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APHID (MICROMYZUS KALIMPONGINSIS) - ANT *(PHEIDOLE SMYTHIESI)* INTERRELATIONSHIP - A PRELIMINARY STUDY

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INTRODUCTION

Associations in general and mutualism in particular, result from usually reciprocal benefits, achieved by the interactions between two or more species. These benefits are often in the form of food, protection or locomotion. It is known that some species of ants interact in such a way with a variety of Hemipteran and Homopteran insects, particularly aphids and coccids (Herzig, 1938, Nixon, 1951 and Way, 1963). That ants feed on the honeydew produced by aphids is well documented. Moreover these ants may alter the life-table parameters of the aphids (El-Ziady, 1960; El-Ziady and Kennedy, 1956), or may influence aphid feeding upon the plants (Banks, 1958). It is also recorded that the presence of ants increase the fitness of aphids and thereby decrease the impact of predators (Banks, 1962; Burns, 1973 and Bradley, 1973).

The rate of excretion by an aphid may be conveniently estimated by multiplying the frequency with the average volume of the droplet excreted during a given time period (Auclair, 1958). Such a quantitative determination of honeydew excretion constitutes a most valuable tool in aphid physiology and is gaining impetus in microbiological research. It may be used in the studies of the rates of aphid feeding, food, assimilation and growth, in nutritional experiments involving food balance and nitrogen economy and in assessing the degree of resistance by the host plants. The species, variety, part and growth stage of the host plant fed upon, may influence honeydew by aphids, tended by ants, has been shown to increase considerably by the former (Banks, *et. al.*, 1958; Herzig, 1937; Mittler, 1958).

From very early times, deposits of honeydew on or near plants have attracted much interest because of their high sugar content. Studies have demonstrated that fresh honeydew is usually a complex mixture of a large variety of chemical compounds, including several sugars, amino-acids and amides, organic acids, alcohols, auxins, salts etc. (Auclair, 1959).

The association of ants, with aphids and related forms, has been worked since the turn of the century, and has been reviewed by Jones, (1929), Herzig, (1937), Nixon (1951), and Wellenstein (1952). An interesting analysis of this association has been presented by Kloft (1959, 1960). Way (1963) has reviewed the mutualism between ants and honeydew-producing Homoptera. The major emphasis has been laid on the beneficial effects derived out of such a mutualistic relationship by aphids. The influence

of ant-tending, upon aphid growth, population dynamics, protection from predators, and honeydew production has been widely studied (Nixon, 1951; Way 1963). However, the benefits derived by the ants in this relationship has been largely overlooked, probably because of the fact that it was thought basic, that the only benefit to the ants would be the honeydew produced by the aphids, which they utilise as their food, ants being obligate honeydew feeders. Way (1953, 1954a, b) showed the ant *Oecophylla longinata* (Latr) tending on coccid, *Saissetia zenzibarensis* Williams, could exist only at relatively low population levels in absence of the coccids. Pontin (1978) has shown that aphids supply enough food for the tending ants, making foraging and preying on other insects for food supply unnecessary, thus offering an energy-economy for food collection.

Keeping the above in view, the aim of this investigation was to substantiate the existing knowledge of the relationship between aphids and ants on the following aspects.

a) to find out the population fluctuations of the aphids as influenced by tending ants.

b) to see the level, or numbers of ants tending each aphid colony through a time period.

c) to find out the amount of honeydew removed by the ants tending the aphid colonies.

d) to see the levels of some biochemicals in the honeydew through the study period.

MATERIALS AND METHODS

Study site

The study was situated in the Umpling area of Shillong (25°34'N and 90°56'E), Khasi Hills district of Meghalaya.

The host plant, *Hedychium coronarium* (Zingiberaceae) is an ornamental plant found in and around Shillong. It has fragrant white or yellow flowers. The plant is seen to proliferate vegetatively from rhizomes shooting forth its aerial parts during monsoons. After the monsoon is over, the plant simply wilts and dies off leaving the underground rhizomes dormant throughout the dry winter periods. This indicates the transitory nature of this monsoonal plant with high conductive efficiency of the essential physiological sap to support the dense population of aphids.

The aphids, *Micromyzus kalimponginsis* (Basu 1967), colonise this plant. These aphids were seen to be distributed along the main veins on the underside of the young and maturing leaves. During the time of flowering, these aphids aggregate on the flowers and flower-buds.

The ants, *Pheidole smythiesi* Forel tend these aphids. They offer protection for these aphids and were also seen to clear the aphid colonies of plant and other debris, thereby

increasing the surface area of the leaves or flowers for aphid feeding. The ants also helped to keep wandering aphids within the colony. In return the ants were provided with the "Honeydew" by the aphids.

Population Analysis

Population counts were made without disturbing the aphid colony nor removal of plant parts keeping in mind the ultimate aim of project. Density of the aphids, together with the tending ants in the experiment site was estimated by counting them on seven stalks and flowers. (At the start of the experiment, plants were in bloom).

On each sampling day, 20 ants tending the aphids were collected. The tending ants were to be used subsequently for the biochemical analysis indicative of our indirect measurement of honeydew. At the same time control ants, which had not attended the aphid colonies, were also collected.

Sampling was done in triplicate, fortnightly throughout the study period for three months.

Ants returning from tending aphid colonies, have markedly distended abdomens. The increase in weight between tending ants, with distended abdomen, full of honeydew and those which have not tended the aphids and having normal size gave a good indication of the amount of honeydew removed from the aphids (Dixon, 1970).

Extraction Technique

For extraction of the honeydew component from the tending and control ant abdomen, the Trichloro Acetic Acid method was followed. Tris-HCl buffer was prepared by dissolving 0.012 M of Tris (Mol. Wt. 121.14) in 100 ml. of distilled water. Then concentrated HCl was added to make the pH of the buffer 7.2 to 7.4.

Twenty tending ant abdomens (average 3.8 mg.) from each collection were taken and homogenised in 1 ml. of the above Tris HCl buffer. For control, 20 ant abdomens (2.1 mg.) were taken to homogenate in 1 ml. of Tris HCl buffer.

To the homogenate 1 ml. of 10% TCA (Trichloro Acetic Acid) was added, mixed and kept at 0°C for 30 minutes. The solution was then centrifuged at 4000 rpm for about 30 minutes. The supernantant was collected.

To the residue, 1 ml. of 5% TCA was added. The aliquot was contrifuged for another 10 minutes at 4000 rmp. The resultant supernantant was pooled with the previous one. With this extract, estimation of carbohydrates and Amino-acids after standard methods.

OBSERVATION AND RESULTS

Aphid-population dynamics

The population counts at the start of the experiment totalled 463 aphids in the 7 stalks, i.e. 66/stalk. this number decreased to 440, average 62/stalk at the next sampling

date. The population reached a peak subsequently, totalling 2766 individuals i.e. 395/stalk. This then took a downward trend though not very low, numbering 1506 individuals i.e. 215/stalk, which at the next sampling, reached 181 aphids and finally the lowest of 110 aphids, both average 26/stalk and 15/stalk respectively (Fig. 1 and Table 1).

Number of tending ants

The corresponding ants tending the aphids were seen to follow a similar pattern, the rise and fall in ant numbers corresponding to the aphid population fluctuations though at a much lower level of magnitude. Initially the total number of ants tending the aphids were found to be 48 (7/stalk), which fell to 22 (3/stalk), and then rose to 79 ants or 11/stalk, which again fell to 27 ants (4/stalk). At the next sampling date the ants totalled 7, or 1/stalk, which was the lowest in the study period. The numbers rose to 13 at the last sampling date (2/stalk) (Fig. 1 and Table 2).

Thus, the two populations were seen to be highly correlated ($r = 0.82$, the aphids showing a positive response to ant tending, significant at $p < 0.01$ level).

Ant biomas and honeydew weights

The weight of the ants tending the aphids did not show any consistent pattern. On the first sampling date, the average weight was seen to be 0.29 mg., which rose to 0.33 mg. It then fell to 0.27 mg. It rose once again to 0.32 mg., only to fall to 0.29 mg. and rise to 0.32 mg. in the subsequent period (Table 3).

The difference between these two weights, at each sampling date, gives a measure of the honeydew uptake by the ants. The honeydew uptake was seen to be inversely related to the aphid and ant population levels, for nearly the whole period of investigation, except in the last two sampling dates, when the honeydew weight and the population structure showed a direct relationship to some measure. At the initial sampling date the honeydew measured was 0.206 mg. which rose to 0.244 mg. then fell to a low level of 0.180 mg., which was incidently the lowest during the study period. At the next sampling date, the level rose to 0.230 mg., then dropped to 0.200 mg. and finally rose slightly to 0.210 mg. at the last sampling (Table 3).

Biochemical analysis of the tending ant abdomen

Sugars : The sugar content in the tending ants (expressed in mg/mg abdomen tissue), was 0.076 mg. initially. This level rose to 0.109 mg. at the next sampling date, (the highest for the study period), then fell subsequently to 0.065 mg. Subsequently it showed a slight rise (0.073 mg.), and then had a decreasing trend for the rest of the study period (0.047 mg. and 0.041 for the last two sampling dates).

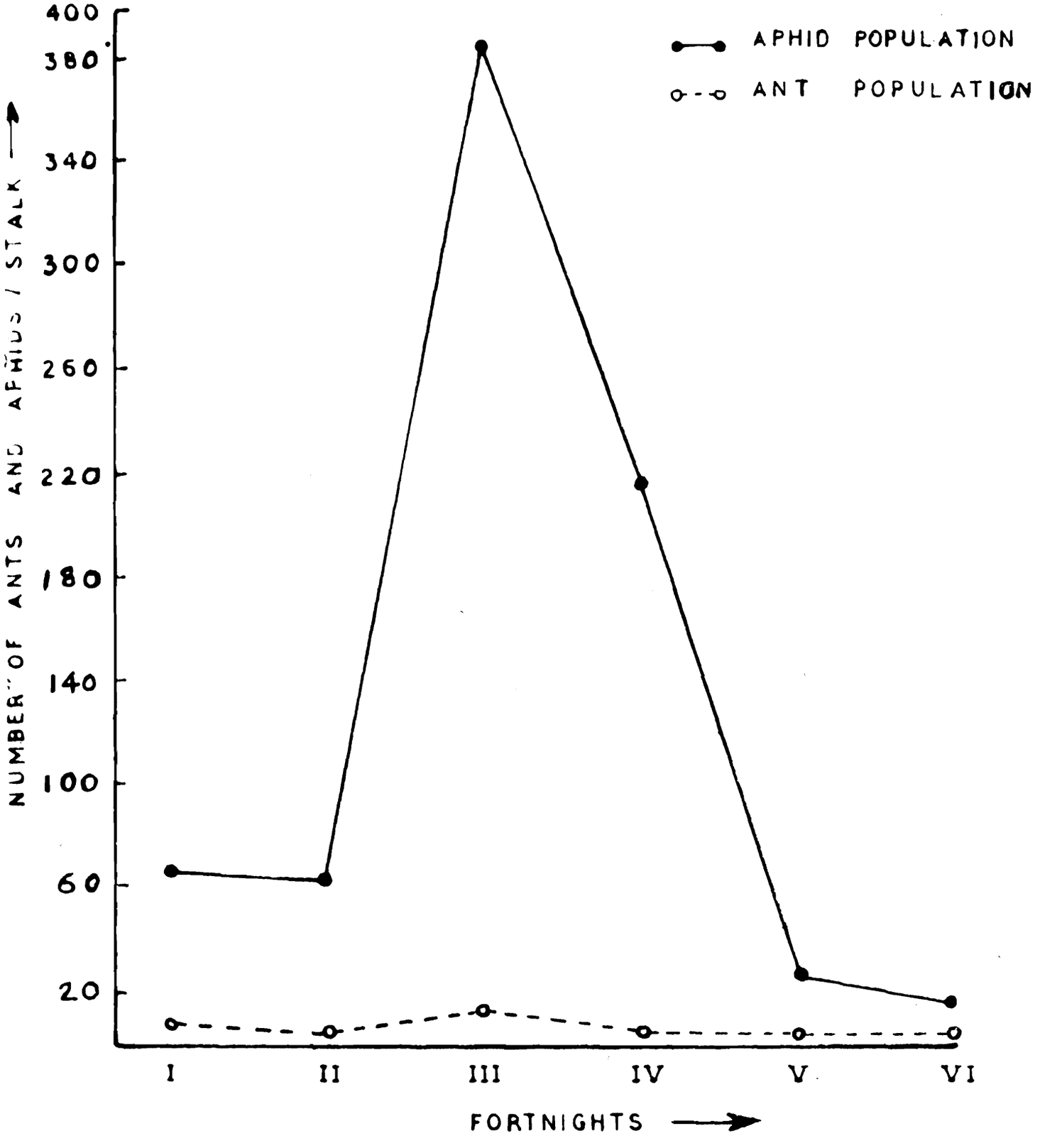

FIG. 1

Fig. 1. Aphid – Ant population dynamics.

Table – 1. POPULATION DATA OF APHIDS

Fortnight	Stalk or leaf number							Total	Average
	1	2	3	4	5	6	7		
I	26	12	43	53	155	116	58	463	66
II	11	2	20	63	135	49	160	440	62
III	480	510	406	240	360	430	340	2766	395
IV	180	240	206	140	430	310	—	1506	215
V	21	—	15	32	40	43	—	181	26
VI	—	—	5	12	93	—	—	110	15

Table – 2. POPULATION DATA OF ANTS

Fortnight	Stalk or leaf number							Total	Average
	1	2	3	4	5	6	7		
I	4	2	6	5	12	13	6	48	7
II	—	1	2	4	7	4	4	22	3
III	15	26	9	5	7	10	7	79	11
IV	4	5	3	5	10	—	—	27	4
V	—	—	1	—	4	2	—	7	1
VI	—	—	—	3	10	—	—	13	2

Table – 3. WEIGHT OF ANTS AND THE RELATIVE CONTENT OF HONEYDEW

Fortnight	Tending Ants		Control Ants			
	Total weight (mg)	Average weight (mg) (a)	Total weight (mg)	Average weight (mg) (b)	Difference of weight between a and b (mg)	Honeydew content (% Body weight)
I	13.92	0.29	0.84	0.084	0.206	710.3%
II	7.22	0.33	0.86	0.086	0.244	73.94%
III	21.04	0.27	0.87	0.087	0.183	67.78%
IV	8.56	0.32	0.87	0.087	0.233	72.81%
V	2.02	0.29	0.59	0.098	0.192	66.21%
VI	4.22	0.32	0.43	0.107	0.213	66.56%

Table – 4. CARBOHYDRATE (TOTAL SUGAR) CONCENTRATION

Fortnight	Sugar concentration mg/20 ant abdomens	Sugar concentration mg/20 ant abdomens	Sugar concentration mg/ Total ant	Sugar concentration mg/mg
I	0.288	0.0144	0.6912	0.076
II	0.414	0.0207	0.4554	0.109
III	0.246	0.0123	0.9717	0.065
IV	0.276	0.0138	0.3726	0.073
V	0.180	0.0090	0.0630	0.047
VI	0.156	0.0078	0.1014	0.041
Control	0.096	0.0048	0.2400	0.046

Table – 5. TOTAL AMINO ACID CONCENTRATION

Fortnight	Amino Acid Conc. mg/20 ant abdomens	Amino Acid Conc. mg/20 ant abdomen	Amino Acid Conc. mg/ Total ant	Amino Acid Conc. mg/mg
I	0.039	0.00195	0.0936	0.0103
II	0.096	0.00480	0.1.56	0.0252
III	0.087	0.00435	0.3436	0.0229
IV	0.159	0.00795	0.2146	0.0412
V	0.243	0.01215	0.0851	0.0639
VI	0.073	0.00360	0.0478	0.0189
Control	0.033	0.00165	0.0825	0.0157

The total amount of sugar taken by the ants was 0.69 mg. at the first sampling date. This amount showed a slight decrease at the next sampling (0.46 mg.), which is more because of the decrease in ant numbers, rather than of a reduction in sugar content. This figure reached a peak thereafter (0.97 mg.), when the sugar removed by the ants was seen to be the highest. Subsequently it showed a decline with 0.37 mg. being removed at the next date. The lowest amount of sugar removed was on the next sampling date, when only 0.063 mg. of sugar was seen to have been removed by the ants. The ant numbers, incidently were also lowest at this date (total of 7 tending ants). On the last sampling date, the amount of sugar removed rose to 0.10 mg. when considered on a total weight basis, the sugar concentration followed the same pattern (Table 4).

Amino-acid : Amino acid concentration in the tending ant abdomen (expressed as mg/mg abdomen tissue), at the first sample date was 0.0103 mg. Thereafter, the amino acid concentration showed an upward trend, recording 0.0252 mg. at the second sampling fell slightly to 0.0229 mg., but increased again to 0.0412 mg. reaching a

peak on the fifth sampling date at 0.0639 mg. Then the level fell drastically, on the last date to 0.0189 mg.

The total amount of amino acids, removed by the ants, was 0.0936 mg on the first day. This, then, rose to 0.1056 mg. on the second day. On the third day, the amount removed was maximum (0.3436 mg.). Thereafter, the amount of amino acids removed, declined to 0.2146 on the third day and 0.0851 on the fourth, inspite of the concentration (mg/mg) being the highest for the whole study period on that date. This was because of the fall in the ants number (total of 7 tending ants). The amount removed on the last day was the lowest (0.0468 mg.), because of the concurrent decrease in amino acid content (0.0189 mg/mg), and the ant number (1/stalk) (Table 5).

DISCUSSION

Mutualistic interactions, resulting in a beneficial effect on both the interacting populations, is an evolutionary adaptation, in order to increase the fitness of both the species, in the face of increasing environmental stress. Aphid-ant associations form no exception to this general rule, and the relationship between these two interacting organisms have evolved to such a degree, that in some cases the relationships have become obligatory.

The present investigation showed that all the colonies of *Micromyzus kalimpengensis* Basu, were tended by ants of the species *Pheidole smythiesi* (Formicidae) only. The aphid population showed a positive correlation to tending ants ($r = 0.82$, $p < 0.01$), even though the numbers of tending ants oscillated at a very low magnitude. The present findings conform to those of earlier workers, such as Muir (1959), Way (1963), Banks (1962), Burns (1973) and Bradly (1973). It is possible that the presence of the ants helped to decrease (or probably, completely removed), the predatory pressure, so that the aphids had no decimation in number due to predators, or parasites.

Honeydew secretion, number of tending ants and aphid populations showed no correlation contrary to expectations. However, since the honeydew measurements were indirect, it is possible that all the honeydew secreted by the aphids were not taken up by the ants, and thus, a strong correlation between these parameters could not be found.

The seasonal fluctuation of honeydew uptake by the tending ants showed an inverse relationship both with the aphid and ant numbers towards the middle of the study period. (i) One reason could be as shown by Herzig (1973), and Mittler (1958) that honeydew secretion of aphids, attended by ants, reveals a decrease in the average droplet volume. (ii) The other could be due to the host plant which had matured by that time; and host plant age has been shown to effect the honeydew secretion (Auclair, 1958; 1959). This is probably the explanation for the lowering of the honeydew excretion when both ant and aphid numbers were more. (iii) The last reason could be attributed to the equidistribution of the honeydew secreted at low levels in relation to the tending ants.

Aphid colonies are a major source of carbohydrate and protein for the ants tending them (Schmidt, 1952). Further, as the colonies provide a steady source of food, foraging by the ants is reduced and this helps in economising energy in the latter (Pontin, 1978). In this study, though comparisions between ants foraging for food, with and without aphid colonies, were not investigated, hence their energy expenditure for food foraging could not be found. However, it has been seen that the ants removed honeydew on an average of 69.72% of their body weight, indicating their extent of dependence on the aphid colonies.

The biochemical analysis of the honeydew removed, showed that the sugar content in tending ant abdomen was significantly higher than in control ants ($t = 2.35$, $p < 0.1$) except on one occasion. However, the number of ants tending the aphid colonies and the sugar content in their abdomen were both low in the last two dates in comparision to other tending ants on earlier occasions. It is tempting to attribute the decrease in ant numbers to the decrease in sugar content, but more data on this is necessary before a clear relationship can be established. An interesting observation however lies in the fact that the sugars removed (indirect measurement of honeydew secretion) is larger as the tending ants increase in their population number. It is probably not farfetched to say that tending ants take advantage of the synchronizing size in aphid numbers to remove and probably store honeydew for use even when aphid population decreases or is totally absent.

Amino acid concentration, again, shows no relation with ant numbers and when the ant numbers were lowest, the amino acid concentration was highest; but this was not consistent throughout. Therefore, sugars, rather than amino acids seem to have a stronger relationship with ant numbers which suggests that ants tend the aphid colonies more for their sugar requirements than for amino acids. It would be worthwhile to investigate this aspect in more detail. However, a similar analysis as for sugar removal could be attributed here indicating a possibility of an equal amount of sugars and amino acid in honeydew.

The study, when considered in totality, suggests that the aphid populations show positive response to ant tending, regardless of the magnitude (or numbers) of the latter. The presence of ants, even in low numbers, seem to be beneficial to the aphids. On the other hand, the study revealed that the ants get a steady supply of sugars and amino acids from the honeydew, which reduces the need for foraging for food.

A study, incorporating energy expenditure of the ants for food foraging, with, and without aphid colonies should be interesting and would provide an insight into the adaptive strategy of the ants, as also the energy benefits to the ants resulting from such associations.

SUMMARY

The present study was undertaken to identify the inherent mechanism in aphid-ant

association. The results revealed a synchrony between the populations of aphids, and ants tending them. The ellution of sugars and amino acids of the honeydew in such tending ant abdomens though did not change significantly over the study period, did however, keep in line with the tending ant population number. These and other indications and the implications of such association has been discussed.

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