



Olfactory behavior and response of household ants (Hymenoptera) to different types of coffee odor: A coffee-based bait development prospect



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ARTICLE INFO

Keywords:

Household ants
Olfactory
Coffee
Behavioral effects

ABSTRACT

Odor sensation is a sensory modality of considerable significance in the foraging behavior and interactional organization of ants. In the food bait technology, smell is the basis of attraction, which, in turn, is the line of bait use and a key parameter for judging efficacy. Yet, the currently available baits possess low attractiveness to many ant pests. Hence, strategies to produce ant bait with increased attractiveness are needed. Despite evidence that coffee has a diverse aroma complex that affects the behavior of honey bees and ants, its attraction to house-invading ants has yet to be investigated. In a series of Y-tube olfactometer bioassays, we examined the behavioral responses of *Tapinoma indicum* (TI), *Monomorium pharaonis* (MP) and *Solenopsis geminata* (SG) to various coffee-induced odor stimuli, comprised of extracts from Arabica, Robusta and Liberica. All coffee extracts showed an influence on the behavior of TI, MP and SG workers, with Arabica showed the most significant influence to the tested ants. The workers of TI, MP and SG were more attracted to the odor of 0.01% Arabica extract (ONE), in comparison with 0.05% Arabica extract (TWO) or 0.10% Arabica extract (THREE). Arabica extract mixed with sugar (S) elicited a significant attraction from workers of all three species in a balanced competition with either unsweetened Arabica extract or water. These results indicated that coffee, particularly Arabica, was attractive to the foragers of TI, MP and SG, thus, the use of coffee as a novel stimulus agent seems plausible in ant bait development.

Introduction

There are > 12,000 known species of ants (Hammond, 2011), many of which are among the most common insects invading or living inside human establishments where they become a nuisance and cause damage (Lee, 2002). The typical house invaders are dolichoderine ants such as *Tapinoma indicum* (Lee, 2002; Man and Lee, 2014), *Monomorium pharaonis* (Osae et al., 2011) and *Solenopsis geminata* (Harris et al., 2005).

Among the species, *T. indicum* is the most nuisance, having the ability to invade any disturbed habitats suitable for its nesting and subsequently forms large-sized colonies (Passera, 1994). Another house-invading ant species, *M. pharaonis* (also known as the Pharaoh ants), attacks a wide range of foodstuffs, clothes, and books (Dumpeert, 1981). This species is also able to chew on silk, rayon, rubber and electrical wiring (Hölldobler and Wilson, 1990). In hospitals, the Pharaoh ant is reputed to carry several pathogenic bacteria (Haack and

Granovsky, 1990; Smith and Whitman, 1992) and may feed on wounds (Anon, 1986), thus, considered as a potential disease vector (Osae et al., 2011). *S. geminata*, on the other hand, is a major threat to agricultural crops (Wilson, 2005), affecting farmers' performance by inflicting irritating stings (Hill, 1987; Nestel and Dickschen, 1990). This type of ant also causes substantial damage to PVC coatings of electrical wiring (Prins, 1985) and drip irrigation tubing (Chang and Ota, 1990).

Efforts to combat such damages rely heavily on the use of chemical insecticides through baiting (Higgins et al., 1997), residual perimeter sprays (Potter and Hillery, 2002), or both (Higgins et al., 1997). In these global strategies against ant pests, baiting forms a very crucial part of the solution (Jordan et al., 2013). This method takes advantage of the social trophallactic and grooming behaviors of ants (Lee, 2008) and relies on the pick-up of bait particles by foraging workers and their transfers to other colony members (Jordan et al., 2013). Baits have been successfully used to control a number of social insect pests (Jordan et al., 2013). However, many bait-based programs are failed due to

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insecticide resistance and insufficient level of attractiveness (Rust et al., 2002; Krushelnycky and Gillespie, 2008). Thus, the ability to produce bait with high attractiveness remains a major challenge for the bait-based ant control strategy. For target ants to pick up and transfer bait particles to the colony, they must be attracted to the bait. Therefore, the first and critical step in the bait use process is attracting the target ants, particularly the foragers (Jordan et al., 2013).

The sense of smell is one of the main sensory modalities for ants (Gronenberg, 2008; Choe et al., 2012). In fact, they primarily perceive the world through smell via the detection of airborne chemicals and touch (Gronenberg, 2008). Since the first line in the bait use process is to entice foragers (Jordan et al., 2013) and those olfactory cues are essential for social organization and behaviors (Gronenberg, 2008), especially orientation (Wolf and Wehner, 2000), these aspects of ant biology should receive the highest attention when seeking new control strategies. Despite substantial efforts to increase bait attractiveness by the addition of insect tissue (Williams et al., 1990), different fruit juices (Lucas and Invest, 1993), sugars, proteins, oils, or a combination of different attractants (Rust et al., 2002), only few available baits are highly attractive to ants (Rust et al., 2002). Since odors can influence the behavior of animals, it is, therefore, an important strategy to target strong-smelling and chemically rich materials to be used as baits.

Coffee is one of the promising candidates as it has over 800 aromatic compounds (Clarke and Macrae, 2012). Many of these constituents such as aliphatics—carbonyl and sulfur contain compounds: alicyclic elements—ketones; aromatic benzenoid compounds—phenols; heterocyclic compounds—furans, hydrofurans, pyrroles, pyridines, quinolines, pyrazines, quinoxalines, indoles, thiophenes, thiophenones, thiazoles and oxazoles are produced during the roasting process (Illy and Illy, 2015; Fisk et al., 2012; Clarke and Macrae, 2012). The increased diversity of compounds results in an aroma complex comprising fruity, earthy, catty, roasty, spicy, buttery, sweet, rotten cabbage-like, honey-like, potato-like, caramel-like, seasoning-like, and vanilla-like smells (Grosch, 1998). These smells hit many scent receptors in both humans and animals including > 900 species of insects as coffee pests. For instance, exposure of rats to coffee aroma deprived them of sleep for a day (Seo et al., 2008). In honey bees, caffeine from coffee stimulated responses in olfactory learning and memory (Wright et al., 2013). Many processes inherent to social living and food processing in bees possess homologs in ants. As bees are genetically related to ants (Johnson et al., 2013) and have far more glomeruli than ants (160 glomeruli) (Flanagan and Mercer, 1989), they may be highly responsive to the aroma of coffee. Despite evidence that coffee has a variety of aromas and that caffeine influences some hymenopterans (bees), where hymenopterans attracted to caffeine in flower nectar. However, there have been no studies on the effects of coffee on ant foraging behaviors with respect to bait technology improvement. The present study was carried out to examine the behavioral responses of household ants towards extracts from different coffee species. The behaviors of these ants in response to coffee exposure at three different concentrations and sugar contents were also probed.

Materials and methods

Test ant species and experimental subjects

T. indicum (TI), *M. pharaonis* (MP) and *S. geminata* (SG) were used in this study. For each of these ant species, colonies were located within the Minden Campus of University of Science Malaysia (Penang, Malaysia, latitudes 5°8'N–5°3'N; longitudes 100°8'E–100°32'E). For each of the three formicine ants, two different colonies were selected as sources of test subjects, and each was marked using tagged wooden stakes. Active workers were collected by placing twenty to thirty traps within each colony starting from 7 am. Each trap consisted of a 1.5-mL Eppendorf tube with the lower bottom removed and was interiorly lined with a thin layer of Fluon (Polytetrafluoroethylene suspension),

adopted from Shirwaikar et al. (2004) in their efforts to prevent the escape of trapped workers. A minute amount of peanut butter or honey held on a small piece of paper was placed inside all traps to serve as feed. After 3 h of collection, the trapped workers were kept in 250-mL plastic containers lined with Fluon coat and labeled according to species, colony number, and date of sampling. The workers' samples were brought to the insectarium of the School of Biological Sciences (Universiti Sains Malaysia). Subsamples were kept in 90% ethanol for species identity confirmation. The laboratory conditions were 27 °C ± 2.0 °C, 75% ± 1% relative humidity (RH), in photoperiod 13:10 h (light:dark) with 1 h of dusk. Workers that had acclimatized to the laboratory environment and were active after a 12 h-starvation period, were used as experimental subjects.

Coffee materials and experimental extracts

Local varieties of *Coffea arabica* (Arabica), *C. canephora* (Robusta) and *C. liberica* (Liberica) obtained from Cap Kuda Coffee Company, Sabah were selected for this study. Coffee beans were roasted with no sugar compounds added. The temperatures ranged from 210 °C to 240 °C were used in the roasting process with the roasting time of 12 to 30 min. Approximately, 150 g of dried and roasted coffee beans were individually crushed using a blender (Pensonic Blender PEN-PB3103; Senheng® Electric Sdn. Bhd., Kuala Lumpur, Malaysia) for 10 min. In order to obtain uniform textures, grounds were sieved twice through a kitchen sieve strainer (250-wire mesh). Fine grounds were used to produce different extracts by Soxhlet extraction in accordance with the published procedures (Cholakov et al., 2013). Briefly, an amount of 50 g of fine Arabica grounds was placed on a paper thimble and extracted with 250 mL of methanol. Five hours after the first downpouring of the methanol from the thimble, the resulting mixture (methanol + extract) was siphoned into a flat-bottomed flask, filtered and transferred into a glass petri dish. The extract was placed in an incubator (Memmert GmbH + Co, KG, Germany) set at 20 °C. After 3 days of evaporation, the extract (referred to as Arabica extract) was kept in a vial and stored in a freezer (− 4 °C) until used. The same quantity of fine grounds and procedures were also performed for Robusta and Liberica grounds; of which the two resulting juices were accordingly designated as Robusta and Liberica extracts.

Another three experimental concentrations of coffee i.e. 0.01%, 0.05% and 0.10% were produced using Arabica, following a slightly modified method of Derraik and Slaney (2005). To prepare 0.01% Arabica solution, an amount of 0.005 g of powdered roasted grounds was placed into a 150-mL glass cup with 50 mL of boiling water. After 10 min, the resulting solution was sieved through a piece of fine-mesh mosquito net as in Satho et al. (2015). This solution was designated as “ONE”. Meanwhile, to prepare 0.05% and 0.10% solutions, the amounts of 0.025 g and 0.05 g of Arabica grounds were used following the same procedures and were referred to as TWO (0.05%) and THREE (0.10%), respectively. To acquire a sugared experimental Arabica, we proceeded as follows. An amount 0.005 g of powdered roasted Arabica grounds was immersed in 50 mL of boiling water in a 150-mL glass cup and was allowed to disintegrate. After 10 min, the resulting solution was filtered and 0.25 g of coarse grain sugar (Gula Prai, MSM Holdings, Malaysia) was added. The solution was referred to as “S” (0.50% of sugar). Another solution was prepared using the same procedures with no sugar added and was referred to as “NS”. A volume of 50 mL cooled boiled water was also prepared as a control (C).

Bioassays setup

A Y-tube olfactometer system previously described by Yusuf et al. (2014) was used to test the attraction of workers of different household ant pests to odors emanating from different coffee extracts. The system comprised of a central tube (7.5 cm long) and two lateral arms (each 5.75 cm long and 10 mm wide). Each arm was connected to a glass

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