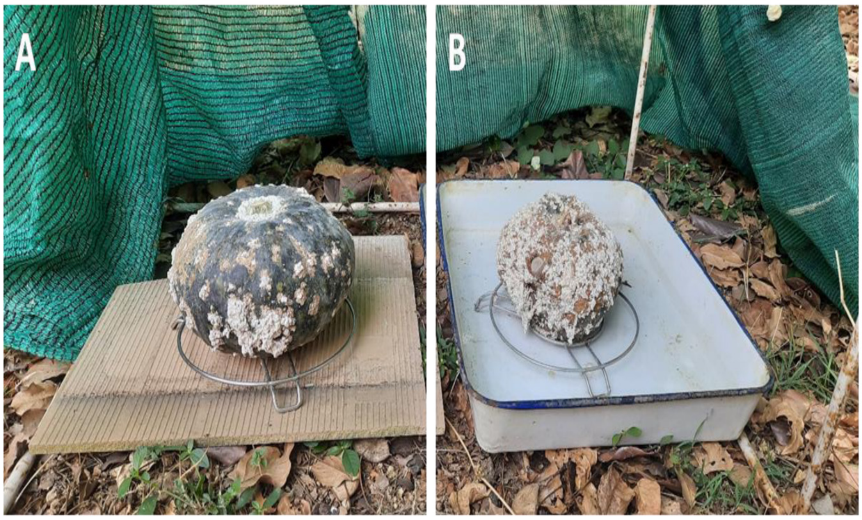

Fig. 5A. Experimental result showing pumpkin survivability in the presence of ants. **5B.** Experimental result showing pumpkin survivability in the absence of ants.

cleaned pumpkins in the laboratory. The mealybug infested pumpkins were individually maintained in a nylon rearing cage (30×30×30 cm). Meanwhile, for regular availability of the prey, fresh pumpkins were infested with *P. citri* whenever required. *P. citri* are easy to maintain in laboratory conditions as they have a high multiplication rate and can be used for studying ant-tending behaviors. Each experiment was replicated 3times with ten trails per replication.

Selection of ant species

For our study, the mealybug infested pumpkins were initially placed in the natural environment, preferably near the ant colonies, to attract them. After a short span, it was observed that among the various ant species that were attracted to the pumpkins, two species of ants namely *T. melanocephalum* (Fabricius) (Hymmenoptera: Formicidae) and *T. albipes* exhibited maximum association with the mealybug infested pumpkins. Therefore, these two ant species were chosen for the following investigations.

Experiment 1 : Ant population attending on the mealybug infested pumpkins in the field trial. An experimental setup was similar to that of Mahmoud *et al.* (2017), with a major objective to compare the number of ants from both *T. melanocephalum* and *T. albipes* species that attended on mealybug infested pumpkin throughout the study period.

Experiment 2 : The maximum spread of mealybugs on pumpkin surface in the presence and absence of mealybug attendant ants in the field trial.

The principal objective of this experiment was to determine the maximum spread by the mealybugs on the pumpkins surface both in the presence and absence of mealybug attendant ants *T. melanocephalum* and *T. albipes* during the field trial study. The endpoint of the experiment were evalivated by cauclating : 1 surface of pumpkin, maximum spread of mealybug on pumpkin surface and the space that was left over on the pumpkin in presence of ants 2 surface area of pumpkin corresponding to maximum spread of mealybugs and the surface area of pumpkin that was covered by honey dew screated by the mealybug in the abasance of ants species quantitative analysis was done using Image-J software.

Experiment 3 : Pumpkin shelf-life in the presence and absence of mealybug attendant ants in the field trial.

The assay was done following a protocol from

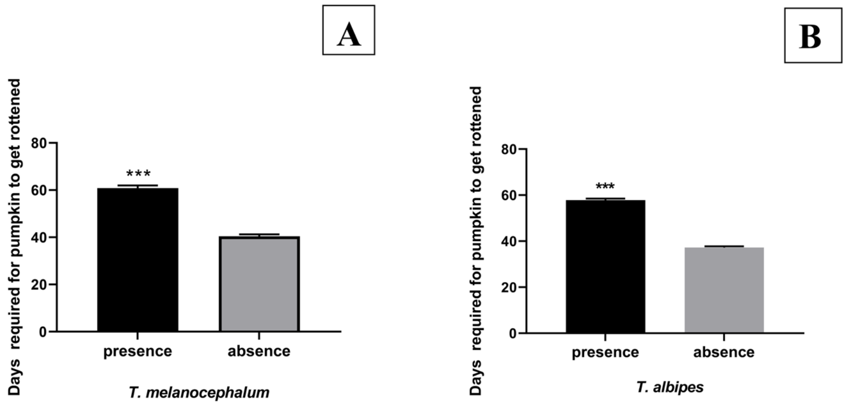

Fig. 6. Mean (± SE) Decay of pumpkin number of days required for the pumpkin to rot in the presence and absence of ants (A) *T. melanocephalum,* (B) *T. albipes* (Non-parametric post-hoc analysis using Mann-Whitney) (*p < 0.05, ** p < 0.01, *** p < 0.001).

Mahmoud *et al.* (2017) with slight modifications. The honeydew secreted by the mealybug acted as a food source and attracted the ants that further established their colony on mealybug infested pumpkins. Observations were drawn regarding the self-life of pumpkins based on the presence and absence of mealybug attendant ants.

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 that attended on mealybug infested pumpkin

Ant-tending similar hemipterans exhibit varying degrees of aggressiveness in a species-dependent manner. Competitiveness between ant species is displayed by the foraging activity of workers (Wilder *et al.* 2013). In our study two ant colonies were observed, namely *T. melanocephalum* and *T. albipes* and analyzed them for their population that attended on the mealybug infested pumpkins. These mealybugs infested pumpkins were placed near the ant colonies. Honeydew secreted by the mealybug colony acted as a food source and attracted the ants that established their colony on the pumpkins. The comparative results of our study showed that between *T. melanocephalum* and *T. albipes* a 30% decrease in the population of *T. albipes* group of ants was found on the mealybug compared to that of the *T. melanocephalum*. Analysis was done using a t-test followed by the post-hoc analysis using Mann-Whitney at ($p < 0.05$) as illustrated in Fig. 1. Interspecies competition between both the ant species was evident by their population size on the pumpkin (Fig. 2). Between the two strains of ants, it was found that *T. melanocephalum* was more aggressive and was present in a larger population than *T. albipes* ants that showed a 30% decrease in their population on the pumpkin. Similar studies have been presented by Pringle (2020). On comparing the total number of days required for the maximum ant population of *T. melanocephalum* and *T. albipes* to spread on the pumpkin, we found that the *T. melanocephalum* required (15% more) 32 days whereas *T. albipes* required 27 days. Similarly, Zhou *et al.* (2017) studied the competitive behavior and foraging activity between *T. melanocephalum* and *S. invicta* for honeydew exploitation. Likewise, Zhou *et al.* (2014) also studied the coexistence pattern between *S. invicta* and *T. melanocephalum* in laboratory conditions on sharing honeydew resources.

Time taken for the maximum spread of mealybugs on pumpkin surface in the presence and absence of mealybug attendant ants in the field trial.

The results of our study showed that in the presence of *T. melanocephalum* the overall pumpkin surface

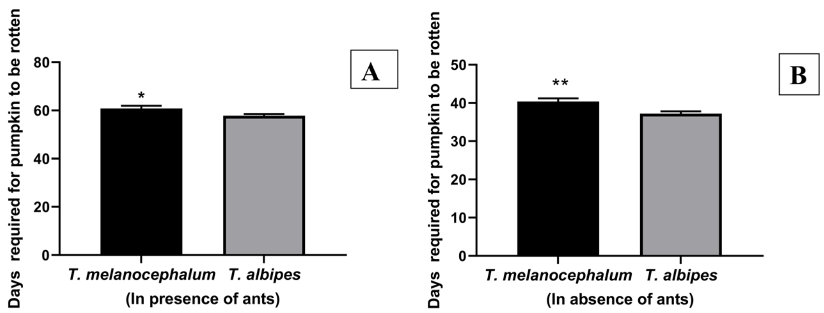

Fig. 7. Mean (± SE) Comparison in the number of days required by the pumpkin to rot in (A) The presence of *T. melanocephalum* and *T. albipes,* (B) In the absence of *T. melanocephalum* and *T. albipes*. (Non-parametric post-hoc analysis using Mann-Whitney) (*p < 0.05, ** p < 0.01; *** p < 0.001).

area was 3159884 (mm^2) in which maximum surface area of pumpkin corsponding to maximum spread of mealybug was 3137686 mm^2 and surface area of pumpkin remained vacant was 22198.11 mm^2 where as in absence of ants the overall pumpkin surface area was 3162475 mm^2 in which maximum surface area of pumpkin corsponding to maximum spread of mealybug was 178491.4 mm^2 and surface area honey dew coverd on pumpkin surface was 224429.8 mm^2. The results of the one-way non-parametric ANOVA using the Kruskal Wallis test showed that there was a significant difference ($p < 0.0001$) both in presence and absence of *T. melanocephalum* as illustrated in Fig. 3 Likewise, in the presence of *T. albipes* ants, the overall pumpkin surface area was 3312647 mm^2 in which maximum surface area of pumpkin corsponding to maximum spread of mealybug was 3280930 mm^2 and surface area of pumpkin remained vacant was 31716.38 mm^2 in their absence, the overall pumpkin surface area was 3314637 mm^2 in which maximum surface area of pumpkin corsponding to maximum spread of mealybug was 178435.5 mm^2 and surface area honeydew coverd on pumpkin surface was 203640.4 mm^2. The results of the one-way non-parametric ANOVA using the Kruskal Wallis test showed that there was a significant difference ($p< 0.0001$) both in presence and absence of *T. albipes* as illustrated in Fig. 4.

It was noticed that the maximum spread of mealybug on pumpkin surface was more in the presence of ants than in the absence of ants. Among the presence of both the ant species. In which *T. melanocephalum* spread the mealybugs on the pumpkin surface faster than *T. albipes*. In absence of ants.

One of the arguments that could be extended in this regard is that due to their smaller body size the *T. melanocephalum* sp. number of ant attending will be more they could simultaneously carry more number of mealybugs including nymphs/crawlers with the larger work load for spreading the mealybugs on the pumpkin surface as compared to that of the *T. albipes* which due to their larger body size number of ant attending will be less compared to *T. melanocephalum* and also *T. albipes* carry less number of mealybugs including nymphs/crawlers with the less workload for spreading the mealybugs on pumpkin surface. However, in the absence of ants, due to the stocking up of honeydew on the host pumpkin, higher mortality of mealybugs was observed resulting in a very low number of total mealybugs post their maximum spread as opposed to the spread observed in the presence of ants.

Survivability of pumpkin in presence and absence of mealybug attendant ants

Ant and mealybug mutualism has been well experimented with and documented in the past. Ants feed on the honeydew secreted by mealybugs present on the pumpkin, which serves as a source of carbohydrate to them (Carroll and Janzen 1973, Detrain *et al.* 2010), and in turn ants promote a hygienic pumpkin surface free from any sooty molds leading to the longevity of pumpkin as well as protection of mealybugs from their natural predators. This also leads to interspecies competition between the ants. Ants exhibit aggressiveness displayed in terms of their increase in colony number, running and jerking movements (Petry *et al.* 2012). The results of our study highlight that the two ant colonies *T. melanocephalum* and *T. albipes* showed variation in the number of days required by the pumpkin to decay. Fig. 5 shows the experimental setup displaying the pumpkin survival status in the presence and absence of ants. When *T. melanocephalum* attended on mealybug infested pumpkins the number of days required for pumpkins to rot was 61 days. Whereas, the number of days required for the decaying of pumpkin in the absence of *T. melanocephalum* was 41 days. Non-parametric post-hoc analysis using Mann-Whitney at $p < 0.05$ revealed that there was a significant difference in the number of days required for the pumpkin to rot between the presence and absence of ant species as illustrated in Fig. 6A. Similarly, when *T. albipes* ants attended on the mealybug infested pumpkin, it was observed that the number of days required for the pumpkins to rot was 57 days as against the number of days needed in the absence which was 37 days. Mann-Whitney analysis showed that the presence of ants increases the decay period of the pumpkin as illustrated in Fig. 6B. A comparative study between the two species is depicted in Fig. 7A. Conclusively, there was a significant difference in the number of days required for the pumpkin to rot and finally decay in the presence and absence of *T. melanocephalum* showing 35% decrease in days. It could be said that the presence of *T. melanocephalum* increased the days required for the pumpkin to decay because they cleared the honeydew and sootymold deposited by the mealybugs, which in turn reduced the fungal growth on pumpkins and prevented it from rotting and decaying. Likewise, the presence and absence of *T. albipes* ants on the mealybug infested pumpkin showed that their presence increased the days required for the decaying of pumpkin by 20 days. However, in the absence of ants, the honeydew secretion of mealybug would remain undisturbed facilitating the attraction of fungal spores and dust particles which further would form a sooty mold on the mealybug infested pumpkin leading to faster rotting of mealybug infested pumpkins by fungus. Similarly, Mahmoud *et al*. (2017) also studied *P. citri* on pumpkins as well as potatoes and ascertained the apt plant that could be used as a host for mass production of *P. citri* in biological control studies. Similar studies were also performed by Asiedu *et al.* (2014) that studied *P. citri* biology. Results of both the studies showed little difference in the mean incubation time required for female egg incubation on pumpkin and potato.

Overall, though the *T. melanocephalum* species aided better in the self-life of pumpkin as well as populated maximally on the mealybug infested pumpkin, it was the *T. albipes* species of ants which owing to their larger body size recorded a faster and larger spread of mealybugs on the pumpkin. Thus, since the presence of ants aids to the longer longevity of the host pumpkins and also in the better expansion of the mealybug population rather than in their absence, the results of our study infer that both *T. melanocephalum* and *T. albipes* ant species exhibit a significant association with *P.citri* mealybugs on the host *Cucurbeta maxima*. As our study brought to light the positive impact held by the ant mealybug association on longevity of host pumpkin, such studies could be taken as a model for prediction studies using citrus mealybugs and also help in structuring pest management programs.

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