Sociobiology 70(2): e7698 (June, 2023) DOI: 10.13102/sociobiology.v70i2.7698

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

In vitro Studies on the Biology and Predatory Potential of Commonly Occurring Ant Species in a Selected Agro-ecosystem of the Himalayan Range, India

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Article History

Edited by
Evandro Nascimento Silva, UEFS, Brazil
Received 21 December 2021
Initial acceptance 03 March 2022
Final acceptance 27 March 2023
Publication date 15 June 2023

Keywords
Camponotus compressus, Polyrhachis thompsoni, Pheidole minor, biocontrol agents.

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Abstract

Four commonly occurring species of queen ants were collected after their nuptial flight from an agroecosystem in Lehri Sarail village of Himachal Pradesh (31°32’03.9” N 76°38’57.7 “E) using light traps. Queens named HAC1, HAC2, HAC3, and HAC4 were placed individually in a test tube with water sources. Once the queen started laying eggs, and a few workers emerged, they were transferred to an artificial formicarium. HAC1 and HAC2 were identified as *Camponotus compressus,* while HAC3 and HAC4 were identified as *Polyrhachis thompsoni* and *Pheidole minor,* respectively. In the study period of one year between July 2020 and July 2021, offspring from all colonies were found to be workers. HAC1, HAC2, and HAC4 produced dimorphic workers, the majors and minors. Studies on colony establishment, biology, and behavior were carried out with colonies from HAC1, HAC2, and HAC3, while a colony from HAC4 failed to establish. All species showed similar types of colony founding, nesting, brood management, necrophorosis, and hibernation. *C. compressus* showed higher fecundity, while percent hatchability was higher in *P. thompsoni*. Percent pupation and adult emergence were higher in *C. compressus* and lower in *P. thompsoni. P. thompsoni* was observed to be more aggressive with a high predation rate, followed by *C. compressus. P. minor* showed aggressiveness initially but was unable to incapacitate the host larva. Understanding ant biodiversity, biology, behavior, predatory potential, etc., will allow us to use the native ants in the agroecosystem as biological control agents. Methods of rearing ants under the artificial setup described here enable laboratory culturing of ants with biocontrol potential and using ants as model organisms to study the bio-efficacy of agrochemicals and pharmaceutical drugs.

Introduction

Ants (Formicidae: Hymenoptera) are eusocial insects with enormous diversity and have colonized almost every region of the earth, contributing to 15-25% of land animal biomass (Schultz, 2000; Keller & Gordon, 2006). Their efficient foraging habits, eusocial lifestyle, abundance, and capability to easily adjust to the local environment make them ecologically more successful. Nearly 333 genera, 17 subfamilies, 13,262 species, and 1,941 subspecies of ants have been reported around the globe (Bolton, 2017). India, with its rich geographical and ecological diversity, is a hot spot for species diversity and has recorded around 828 ant species under 100 genera grouped into ten subfamilies. They live in varied habitats like trees, twigs, barks, cavities of nuts, rotten logs, fallen branches, soil, rocks, etc. They are associated with various plants and animals and possess varied feeding habits. Ants show a symbiotic relationship with other organisms like plants, insects, and other species of ants. They have coevolved with other species over a long time, enabling them to adapt to imitative, parasitic, and mutualistic relationships (Holldobler & Wilson, 1990; Coovert, 2005). Ants are good biological

Open access journal: http://periodicos.uefs.br/index.php/sociobiology
ISSN: 0361-6525

indicators as they establish mutualistic behavior with the living organisms in the environment. Ants show polymorphism and assist one another to survive (Watanasit et al., 2000). Ants live in colonies with a large number of individuals. They tend to grow as large colonies under ideal environmental conditions. Ants are social insects, and only the queen can reproduce, and workers carry out all other activities like brood care, foraging, food supply, nest maintenance, and defense (Wilson, 1971). The fecundity and the queen's oviposition rate determine colony growth (Passera, 1990; Marinho, 1998). The queen produces diploid workers (females incapable of mating) from fertilized eggs and haploid males from unfertilized eggs. After a successful colony establishment, queens also have fertile female offspring, which then disperse to form a new colony (Autuori, 1940; Bazire-Benazet, 1957). However, in a few species of ants like *Atta* spp. and *Acromyrmex* spp., workers are not completely sterile but only produce males when the queen is absent (Camargo et al., 2005; Dijkstra & Boomsma, 2006; Shimoji et al., 2018). Ants are omnivores and play various significant roles in the agroecosystem. They act as bioindicators (Majer, 1983), biocontrol agents (Offenberg, 2015), plant pollinators (Garcia et al., 1995), restorers of soil health (Jones et al., 1994; Lavelle, 1997), conservators of biodiversity (Keller & Gordon, 2006), diagnostic tools for orchards (Cerdà, 2009; Diamé et al., 2015) and in some cases as crop and stored grain pests.

Studies on the biology and behavior of ants *in vitro* are scarce compared to other economically important insects. Hence, the current investigation aimed to study ants' biology, behavior, and adaptability under laboratory conditions. This will enable us to design methods to mass culture ants with predatory potential in the laboratory and release them as biocontrol agents. In addition, ants can be used as model organisms to screen the effect of agrochemicals and drugs, thus helping us to identify safe molecules for pest management and human health care.

Materials and Methods

Study site, sample collection, and colony establishment under controlled conditions

The study area was an agricultural field of Lehri Sarail village, Bilaspur District, Ghumarwin Tehsil of Himachal Pradesh (31°32'03.9" N 76°38'57.7" E). Light traps set on farm buildings were used to lure queen ants. Queen ants were collected in 30 ml screw-capped glass vials after their nuptial flight. Immediately after collection, the queens were individually released into the air-filled space of a 15 ml glass test tube setup half-filled with water and separated by the cotton plug. This setup provides a long-term supply of drinking water essential for survival and egg-laying by the queens (Antkeepers, 2021). A piece of paper was also placed in a test tube in case the queen ant needed an extra dry surface to place the brood. Then the open end of the test tube was sealed with a cork. This setup also resembles a claustral chamber, which the queen makes and seals herself till the first worker arrives. The test tube was placed in dark to provide conducive environment for queen ants to lay eggs. After the arrival of first 20 workers, colonies were transferred into small formicarium set up made of 250 ml plastic bottle (5 cm diameter, 16 cm height) attached with a circular box (8.5cm diameter and 5.5 cm height) acting as foraging area. Minute holes were made on the plastic bottle for proper ventilation. Plate 1 shows the setup used for rearing ant colonies. The ants were transferred into the formicarium by their choice without being forced by covering the formicarium with black paper. This induced the ants to move to the fresh dark recipient chamber from the older tube. Initially pre-killed, crushed cockroach and sugar crystals were placed in the foraging area to fulfil protein and carbohydrates requirements of the ants. Plastic straws (0.5 mm diameter) were used for connecting the formicarium with the foraging area. Loose sand (1 cm height) was spread into the foraging area. Insect pests abundantly found in the agroecosystem were provided as food for the ants. Host insects were collected from plants where no pesticides were sprayed. They were killed by freezing, then they were cut into small pieces, and served as meal to ants. The ant rearing setup was adapted from previous studies with modifications (Cardoso et al., 2011).

Morphology and taxonomical identification of ant species under study

Morphological features such as body size, color, head, thorax and their appendages, and abdominal modifications of the queen and the workers from the newly established colony were recorded. To identify the ant species, a single worker ant from each colony was sacrificed using ethyl acetate. Care was taken to avoid any physical damage to keep the ant's morphological features intact. The killed ant specimens were placed in 2 ml plastic vials and preserved using 70% ethanol. Then, they were sent for identification to Professor Dr. Himender Bharti, an expert ant taxonomist recommended by Zoological Survey of India.

Studies on the biology, behavior and predatory potential of ants

The biology and behavior of ants were studied in captivity. The well-established colonies were observed twice a day for a period of one year from 15 July 2020 to 15 July 2021. We avoided disturbing the ant colony while recording data. The adult fecundity and the number of larvae and pupae were recorded at weekly intervals. To study the duration of the developmental stages viz., egg, grubs, pupa and adult we observed five individuals in each colony and recorded the data. The first meal was provided three days after adult emergence from pupa. Sugar crystal and cricket leg were offered to ants in every meal and the test tubes were cleaned regularly to avoid microbial growth.

To study the predatory potential of different ant species, 4th instar larvae of rice moth, *Corcyra cephalonica* was used as prey. Each treatment was replicated three times. Test tubes containing rice moth larvae alone served as control. In each replication, five worker ants were released into a test tube containing a rice moth larva and secured. The tubes were observed every thirty minutes up to six hours of release to study the predatory behavior of the ants recording parameters such as antennal touch on the prey, biting of prey and killing the prey (Morris & Perfecto, 2016). A completely randomised design (CRD) was used for the lab experiments. Data on activity count were transformed into square root transformation (X+ 0.5) as per the method developed by Poisson for statistical analysis (Snedecor & Cochran, 1967). Data from laboratory experiments were subjected to analysis of variance (ANOVA). The means were separated by Duncan's New Multiple Range Test (DMRT) (Gomez & Gomez, 1984). We used SPSS version 22.0 for all statistical analysis.

Results

A total of four queen ants were collected after their nuptial flights in July 2020 and were named as HAC1, HAC2, HAC3 and HAC4, where HAC stands for queens collected from Himachal. They were transferred individually into 15 ml test tube with water source. Once the queen laid eggs and few workers emerged, they were transferred to a bigger formicarium set up. Observations on the morphological features of the queen and the newly emerged workers were recorded (Table 1). The morphological features were compared with the data available in the database for species identification. Morphological observations revealed that the queens of all four colonies were considerably larger in size, and measured about 1.5 cm, (HAC1, HAC2), 0.8 cm (HAC3), 0.5 cm (HAC4). The queens were also found to possess wings initially. Both HAC1 and HAC2 were identified as *Camponotus compressus* (Plate 2a, b), while HAC3 and HAC4 were identified as *Polyrhachis thompsoni* (Plate 3) and *Pheidole minor* (Plate 4), respectively. Among the four colonies collected, HAC1, HAC2 and HAC3 were well established, whereas the colony from HAC4 failed to establish, due to the death of the queen. Hence, studies on the behavior and biology of the ant species were carried with the colonies established from HAC1, HAC2 and HAC3. The colonies were observed twice daily, although data for population counts of various bio stages were tabulated at weekly intervals (Table 2). In all three study colonies, the queen started to lay eggs within a week after their captivity inside the artificial test tube setup. The queen solely took care of the brood until the first worker adult emerged. As the workers emerged, they started to take care of the brood and the queen. The workers fed them, cleaned the hive and protected the colony. Most of the colony founding characters and brood behavior were similar in the colonies of *Camponotus compressus* (HAC1 and HAC2) (Plates 5a-h, 6a-d) and *Polyrachis thompsoni* (HAC3) (Plates 7a-e). The ants emerging from all three established colonies in the specified study period (15 July 2020 – 15 July 2021) were found to be workers. The biology parameters of the three well established ant colonies namely HAC1, HAC2, HAC3 in one year period is shown in Table 3 and Figure1. The fecundity of *Camponotus compressus* queens (HAC1 and HAC2) was 416 and 453 eggs respectively in one year study period, while the fecundity *Polyrachis thompsoni* HAC3 was around 146 eggs. The duration of the egg stage of *Camponotus compressus* (HAC1, HAC2) was approximately eight days, while that of *Polyrachis thompsoni* (HAC3) was slightly higher and was around 12.8 days. The percent egg hatchability of HAC1, HAC2 and HAC3 were 66.11%, 73.73% and 80.82% respectively. This resulted in 275, 334 and 118 larvae in HAC1, HAC2 and HAC3, respectively. The larval duration of offsprings from HAC1, HAC2 and HAC3 were 26.6, 27, 30.8, days respectively. Out of 275, 334, 118 larvae from HAC1, HAC2 and HAC3 only197 (71.63%), 245 (73.35%) and 75 (63.56%) pupated, respectively. Based on the morphology we found that all the adults that emerged out of pupa were workers. However, in *C. compressus*, the workers were dimorphic. The larger ones were referred as the 'majors' or 'super majors' and smaller ones were referred as 'minors'. In *P. thompsoni* only one type of worker was present. The total number of workers that emerged from the colonies formed by HAC1, HAC2 and HAC3 were 143, 171 and 58, respectively. The estimated percentage adult emergence was 72.59%, 69.80% and 63.56%, respectively. In the case of colonies HAC1 and HAC2 the ratio of major to minor worker was 1:19.43 and 1:20.5. The longevity of the workers from HAC1, HAC2 and HAC3 was 35.0, 36.6 and 32.6 days, respectively. Studies on predatory potential were carried out using all three ant species collected, namely *Camponotus compressus* (HAC1 and HAC2), *Polyrhachis thompsoni* (HAC3) and *Pheidole minor* (HAC4). In the case of antennal touch there was no significant difference between the number of times each ant species touches the prey throughout the entire time of observation (Table 4, Figure 2). However, it was noticed that antennal touch was more frequent within one hour of release of the ants with the prey. Among the three ant species, it was observed that *Polyrachis thompsoni* bit the prey a greater number of times with an average of 17.33 times as compared to the other ant species under study within half an hour of release with the prey. *Phedolie minor* and *Camponotus compressus* bit the prey on average 5.00 and 0.67 times, respectively. The frequency of biting gradually decreased after half an hour with no significant difference among the ant species under study (Plate 8 a-c, Table 5, Figure 3). Among the three ant species tested *Polyrachis thompsoni* killed the prey larvae more efficiently within four hours, followed by *Camponotus compressus,* which killed the host larvae at five hours after release. Though *Phedolie minor* showed predatory behavior like biting, they were not efficient enough to kill the prey (Table 6, Figure 4).

Discussion

All four queen ants HAC1, HAC2, HAC3 and HAC4 used in this study were collected using light traps in farm building. Nene et al. (2017) described that *Oecophylla longinoda* queen catch was enhanced with the presence of light during night as they get attracted towards light. The queens were later transferred individually into a 15 ml glass test tube that were half-filled with water and separated by cotton plug. Test tube setup was made to create starting environment for colony founding. This setup kept the queens hydrated. In all the three species under study, the queen started to lay eggs within a week of their captivity under artificial test tube setup. Previous studies also reported that the queen starts laying eggs within a week of their nuptial flight. The Incubation period of the eggs usually varied between 40 hours and 2 weeks depending on the species (Narendra & Kumar, 2006; Verza et al., 2017).

In the current study, the queens were left undisturbed until they laid eggs and after at least 20 workers emerged, they were transferred to a transparent formicarium setup which included a nesting and brood area, foraging area, water source and dump yard. Miller (1929) reported that an artificial nest should include (i) a water source to keep ants hydrated, (ii) nesting area where queen can lay eggs and workers can take care of brood and (iii) a foraging area where food is supplied to the colony.

Within a short time, queen ants accepted the test tube setup and started to clean their antennae using their mouth parts and started to drink water from moist cotton plug inside the test tube. These tubes were stored in a box in a dark corner of the room to avoid disturbance and the first observation was made 12 hours after capturing.

All the queen ants HAC1, HAC2, HAC4 except HAC3 possessed wings initially during collection. They shed off their wings within 12 hours of collection. The dealate queen looked like a big ant but had visible wing scars on her thorax. Morphological observations revealed that queen of HAC3 also had scars evidencing that they possessed wings initially. Queens HAC1 and HAC2 were the largest followed by HAC3 and HAC4 respectively. Workers were comparatively smaller and were wingless. Dimorphic workers were found in HAC1, HAC2 and HAC4. Laciny et al (2019) described that caste specific modulation in the morphometry of ants occurs to suit their life functions. Taxonomic identity revealed that the commonly occurring ants in agroecosystem collected for study included *Camponotus compressus* (HAC1, HAC2), *Polyrhachis thompsoni* (HAC3) and *Pheidole minor* (HAC4). Bharti et al. (2016) reported that *Camponotus*, *Polyrhachi* and *Pheidole* were the dominant genera documented in India, each with 83, 71 and 58 species contributing respectively to 10%, 8.5% and 7.0% of the known Indian species.

Queens of all three species under study solely took care of the brood until the first worker adult emerged. Lone and Sharma (2008) reported that *Camponotus* species have a life cycle similar to other social ants. Nuptial flight takes place during June to July when males and queens emerge from their nest for mating. Male dies during mating and queen explores a suitable site for nest making. Queen rips her wings off before laying eggs. Once they start laying eggs, the queen never leave the nest and take care of the brood until the adult worker emerges and takes up the function. The larvae became adult by completing its metamorphosis within the nest. The adults are divided into two forms, the winged reproductive and wingless sterile castes (Holldobler & Wilson, 1990; Kadu, 2012). Also, the colony founding characters and brood behavior were similar in both *Camponotus compressus* and *Polyrachis thompsoni*. Queen of all the colonies laid elliptical translucent eggs of size 1mm in length and they placed all the eggs together while taking care of them. Tiny larvae hatched from the eggs within two weeks. Queen ant solely took care of the offspring and fed them with nutrition stored inside her body. No food material was given to queen during the founding stage. Larvae turned into pupa by spinning light brown cocoon. Emerging adults had small and softer body which hardens with time, and size of the gaster of worker ants becomes bigger and comparatively translucent as ant feeds, and stores food into the gaster. The offspring that emerged from all three colonies in the study period of one year were found to be workers. Fertile males and females will appear only after a considerable size of the colony has been established. Both the genera showed trophallaxis (mouth to mouth feeding). When sugar crystals and protein source were provided as feed in the foraging area, workers fed themselves and fed the queen and larvae. Worker ants of both genera maintained colony hygiene by dumping their garbage in the corner 'bathroom area' of the colony. At the death of a worker ant, its body is carried by other worker ants and dumped with the waste material and the process is termed as necrophoresis. When disturbed, all the worker ants quickly grab as much as brood they can and try to place the brood in a safer area. Colonies with smaller number were not much aggressive, however aggressiveness increased with the colony size. When the daily minimum temperatures dropped below 4 °C the colonies went into hibernation, although one or two workers in each colony showed activity required for colony maintenance**.** Hibernation continued until the daily minimum temperature was equal to or above 18 °C which prevailed for 19 weeks starting from the second fortnight of November 2020 up to the third week of March 2021.

Studies on biology and reproductive potential revealed that fecundity of *Camponotus compressus* queens were higher as compared to *Polyrachis thompsoni,* though this was counterbalanced by percent hatchability which was higher in *P. thompsoni.* Both egg and larval duration were higher in *P. thompsoni.* Percent pupation and adult emergence as well as adult worker longevity were comparatively higher in *C. compressus. P. thompsoni* had only one type of worker while *C. compressus* had dimorphic workers. The bigger workers called as majors had larger head and strong mandibles suited for defense and colony protection. But majors were relatively less in numbers as compared to smaller workers 'the minors' (1:20). Minors actively participated in brood maintenance, foraging and housekeeping. Laciny et al., (2019) reported morphological and behavioural differences leading to caste specific variation in tribe Camponotini. Colonies founded by *Polyrachis thompsoni* (HAC3) had only one type of worker. Another differential observation was that the worker ants of *P. thompsoni* grabbed the larvae and tried to cover the inner walls of the test tube with the larval silk. *Polyrachis* spp. lacks metapleural gland, and rely on grooming behaviour and utilisation of larval silk for protecting their colonies from parasitise and pathogens. Long term observations will provide us more information of colony establishment and biology including ratio of reproductives produced etc., and colony behaviour under laboratory conditions.

Studies on predatory potential using parameters such as antennal touch, prey biting and host killing revealed that no significant difference occurred among the ant species under study with respect to antennal touch. However, it was observed that antennal touch was more initially after release of ants with the host species evidencing that antenna acts as an important organ to sense the host suitability. Among the three species *P. thompsoni* bit the prey a greater number of times as well as killed it efficiently within 4 hours of its release. Though *P. minor* ranked next with respect to the frequency of biting of prey and *C. compressus* showed least biting, *P. minor* was not efficient enough to kill host. Contrastingly *C. compressus* killed the prey within 5 hours of release, ranking next to *P. thompsoni.* Antennal touch, biting of prey and killing of prey were used as parameters to evaluate predatory potential of two species of ants *viz., Solenopsis picea* and *Wasmannia auropunctata* on coffee berry borer, *Hypothenemus hampei* (Morris & Perfecto, 2016). Further research including a variety of host insects in bioassays will help us understand specificity in host preference by the predatory ant species. This will enable us to identify if the ant species are generalist or specific predators and can be used for biocontrol of targeted pests. The above studies also open up avenues to mass culture ants in laboratory and to use them as model organisms to study the effect of agrochemicals and pharmaceutical drugs for pest and disease management in field of agriculture, and human medicine.

Table 1. Morphology and identity of ant species found in the study area.

Code	Species identified	Morphological features	Castes observed in colony established under captivity	
			Queen	Worker(s)
HAC1 HAC2	*Camponotus compressus**	Body length	1.5 cm	Majors: 1.1 cm; Minors: 0.7 cm
		Body color	Dark Black	Majors: Dark Black; Minors: Dark Black
		Head and its appendages	Antennae, compound eyes, mandibles were clearly visible.	Majors: Head was bigger than minors. Antennae, compound eyes, mandibles were visible. Minors: Antennae, compound eyes, mandibles were visible.
		Thorax and its appendages	Wings were present and after wings were shed scars were visible. Coxa and tarsus were red and femur and tibia were black.	Both majors and minors were wingless with red coxa and tarsus and black femur and tibia
		Abdomen and modifications	Large abdomen without sting	In both Majors and minors abdomen was equal to size of the head, sting was absent
HAC3	*Polyrhachis thompsoni*	Body length	0.8 cm	0.6 cm
		Body color	Black	Black
		Head and its appendages	Head contains antennae, compound eyes and a pair of mandibles that were clearly visible	Head contains antennae, compound eyes and a pair of mandibles that were clearly visible.
		Thorax and its appendages	Wings were present and after shedding wing scars were visible. Thorax was more or less flat, and a pair of spikes emerged from posterior end of thorax.	Wingless. Thorax is more or less flat and a pair of spikes emerged from posterior end of thorax.
		Abdomen and modifications	Two large spikes emerged from petiole. Abdomen was globulose in shape and sting was absent	Two large spikes emerged from the petiole. Abdomen was globulose in shape and sting was absent
HAC4	*Pheidole minor**	Body length	0.5 cm	Majors: 0.4 cm; Minors: 0.2 cm
		Body color	Black	Majors: dark brown; Minors: dark brown
		Head and its appendages	Small antennae, compound eyes and mandibles were clearly visible	Majors: Head was the largest part of the body. Antennae, eyes and mandibles were clearly visible. Minors: Small antennae, small eyes and mandibles were clearly visible
		Thorax and its appendages	Wings were present and after shedding wing scars were visible.	Majors: Wingless; Minors: Wingless
		Abdomen and modifications	Abdomen was dorsoventrally flattened and stingless	In both majors and minor abdomen were spherical, smaller than head and stingless.

*Workers of this species were dimorphic in nature.

2. Data on number of various bio stages at weekly intervals in the ant colonies under study.

Date of observation	No. of Queen			No. of eggs#			No. of larvae#			No. of pupae#			No. of adults#		
	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3
15-Jul-20	1	1	1	3	5	0	0	0	0	0	0	0	0	0	0
22-Jul-20	1	1	1	9	12	0	2	2	0	0	0	0	0	0	0
29-Jul-20	1	1	1	7	12	5	5	5	0	2	2	0	0	0	0
05-Aug-20	1	1	1	10	10	2	5	10	4	4	4	0	0	0	0
12-Aug-20	1	1	1	6	9	3	6	8	1	5	8	0	4	4	0
19-Aug-20	1	1	1	12	12	0	3	8	2	6	7	0	3	6	0
26-Aug-20	1	1	1	10	10	0	9	10	0	1	7	3	4	6	0
02-Sep-20	1	1	1	18	10	0	8	7	0	7	5	2	0	5	0
09-Sep-20	1	1	1	20	13	10	10	6	0	5	4	0	6	4	3
16-Sep-20	1	1	1	22	15	10	15	10	10	6	5	0	4	3	2
23-Sep-20	1	1	1	0	7	0	15	10	5	11	8	0	3	3	0
30-Sep-20	1	1	1	25	14	0	0	6	0	12	9	2	9	6	0
06-Oct-20	1	1	1	0	11	0	18	12	0	0	3	7	10	7	0
13-Oct-20	1	1	1	0	0	10	0	10	0	15	8	4	3	2	6
20-Oct-20	1	1	1	0	0	2	0	0	2	0	9	0	12	7	5
29-Oct-20	1	1	1	0	0	0	0	0	7	0	0	0	0	7	2
07-Nov-20	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
15-Nov-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
22-Nov-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
29-Nov-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
06-Dec-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
13-Dec-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
20-Dec-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
27-Dec-20*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
03-Jan-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
10-Jan-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
17-Jan-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
24-Jan-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
31-Jan-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
07-Feb-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
14-Feb-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
21-Feb-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
28-Feb-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
06-Mar-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
13-Mar-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
20-Mar-21*	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
27-Mar-21	1	1	1	1	0	0	0	0	0	0	0	0	0	0	7
03-Apr-21	1	1	1	10	10	4	1	0	0	0	0	0	0	0	1
10-Apr-21	1	1	1	10	10	5	8	9	2	0	0	0	0	0	0
17-Apr-21	1	1	1	22	20	10	9	9	7	4	7	0	0	0	0
24-Apr-21	1	1	1	18	20	2	14	15	9	7	6	0	4	5	0
01-Apr-21	1	1	1	10	10	5	12	17	0	11	12	5	5	5	0
08-May-21	1	1	1	10	10	0	9	8	6	10	13	8	8	10	0
15-May-21	1	1	1	20	20	10	6	8	0	6	4	2	6	10	9
22-May-21	1	1	1	20	12	20	12	17	5	4	6	3	4	2	2
29-May-21	1	1	1	20	23	0	12	9	12	8	15	0	3	5	5

2. Data on number of various bio stages at weekly intervals in the ant colonies under study. (Continuation)

Date of observation	No. of Queen			No. of eggs#			No. of larvae#			No. of pupae#			No. of adults#		
	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3	HAC1	HAC2	HAC3
05-Jun-21	1	1	1	10	11	10	13	20	6	9	7	0	6	12	0
12-Jun-21	1	1	1	15	18	3	9	10	9	11	16	2	8	5	0
19-Jun-21	1	1	1	21	25	18	11	15	1	9	7	8	9	12	0
26-Jun-21	1	1	1	20	25	5	12	23	15	8	12	8	7	6	9
03-Jul-21	1	1	1	25	37	5	16	24	3	10	20	0	6	10	7
10-Jul-21	1	1	1	22	22	7	17	26	4	13	20	0	8	15	0
15-Jul-21	1	1	1	20	40	0	18	20	7	13	21	12	11	14	0
Total number of bio stages at the end of one year	**1**	**1**	**1**	**416**	**453**	**146**	**275**	**334**	**118**	**197**	**245**	**75**	**143**	**171**	**58**

*Hibernation period; #No. newly added.

Table 3. Studies on biology of ants under captivity.

Parameters	Biology of Ant colonies under captivity		
	HAC1	HAC2	HAC3
No. of Eggs#	416	453	146
Egg duration*	8.2 ± **0**.40	8.4 ± **0**.25	12.8 ± **0.58**
No. of larvae	275	334	118
Percent hatchability	66.11%	73.73%	80.82%
Larval duration *	26.6 ± 0.75	27 ± 0.84	30.8 ± 0.37
Percent Pupation	71.63%	73.35%	63.56%
No. of Pupa	197	245	75
Pupal duration*	9.2 ± 0.49	9.4 ± 0.24	12 ± 0.55
Percent adult emergence	72.59%	69.80%	63.56%
No. of adults	143	171	58
No. of Big workers	7	8	NA
No. of small workers	136	164	58
Ratio of big: small workers	1:19.43	1:20.5	NA
Longevity of worker *(minors)	35 ± 1.38	36.6 ± 1.03	32.6 ± 0.87

* Mean of five replicates; # Fecundity of the queen in one year period.

Table 4. Predatory behavior of ants species under study: antennal touch on the prey.

Ant species	No. of Antennal touch on the prey*											
	0.30 hrs	1.00 hrs	1.30 hrs	2.00 hrs	2.30 hrs	3.00 hrs	3.30 hrs	4.00 hrs	4.30 hrs	5.00 hrs	5.30 hrs	6.00 hrs
Camponotus compressus	1.67 (1.47)[a]	0.33 (0.91)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.33 (0.91)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]
Polyrhachis thompsoni	1.00 (1.22)[a]	1.00 (1.22)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]
Pheidole minor	1.33 (1.35)[a]	0.67 (1.08)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.33 (0.91)[a]	0.33 (0.91)[a]	0.33 (0.91)[a]	0.33 (0.91)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]

*Mean of three replications, Figure in Parentheses are $\sqrt{(x+0.5)}$ transformed values
In column, means followed by a common letter are not significantly different by DMRT (P=0.05)

Table 5. Predatory behavior of ants species under study: Biting of prey.

Ant species	No. of times ant bites the prey*											
	0.30 hrs	**1.00 hrs**	**1.30 hrs**	**2.00 hrs**	**2.30 hrs**	**3.00 hrs**	**3.30 hrs**	**4.00 hrs**	**4.30 hrs**	**5.00 hrs**	**5.30 hrs**	**6.00 hrs**
Camponotus compressus	0.67 (1.08)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	1.33 (1.35)[a]	0.33 (0.91)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]
Polyrhachis thompsoni	17.33 (4.22)[b]	0.00 (0.7)[a]	0.33 (0.91)[a]	0.00 (0.7)[a]	1.00 (1.22)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]
Pheidole minor	5.00 (2.35)[ab]	1.33 (1.35)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]

*Mean of three replications, Figure in Parentheses are √ (x+0.5) transformed values
In column, means followed by a common letter are not significantly different by DMRT (P=0.05)

Table 6. Predatory behavior of ants species under study: Host killing.

Ant species	Host killing*											
	0.30 hrs	**1.00 hrs**	**1.30 hrs**	**2.00 hrs**	**2.30 hrs**	**3.00 hrs**	**3.30 hrs**	**4.00 hrs**	**4.30 hrs**	**5.00 hrs**	**5.30 hrs**	**6.00 hrs**
Camponotus compressus	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.33 (0.91)[a]	0.67 (1.08)[b]	0.67 (1.08)[b]	0.67 (1.08)[b]
Polyrhachis thompsoni	0.00 (0.7)[a]	0.33 (0.91)[a]	0.67 (1.08)[a]	0.67 (1.08)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	1.00 (1.22)[b]	1.00 (1.22)[b]	1.00 (1.22)[b]	1.00 (1.22)[b]	1.00 (1.22)[b]
Pheidole minor	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]	0.00 (0.7)[a]

*Mean of three replications, Figure in Parentheses are √ (x+0.5) transformed values
In column, means followed by a common letter are not significantly different by DMRT (P=0.05)

Plate 1. Formicarium setup

Plate 2. *Camponotus compressus* queen

2a) *Camponotus compressus* queen with wings

2b) *C. compressus* queen after shedding of wings

Plate 3. ***Polyrhachis thompsoni*** **(Queen)**

Plate 4. ***Pheidole minor*** **(queen and workers)**

Plate 5. Colony establishment and behaviour of *Camponotus compressus*

5a) *C. compressus* queen drinking water

5b) *C. compressus* queen with eggs and larvae

5c) *C. compressus* queen with first worker

5d) *C. compressus* brood care

5e) *C. compressus* queen trophallaxis showing fully open mandibles

5f) Trophallaxis in *C. compressus*

5g) *C. compressus* cleaning itself

5h) Colony established by *C. compressus*

Plate 6. Biostages of *Camponotus compressus*

6a) *Camponotus compressus* queen with eggs

6b) *Camponotus compressus* larvae

6d) *Camponotus compressus* adults (queen and workers)

Plate 7. Colony establishment, behaviour and biostages of *Polyrachis thompsoni*

7a) *P. thompsoni* queen with brood

7c) *P. thompsoni* web spinned from larval silk

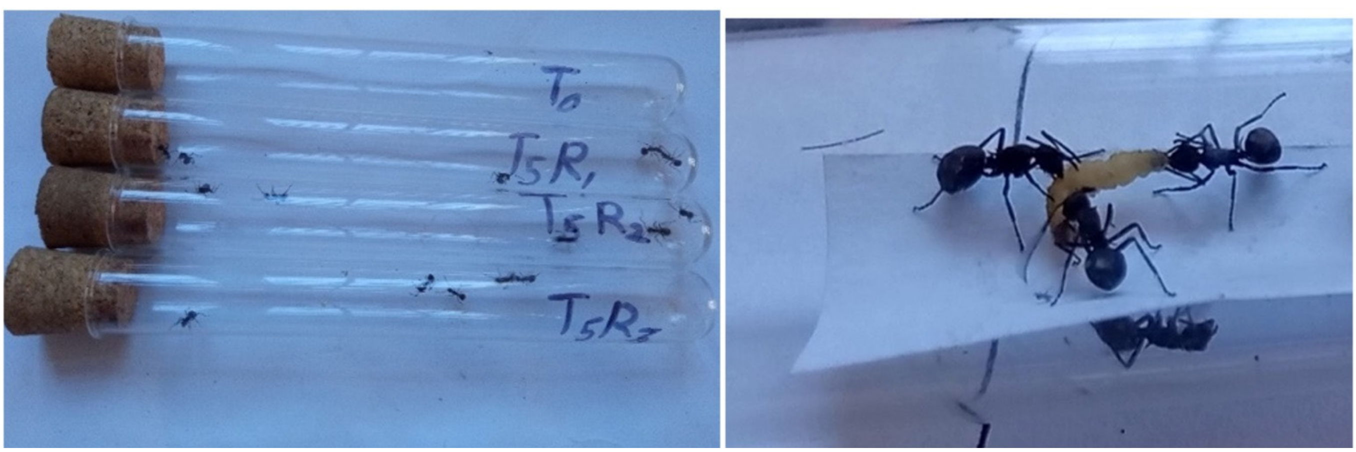
7b) *P. thompsoni* with queen, eggs, pupa, workers

7d) *P. thompsoni* with freshly dead worker

7e) *P. thompsoni* feeding on cockroach

Plate 8. Predatory potential of ant species against *Corcyra* larvae

8a) Predatory potential of *Camponotus compresssus* against *Corcyra* larvae



8b) Predatory potential of *Polyrachis thompsoni* against *Corcyra* larvae

8c) Predatory potential of *Pheidole minor* against *Corcyra* larvae

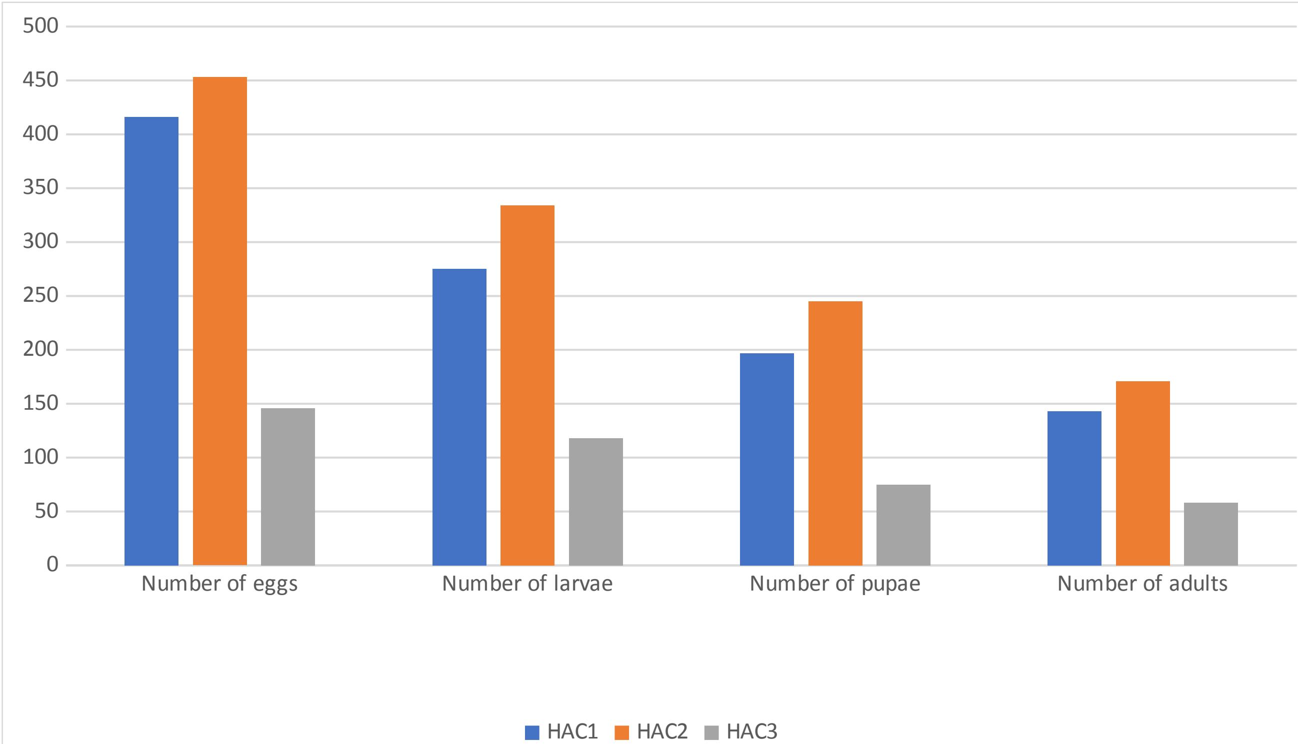


Fig 1. Studies on population of various biostages in ant colonies established by ant species HAC1, HAC2 and HAC3 in one year period (July 2020 – July 2021).

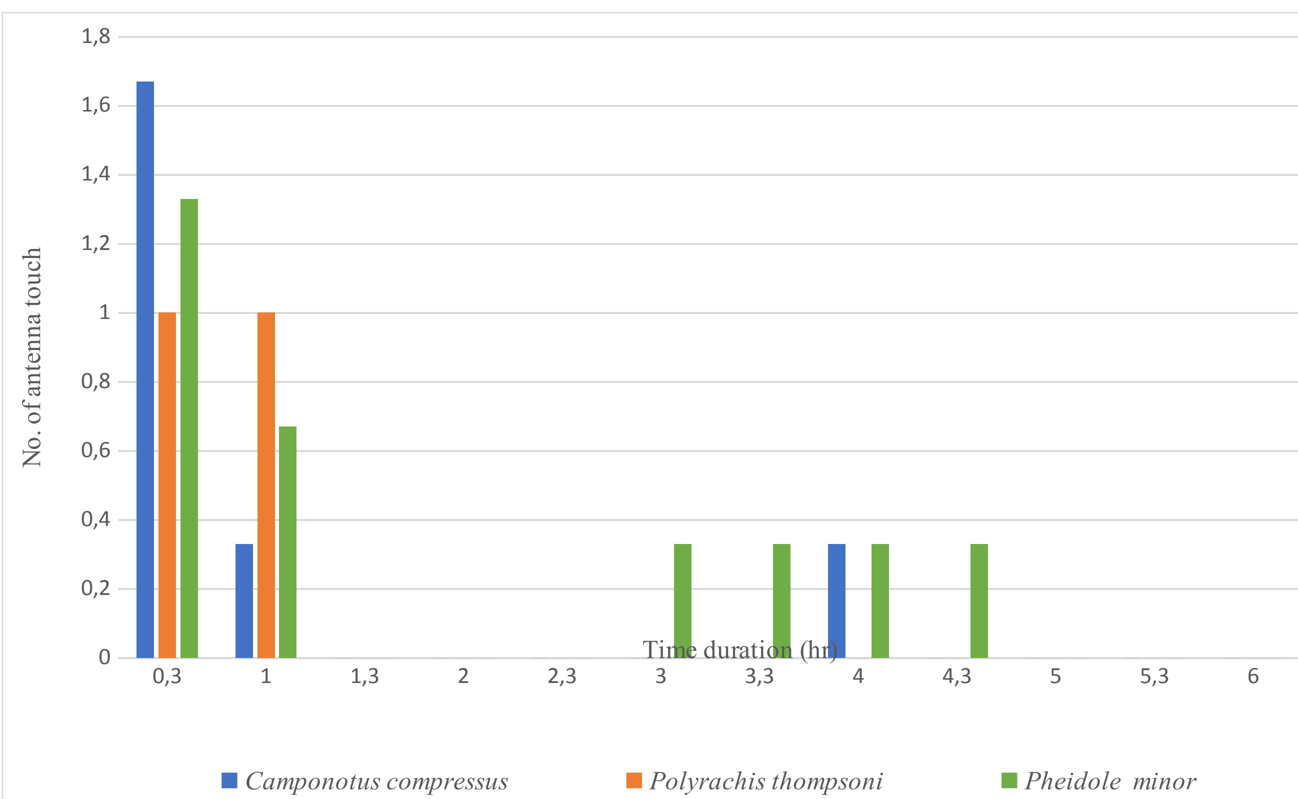


Fig 2. Predatory potential: Antennal touch.

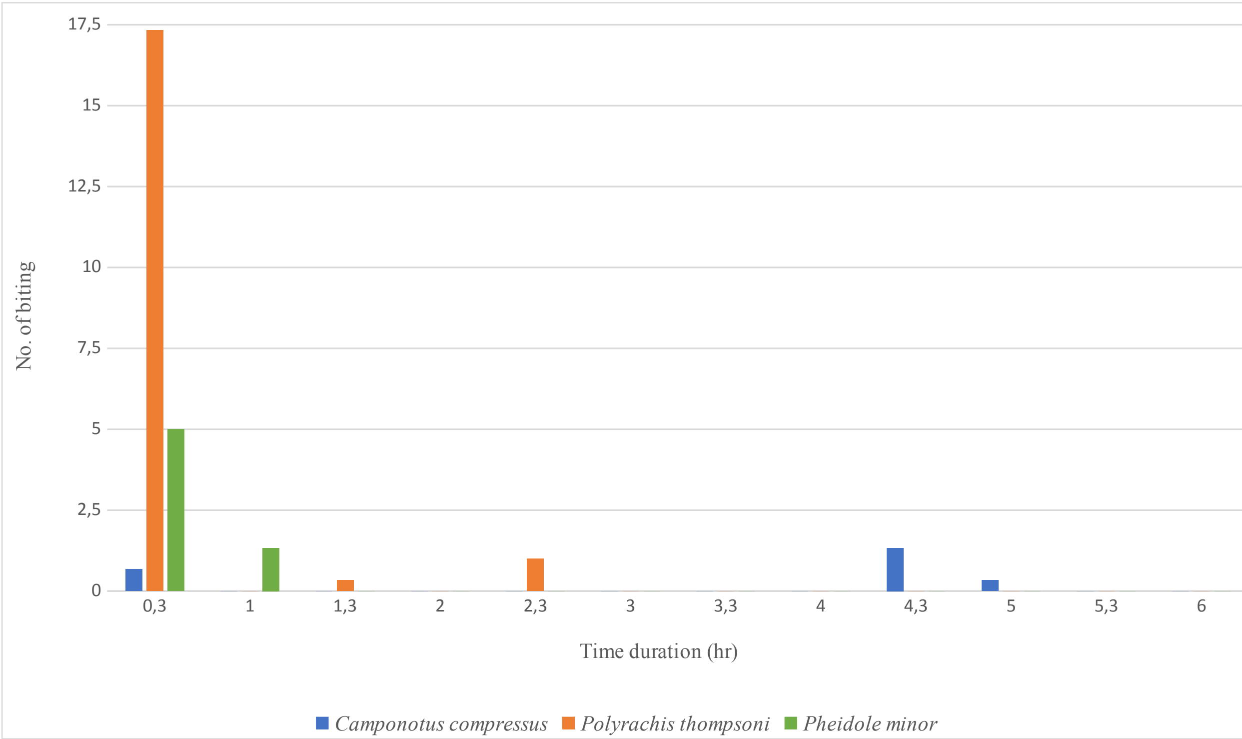


Fig 3. Predatory Potential: Biting of Prey.

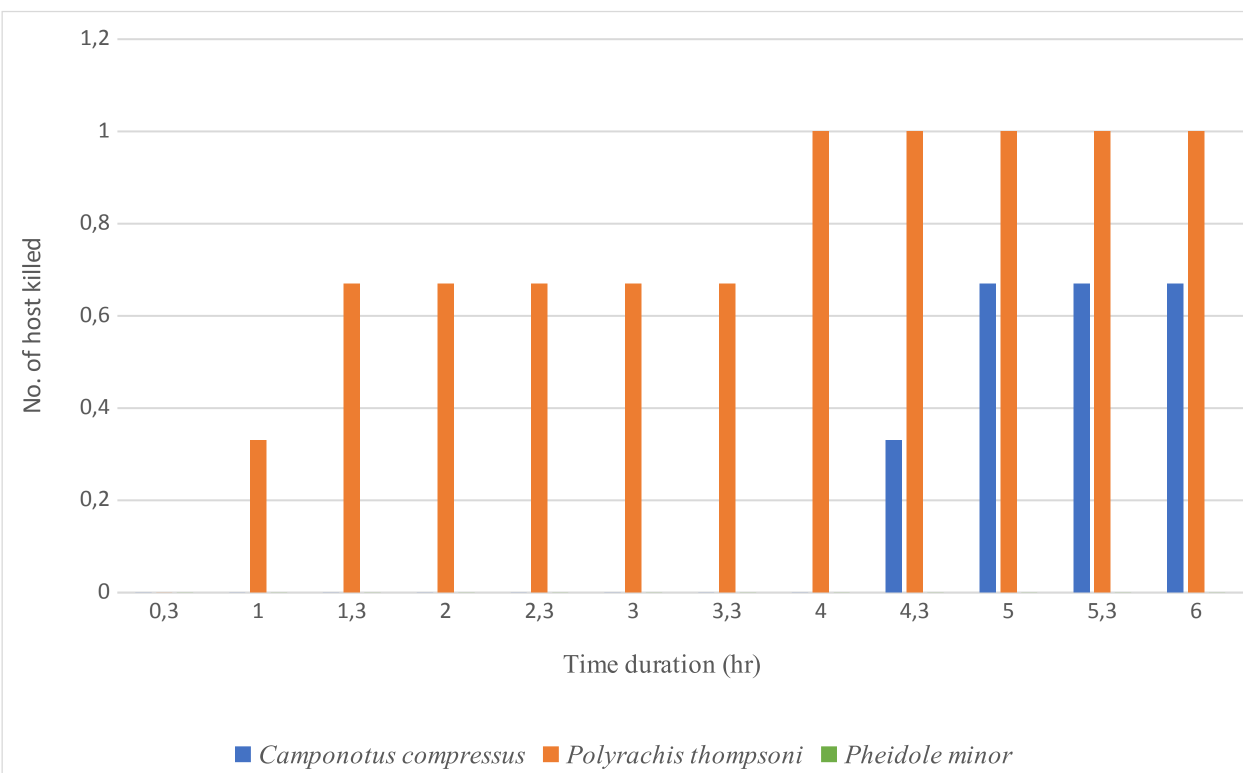


Fig 4. Predatory potential: Killing of prey.

Acknowledgment

The authors gratefully thank the generous support offered by Professor. Dr. Himendar Bharti, Punjabi University, Patiala, India for the help rendered in identification of the ant species. The authors also thank Lovely Professional University, Punjab, India for providing necessary support to carry out the study. The authors sincerely acknowledge the help rendered by Dr. R. Nagarajaprakash, Associate Professor and Research co-ordinator- Sponsored research, Centre for Research, Dhanalakshmi Srinivasan University for his support in improving the manuscript technically and grammatically. The authors also wish to thank the anonymous referees for their suggestions in improving the manuscript.

Funding: The authors declare that the current study did not receive any financial aid.

Authors' Contribution

Dr. Pathma Jayakumar conceptualized the research, reviewed literature, designed and supervised the experiments, drafted, edited and approved the final version of manuscript. Mr. Kushal Thakur reviewed the literature, conceptualized and carried out the experiments and recorded the data. Mr. Bhushan L Sonawane reviewed the literature, recorded and analysed the data. All the authors approved the final version of the manuscript.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: Not applicable.

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