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Behavioral responses of household ants (Hymenoptera) to odor of different coffee species and formulations: Sustainability approach for green pest management strategies

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9 *Running Head:Olfactory behavior of ants on coffee*

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SUMMARY

37   Odor sensation is a sensory modality of considerable significance in the foraging behavior and

38   interactional organization of ants. In the food bait technology, smell is the basis of attraction,

39   which in turn, is the initial line of bait use and a key parameter for judging efficacy. Yet, baits

40   that are currently available possess low attractiveness to many ant pests. Hence, strategies to

41   produce ant bait with increased attractiveness are needed. Despite evidence that coffee has a

42   diverse aroma complex that affects the behavior of honey bees, which share many social and

43   genetic traits with ants, its attraction to formicine foragers have yet to be investigated. In a series

44   of Y-tube olfactometer bioassays, we examined the behavioral responses of *Tapinoma indicum*

45   (TI), *Monomorium pharaonis* (MP) and *Solenopsis geminata* (SG) to various coffee-induced

46   odor stimuli, comprised of extracts from Arabica, Robusta and Liberica. All coffee species

47   showed an influence on the behavior of TI, MP and SG workers, but the clearest effect was seen

48   with Arabica. The workers of TI, MP and SG were more attracted to the odor of 0.01% Arabica

49   extract (ONE), in comparison with 0.05% Arabica extract (TWO) or 0.05% Arabica extract

50   (THREE). Arabica extract mixed with sugar (S) elicited significant attraction from workers of all

51   three species when in balanced competition with either unsweetened Arabica extract or water.

52   These results indicate that coffee, particularly Arabica is attractive to the foragers of TI, MP and

53   SG, and can encourage the use of coffee in bait development. As coffee is already accepted in

54   most human societies, the development of coffee-based bait may be an attractive option.

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56   Key words:household ants, olfactory, coffee, behavioral effects

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INTRODUCTION

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61   There are more than 12,000 known species of ants (Hammond, 2011), many of which are among

62   the most common insects invading or living inside human establishments where they become a

63   nuisance and cause damage (Lee, 2002; Coggins, 2013). This is typical of formicine ants such as

64   *Tapinoma indicum*, regarded as household pests that invade health care facilities in Southeast

65   Asia (Lee, 2002; Man and Lee, 2014); *Monomorium pharaonis,* a serious threats to homes,

66   hospitals, offices and business establishments—hotels, supermarkets, restaurants   (Osae,

67   Cobblah, Djankpa, Lodoh, and Botwe, 2011); and *Solenopsis geminata*, a threat to birds,

68   livestock, agricultural ecosystems and people (Harris, et al. 2005).

69   *T. indicum* has an increased ability to invade disturbed habitats as they are able to rapidly

70   find suitable habitats for nesting and produce large-sized colonies (Passera, 1994). When they

71   invade houses, *M. pharaonis* also known as the Pharoah ants, attack a wide range of foodstuff,

72   clothes, books (Dumpert, 1981) and can chew on silk, rayon, rubber and electrical wiring

73   (Hölldobler and Wilson, 1990). In hospitals, the Pharoah ants are reputed to carry several

74   pathogenic bacteria (Haack and Granovsky, 1990; Smith and Whitman, 1992) and may feed on

75   wounds (Anon, 1986), thus considered a potential disease vector (Osae, et ak. 2011). *S.*

76   *geminata*, on the other hand, is a major threat to agricultural crops (Wilson, 2005), affecting

77   farmers' performance by inflicting irritating stings (Hill, 1987; Nestel and Dickschen, 1990).

78   This type of ants also causes substantial damage to PVC coatings of electrical wiring (Prins,

79   1985) and drip irrigation tubing (Chang and Ota, 1990).

80   Efforts to combat such damages rely heavily on the use of chemical insecticides through

81   baiting (Higgins, Bell, Silcox, and Hollbrook, 1997), residual perimeter sprays (Potter and

82   Hillery, 2002), or both in combination (Higgins, Bell, Silcox, and Hollbrook, 1997). In these

83   global strategies against ant pests, baiting form a very crucial part of the solution (Benett et al.,

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84   2013). This method takes advantage of the social trophallactic and grooming behaviors of ants

85   (Lee, 2008) and relies on the pick up of bait particles by foraging workers and their transfers to

86   other colony members (Benett et al., 2013). Baits have been used successfully to control a

87   number of social insect pests (Stanley, 2004; Benett et al., 2013). However, many bait-based

88   programs failed due to insecticide resistance and insufficient level of attractiveness (Rust,

89   Reierson, and Klotz, 2002; Krushelnycky and Gillespie, 2008). Thus, the ability to produce bait

90   with high attractiveness remains a major challenge for the bait-based ant control strategy. For

91   target ants to pick up and transfer bait particles to the colony, they must be attracted to the bait.

92   Therefore, the first and critical step in the bait use process is attracting the foragers (Benett et al.,

93   2013).

94   The sense of smell is the main sensory modality for ants (Gronenberg, 2008). In fact, they

95   primarily perceive the world through smell via the detection of airborne chemicals and touch

96   (Dodgson, 2015). Since the first line in the bait use process is to entice foragers (Benett et al.,

97   2013) and that olfactory cues are essential for social organization and behaviors (Gronenberg,

98   2008), especially orientation (Wolf and Wehner, 2000), these aspects of ant biology should

99   receive the highest attention when seeking new control strategies. Despite substantial efforts to

100   increase bait attractiveness by the addition of insect tissue (Williams, Lofgren, and Vander Meer,

101   1990), different fruit juices (Lucas and Invest, 1993), sugars, proteins, oils, or a combination of

102   different attractants (Rust and Choe, 2012), very few baits are available that are very attractive to

103   ants (Rust, Reierson, and Klotz, 2002; Krushelnycky and Gillespie, 2008). Therefore, it is an

104   important strategy to target strong-smelling and chemically rich materials.

105   A special characteristic of ants is that they have a remarkably sophisticated sense of smell

106   (Choe, Ramírez, and Tsutsui, 2012; Harris-Lovett, 2015) and seem to be able to sniff most of the

107   substances that humans can smell (Dodgson, 2015). They have a brain with around 250,000

108   nerve cells (John and Sarah, 2010) and use special neurons associated with tiny hairs on their

109   antennae to smell odors (Roces, 1994; Dupuy, et al., 2006). An ant's antennal lobe possesses

110   over 400 glomeruli (Zube, et al., 2008; Gronenberg, 2008). There is a close association between

111   a number of glomeruli and the smell detection capacity. Indeed, a large number of odorant

112   receptors and glomeruli allows for complex combinatorial olfactory codes to detect and

113   discriminate a wide range of odorant stimuli (Kelber, et al. 2009) with precision (Gronenberg,

114   2008). Therefore, any material that has high aroma potency may be a valuable candidate as an

115   attractant for ants.

116   There are more than 1000 chemicals in coffee including over 800 aromatic compounds

117   (Clarke and Macrae, 2012). Many of these constituents such as aliphatics—carbonyl and sulfur

118   contain compounds: alicyclic elements—ketones; aromatic benzenoid compounds—phenols;

119   heterocyclic compounds—furans, hydrofurans, pyrroles, pyridines, quinolines, pyrazines,

120   quinoxalines, indoles, thiophens, thiophenones, thiazoles and oxazoles are produced during the

121   roasting process (Illy and Illy, 2015; Fisk, Kettle, Hofmeister, Virdie, and Kenny, 2012; Clarke,

122   and Macrae, 2012). The increased diversity of compounds results in an aroma complex

123   comprising fruity, earthy, catty, roasty, spicy, buttery, sweet, rotten cabbage-like, honey-like,

124   potato-like, caramel-like, seasoning-like, and vanilla-like smells (Grosch, 1998). These smells hit

125   many scent receptors in both humans and animals including insects. For instance, exposure of

126   rats to coffee aroma deprived them of sleep for a day (Seo et al., 2008). In honey bees, caffeine

127   from coffee stimulated responses in olfactory learning and memory (Wright et al., 2013). Many

128   processes inherent to social living and food processing in bees possess homologs in ants. As

129   bees are genetically related to ants (Johnson et al., 2013) and have far more glomeruli than them

130   (160 glomeruli) (Flanagan and Mercer, 1989), they may be highly responsive to the aroma of

131   coffee. Despite evidence that coffee has a variety of aromas and that coffee influences some

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132   hymenopterans (bees), there have yet to be any studies on the effects of coffee on ant foraging

133   behaviors with respect to bait technology improvement. The present study was carried out to

134   examine the behavioral responses of formicine ants towards extracts from different coffee

135   species. The behaviors of these ants in response to coffee exposure at three different

136   concentrations and sugar contents were also probed.

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138   **MATERIALS AND METHODS**

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140   **Test ant species and experimental subjects**

141   *T. indicum* (TI), *M. pharaonis* (MP) and *S. geminata* (SG) were selected in this study. For each

142   of these ant species, colonies were located within the Minden Campus of University of Science

143   Malaysia (Penang, Malaysia, latitudes 5°8'N - 5°3'N; longitudes 100°8'E - 100°32'E; Ahmad et

144   al., 2006). For each of the three formicine ants, two different colonies were selected as sources of

145   test subjects, and each was marked using tagged wooden stakes. Active workers were collected

146   by placing twenty to thirty traps within each colony starting from 7 am. Each trap consisted of a

147   1.5-mL Eppendorf tube with the lower bottom removed and was interiorly lined with a thin layer

148   of Fluon (Polytetrafluroethylene suspension), adopting Shirwaikar, Issac, and Malini, (2004) in

149   their efforts to prevent the escape of trapped workers. A minute amount of peanut butter or

150   honey held on a small piece of paper was placed inside all traps to serve as feed. After 3 hours of

151   collection, the trapped workers were kept in 250-mL plastic containers lined with Fluon coat and

152   labeled according to species, colony number, and date of sampling. The workers' samples were

153   brought to the insectarium of the School of Biological Sciences (Universiti Sains Malaysia.

154   Subsamples were kept in 90% ethanol for species identity confirmation. The laboratory

155   conditions were 27°C ± 2.0°C, 75% ± 1% relative humidity (RH), in photoperiod 13:10 hours

156   (light:dark) with 1 hour of dusk. For all species, workers that had acclimatized to the laboratory

157   environment and were active after a 12h-starvation period, were used as experimental subjects.

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159   **Coffee materials and experimental extracts**

160   *Coffea arabica* (Arabica)*, C. canephora* (Robusta) and *C. liberica* (Liberica) varieties cultivated

161   in Malaysia were selected for this study. Samples of 150 g of dried roasted coffee bean seeds

162   from these different species were individually crushed using a blender (Pensonic Blender PEN-

163   PB3103; Senheng® Electric Sdn. Bhd., Kuala Lumpur, Malaysia) for 10 minutes. In order to

164   obtain uniform textures, grounds were sieved twice through a kitchen sieve strainer (250-wire

165   mesh). Fine grounds were used to produce different extracts by Soxhlet extraction in accordance

166   with the published procedures (Cholakov, Toteva, Nikolov, Uzunova, and Yanev, 2013). Briefly,

167   an amount of 50 g of fine Arabica grounds was placed on a paper thimble and extracted with 250

168   mL of methanol. Five hours after the first down-pouring of the methanol from the thimble, the

169   resulting mixture (methanol + extract) was siphoned into a flat-bottomed flask, filtered and

170   transferred into a glass petri dish. The extract was placed in an incubator (Memmert GmbH + Co,

171   KG, Germany) set at 50°C. After 3 days of evaporation, the extract which was kept in a vial,

172   stored in a fridge (-4°C) can validly be referred to as Arabica extract. The same quantity of fine

173   grounds and procedures delineated above for Arabica were also performed for Robusta and

174   Liberica grounds; the two resulting juices were designated as Robusta and Liberica extracts

175   correspondingly.

176   Three other experimental concentrations of coffee were produced using Arabica, following

177   a slightly modified method previously published (Derraik and Stanley 2005). An amount of

178   0.005 g of powdered roasted grounds was placed into a 150-mL glass cup with 50 mL of boiling

179   water. After ten minutes, the resulting solution was sieved through a piece of fine-mesh mosquito

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180   net as done in other experiments (Satho et al., 2015); this 0.01% Arabica solution was designated

181   as "ONE". Solutions generated by submerging amounts of 0.025 and 0.05 g of Arabica grounds

182   each in 50 mL of boiling water for the same extraction time (10 minutes) were referred to as

183   Arabica extracts TWO (0.05%) and THREE (0.10%) respectively. To acquire a sugared

184   experimental Arabica, we proceeded as follows. An amount 0.005 g of powdered roasted

185   Arabica grounds was immersed in 50 mL of boiling water in a 150-mL glass cup and allowed to

186   disintegrate. After ten minutes, the resulting solution was filtered, and 0.25 g of sugar was added

187   to it. The infusion collected after complete dissolution of the sugar and filtration was referred to

188   as Arabica extract with sugar or "S" (0.50% of sugar). Another solution obtained by the same

189   procedures described for the above infusions, but without sugar is dubbed Arabica extract

190   without sugar or "NS". Cool boiled water acted as a control (C). When necessary, additional

191   extract preparations were carried out to obtain enough quantities of experimental extracts for the

192   bioassays.

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195   **Bioassays setup**

196   A Y-tube olfactometer system that has been described elsewhere (Yusuf, Crewe, and Pirk, 2014)

197   was used to test the attraction of workers of different formicine ant pests to odors emanating

198   from different coffee extracts. The system comprised a central tube (7.5 cm long) that branches

199   into two lateral arms (each 5.75 cm long and 10 mm wide). Each arm was connected to a glass

200   chamber (200-mL capacity) that held a given test coffee extract. Each glass chamber had a screw

201   top containing inlets for the incoming air and outlets for odors to exit the Y-tube. A charcoal-

202   filtered air stream was passed into each arm at a flow rate of 250 mL/min to drive experimental

203   odors towards the decision area. A mesh screen was placed at each of the end points of the

204   olfactometer to prevent the escape of workers from the test arena. Bioassays were conducted

205   under laboratory conditions (temperature: $27.0 \pm 0.1 \circ C$, relative humidity: $80.5 \pm 8.5\%$, and

206   photoperiod: 14:10 h) between 7 am and 10 am or 4 and 5 pm, adopting the practice of previous

207   researchers (Yusuf, et al., 2014) in their attempts to cover the optimal foraging periods of test

208   ants.

209   A first experiment was carried out to examine the attractiveness of extracts from three

210   coffee species to *T. indicum*. To determine whether TI is attracted to Arabica in the presence of

211   Robusta, thirty workers were introduced individually into the central arm and given 5 min to

212   choose between the stimuli in the two arms, each holding either Arabica or Robusta extracts. The

213   same procedures and numbers of TI worker replicates as reported above were repeated but the

214   two arms contained one the following solutions: (i) Arabica and (ii) Liberica. To establish

215   whether TI was enticed by Robusta in the presence of Liberica, thirty TI worker replicates were

216   individually given the opportunity to choose between two stimuli: (i) an arm holding Robusta

217   and (ii) an arm containing Liberica. The same settings, treatments, procedures, and numbers of

218   replicates, were also carried out for MP, and SG.

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220   **Bioassay 2**

221   To determine whether coffee attractiveness to ants depends on coffee concentration, the

222   behavioral responses of TI workers to Arabica extracts were evaluated for three different

223   concentrations in a series of two-choice comparative trials. In the first trial, a single TI worker

224   was released from the central arm of the Y-tube and was allowed to select one of the following

225   conditions: (i) an arm holding 0.01% Arabica extract (ONE) and (ii) an arm holding 0.05%

226   Arabica extract (TWO). In the second trial, an individual TI worker was given a choice between

227   (i) an arm holding ONE and (ii) an arm holding 0.10% Arabica extract (THREE). In the third

228   trial, a single TI worker was given opportunity to choose between an arm holding TWO and the

229   other with THREE. For each trial, thirty TI workers were individually tested and in each case, a

230   single worker was given 5 min to select between the two treated arms. The same three

231   comparative trials performed for TI were also done for MP and SG, with thirty test worker

232   replicates in each case.

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234   **Bioassay 3**

235   To establish whether sugar has an influence on the attractiveness potential of coffee on ants, the

236   behavioral responses of TI workers to three Arabica extracts with different sugar contents were

237   tested in a sequence of two-choice comparative bioassays, using similar designs and procedures

238   mentioned in Experiment 2. In a first bioassay, an individual TI worker was introduced into the

239   central arm and permitted to choose between the two arms each containing one of the following

240   Arabica extracts: (i) Arabica extract mixed with 0.50% of sugar (S) and ii) Arabica extract

241   without sugar (NS). In the second bioassay, a single TI worker was given a choice between (i) an

242   arm containing S and (ii) an arm holding water (Control: C). In the third bioassay, a single TI

243   worker had to choose between an arm containing NS and an arm with C. Thirty TI workers were

244   tested for each of the three bioassays. The same numbers of bioassays, worker replicates,

245   procedures and treatments as described for TI were also conducted for MP and SG.

246
247   **Data collection and analyses**

248   In all bioassays, each worker was given a 5-min period to select between the arms. A choice for

249   the left or right arm of the olfactometer was recorded when the worker went 1 cm past the Y

250   junction and stayed there for at least 1 minute or when it frequently visited an arm. For all two-

251   choice bioassay data obtained, a preference index (PI) was computed using a formula reported

252   by other researchers (Carsson et al., 1999; Nyasembe et al., 2012). The formula was expressed as

253   PI = [(SS – NSS) / (SS + NSS)] x 100, where SS is the number of ants responding to the first

254   stimulus and NSS is the number of them reacting to the second one. A PI of 0% would mean that

255   equal number of experimental workers were attracted to each of the two arms of the Y-tube

256   olfactometer. A positive value of the PI signifies that more workers responded to the first extract

257   whereas a negative value indicates more were attracted to the second extract. As established by

258   Anaclerio et al. (2012), in all bioassays, only workers that responded were included in data

259   collection and analysis. The PI was used as a measure of the ants' behavioral response to the

260   tested stimulus. Behavioral responses of workers belonging to the different tested ant species to

261   each experimental coffee stimulus were compared using the Chi-square test (SPSS Statistics, *v.*

262   20) with $P < 0.05$ as an indicator of statistical significance.

263

264   **RESULTS**
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266   **Behavioral responses to stimuli from different coffee species**

267   When presented with Arabica and Robusta, TI is attracted to both odor stimuli, but Arabica

268   seems to elicit more attraction from its workers. However, there is no significant difference in

269   the preference index between the two smells ($\chi2 = 1.096$, *df* $= 1$, $P > 0.05$). In the second

270   experiment, a comparison is made between the attractiveness of the extracts of Arabica and

271   Liberica. The TI workers tend to be more attracted to the odor stimulus from Arabica than that

272   from Liberica, but the difference is not significant ($\chi2 = 2.25$, *df* $= 1$, $P > 0.05$). When exposed to

273   the smells from Robusta and Liberica, TI workers have a tendency to exhibit a preference for the

274   first odor over the second one, but the attractiveness did not differ significantly between the

275   coffee species ($\chi2 = 0.20$, *df* $= 1$, $P > 0.05$) (Fig. 1A).

276   When given similar options to walk towards the two sources of coffee odor, MP workers

277   are attracted to both odors, but behavioral responses reveals different patterns according to the

278   coffee type. Arabica has a tendency to attract more workers than Robusta, as shown by the

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279   positive PI, but the difference in attractiveness between the two coffee species is insignificant ($\chi2$

280   = 1.096, df = 1, P > 0.05). When one arm holding Arabica and another with Liberica are

281   presented to MP, they head to both odor sources, but the workers are more susceptible to

282   approach Arabica than Liberica. However, the attractiveness level of the two coffee odor is

283   statistically similar ($\chi2$ = 2.25, df = 1, P > 0.05). In the choice bioassay involving Robusta and

284   Liberica, the MP workers visit both coffee odor sources, but they tend to visit the arm holding

285   Liberica more. However, there is no significant difference in the attractiveness level between the

286   Robusta and Liberica stimuli ($\chi2$ = 1.818, df = 1, P > 0.05), as illustrated by the negative PI (Fig.

287   1B).

288   When there is an equal chance for walking towards a source of coffee either the Arabica

289   odor and Robusta smell, SG workers tend to be more attracted to the first odor. However, there

290   are no significant differences in the attraction level between the two sources ($\chi2$ = 6.66, df = 1, P

291   < 0.05). In the two-choice bioassay comprising Arabica and Liberica, SG workers walk towards

292   both coffee odor sources, but they tend to prefer Arabica, but the attractiveness does not differ

293   between the two odors ($\chi2$ = 2.25, df = 1, P > 0.05). There was no difference in the attractiveness

294   level between Robusta and Liberica coffee ($\chi2$ = 0.20, df = 1, P > 0.05) (Fig. 1C).

295

296   **Behavioral responses following Arabica exposure at three different concentrations**

297   In the two-choice experiment involving the two lowest Arabica concentrated extracts (ONE and

298   TWO), TI workers are significantly attracted to extract ONE ($\chi2$ = 4.464, df = 1, P < 0.05).

299   When presented with extract ONE and THREE, TI workers prefer the first over the second

300   extract ($\chi2$ = 11.439, df = 1, P < 0.05). However, no significance in the level of attraction to TI

301   workers is observed between TWO and THREE ($\chi2$ = 1.434, df = 1, P > 0.05) (Fig. 2A).

302   When provided with similar chances to walk towards the Arabica ONE or the Arabica

303   TWO, MP is attracted to both, but significantly more attracted to the less concentrated extract

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304   ($\chi2$ = 3.258, df = 1, P < 0.05). Similarly, Arabica ONE elicit greater attractiveness to MP

305   workers when presented with Arabica THREE in a two-choice bioassay ($\chi2$ = 17.802, df = 1, P<

306   0.05). However, no significant attraction of MP is found when workers are furnished with even

307   options to choose between two arms containing Arabica TWO and Arabica THREE ($\chi2$ = 1.818,

308   df = 1, P > 0.05) (Fig. 2B).

309   No significant differences in the attraction to SG workers are found between Arabica ONE

310   and TWO ($\chi2$ = 0.804, df = 1, P > 0.05), Arabica TWO and THREE ($\chi2$ = 15.05, df = 1, P <

311   0.05) or Arabica ONE and THREE ($\chi2$ = 0.089, df = 1, P > 0.05) (Fig. 2C).

312

313   **Behavioral responses following Arabica exposure at sugar contents**

314   In the two-choice bioassay comprising the two less sweet Arabica extracts, Arabica S shows

315   greater attractiveness to TI workers ($\chi2$ = 16.39, df = 1, P < 0.05). A similar pattern of preference

316   is observed when comparing Arabica S and water ($\chi2$ = 20.854, df = 1, P < 0.05). A significant

317   attraction of TI workers by water is noticed when they are given a choice between an arm

318   containing Arabica NS and water ($\chi2$ = 11.439, df = 1, P < 0.05) (Fig. 3A).

319   Arabica S extract generated significant attraction in MP workers when in competition with

320   Arabica NS ($\chi2$ = 24.23, df = 1, P < 0.05) and with water ($\chi2$ = 19.289, df = 1, P < 0.05). In the

321   two-choice bioassay involving water and the sweetest Arabica extract, MP workers exhibited

322   greater preference for water ($\chi2$ = 17.802, df = 1, P < 0.05) (Fig. 3B).

323   In the two-choice experiment, both Arabica extracts (S and NS) were attractive to SG, with

324   S extract inducing greater attraction ($\chi2$ = 12.577, df = 1, P < 0.05). A similar attractiveness

325   pattern is seen when the S extract is paired with water ($\chi2$ = 22.5, df = 1, P < 0.05). When given

326   opportunities to select between water and the sweetened extract, MP workers approach both

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327   Arabica infusions, but the latter extract is significantly less attractive than the first one ($\chi2$ =

328   16.39, df = 1, P < 0.05) (Fig. 3C).

329

330   **DISCUSSION**
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332   The most important observation of this study is that coffee influenced the behavior of *Tapinoma*

333   *indicum*, *Monomorium pharaonis* and *Solenopsis geminata*. That influence also showed different

334   patterns according to species, coffee concentration, and sugar content.

335   The fact that coffee elicited attraction to the workers of formicine ants, irrespective of

336   species seems in agreement with an earlier work from Wright et al. (2013), who substantiated the

337   ability of the main coffee ingredient—caffeine to manipulate the olfactory and behavioral

338   responses of hymenopterans. Bees and ants are hymenopterans from the aculeate subclade, a

339   group characterized by an increased level of sociality (Wilson, 1971; Bradley et al., 2009).

340   Besides ecological and behavioral traits, ants and bees are genetically related (Johnson et al.,

341   2013) and have marked similarities in their sensory system and functioning (Gronenberg, 2008).

342   Their antennae are equipped with a variety of types of sensory sensilla, many of which

343   specialized for chemoreceptive functions (Kropf, Kelber, Bieringer, and Rössler, 2014;

344   Barsagade, Tembhare, and Kadu, 2013). In both formicine and honeybees, the olfactory sensilla

345   hold olfactory receptor neurons that are connected to glomeruli, the functional unit of the

346   antennal lobe (Anton and Homberg, 2013; Gronenberg, 2008). This structure is the first relay

347   station for the processing of olfactory information (Zube, et al., 2008). Ants possess over 400

348   glomeruli (Gronenberg, 2008), representing about twice the number of glomeruli in honeybee

349   (Flanagan and Mercer, 1989). Given this, it is tempting to advocate that all the tested ant species

350   have behaviorally responded to the coffee odor because they all possess coffee odorants-specific

351   olfactory receptors. It is possible that the discrepancies in behavioral response intensities

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352 observed between the *T. indicum*, *Monomorium pharaonis,* and *Solenopsis geminata* owe to the

353 differences in glomeruli abundance and/or coffee aroma-mediated olfactory detection capacity.

354 In favor of this insinuation, it has been documented that the number of glomeruli differs across

355 formicine ant species (Goll, 1967; Nishikawa et al., 2008; Zube, et al., 2008). The evidence also

356 exist that demonstrate a close link between glomerulus abundance and smell detection

357 competence (Gronenberg, 2008; Kelber, et al., 2009) as well as the quantity of odors that can be

358 discriminated (Gronenberg, 2008).

359   Within the tube olfactometer, we showed a major effect of coffee concentration on the

360 behavioral responses of the workers of *T. indicum*, *Monomorium pharaonis* and *Solenopsis*

361 *geminata*. Differences in behavioral reaction to chemical cues have often been associated with

362 the concentrations of chemical compounds. Youngsteadt et al. (2007) examined the

363 attractiveness of a blend of five compounds from *Peperomia macrostachya* seeds, one

364 component—geranyl linalool from the same seeds and a solvent (control) on the formicine ant,

365 *Camponotus femoratus*. They found that workers preferred the blend and its attractiveness

366 gradually decreased in the order of geranyl linalool > solvent. The abating attraction level seen

367 when proceeding from blend to geranyl linalool to solvent, distinctly indicates an odor

368 concentration-dependent attractiveness. Coffee aroma is perceived via smelling and

369 the perception of coffee smell is dependent upon both the concentration of the compound and its

370 odor threshold (Coffee Research Org, 2016). There are more than 800 volatile aromatic

371 compounds in coffee (Coffee Research Org, 2016) that occur naturally (Illy and Illy, 2015) and

372 during roasting (Farah, 2012). The flavor and aroma of coffee have been well-documented

373 (Clarke, 1985; Blank, et al., 1991). For instance, damascenone and 3-mercapto- 3-

374 methylbutylformate give a honey-like, fruity and catty odor respectively. 3-Methyl-2-buten-1-

375 thiol and 2-Isobutyl-3-methoxypyrazine are responsible for the amine-like and earthy smells. The

15

376   phenolic, buttery and spicy scents originate from Guaiacol, 2,3-Butanedione and 4-vinylguaiaco

377   accordingly. The methional, vanillin, and furaneol are responsible for the potato-like, vanilla and

378   caramel-like odors. Sotolon and abhexon have a seasoning-like smell.

379   The present choice experiment was conducted with three different Arabica concentrations:

380   0.01%, 0.05%, and 0.10%. For all species, workers tended to prefer 0.01% and 0.05% over

381   0.10%. All these extracts came from Arabica and therefore contain the same chemicals. Hence,

382   the observed differences in the attractiveness level between the low (0.01% and 0.05%) and the

383   highest (0.10%) concentrations may have occurred due to at least two reasons. First, the

384   concentration 0.10% may contain a greater amount of odorous compounds than the two other test

385   concentrations (0.01% and 0.05%), and it is likely that the increased presence of such

386   compounds has produced a strong smell that was repulsive to the workers. A second possible

387   explanation for the discrepancy in the Arabica attraction between the highest and low

388   concentrations could be related to the difference in color stimulus. Optically, the 0.01%

389   concentration had a light brown color, while the 0.10% was dark brown. Formicine ants are

390   known to be capable of differentiating between colors (Camlitepe and Aksoy, 2010) and do not

391   like strong colors (Science Buddies, 2011). It is possible that the dark brown color of the 0.10%

392   Arabica matched the appearance of potential predators i.e., *Anoplolepis gracilipes*—a predator

393   (Konopik, et al., 2014) common in the areas where the experimental workers originated from—

394   and acted as a repulsive signal to workers.

395   There was an obvious association between the presence of sugar in Arabica extract and the

396   behavioral responses of the tested ants. Significant attractions to sugared Arabica were observed

397   for workers of TI, MP and SG when unsweetened Arabica or water was the other option offered

398   to them. Similar observations were reported previously in formicine ants. Dieng et al. (2016), in

399   an experiment, worked with the black crazy ant exposed its workers to four extracts of tea with

16

400   different sugar contents and found a significantly greater preference for two sweetest meals over

401   those without sugar or with the least amount of sugar. In related work, Stanley and Robinson

402   (2007), when dealing with *Paratrechina bourbonica* exposed its workers to five baits and

403   reported sugared solutions among the most attractive baits. Meanwhile, Nyamukondiwa and

404   Addison (2014) examined food preferences of the Argentine ant, the black cocktail ant and

405   *Anoplolepis custodiens* in the wild. They observed that their foragers had a preference for

406   sugared water and honey. To shed light on the macronutrient preference and consumption of the

407   formicine *Camponotus pennsylvanicus*, Cannon (1998) used simple sugars with various levels of

408   sweetness. He reported a preference for sucrose and fructose—known to be sweeter than glucose

409   (WordPress.com, 2009). In the sugar effect study, three extracts were used: Arabica with 0.50%

410   sugar or "S", Arabica without sugar or "NS" and water (Control or C). The extract "S" was

411   generated by dissolving 0.25 g of sugar (also known as table sugar or sucrose (WordPress.com,

412   2009) in 50 mL of a 0.01% Arabica solution. Generally, formicine ants including TI (*Tapinoma*

413   *indicum)* (Lee, 2008), MP (*Monomorium pharaonis*) (Sorensen and Vinson, 1981) are naturally

414   highly active (Harris, de Ibarra, Graham, and Collett, 2005). For most social insects including

415   formicine ants, sugar is a ready-to-use energy source (Chong, et al., 2002). In most ant species,

416   sugars have positive effects, supplying energy for various activities (Kay, et al., 2004). Based on

417   these reports, the observed strong attraction of sugar to TI, MP and SG foragers may have been

418   due to the need of energy supply for their foragers. In support of this, it has been reported that

419   active ant workers are constantly in an energy deficient state (Sorensen and Vinson, 1981).

420   Besides giving insight into the behavioral ecology of three different formicine species, the

421   results of the current study illustrated the potential of coffee, especially Arabica, as a valuable

422   source in baiting strategy against ant pests.

423

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427

428   **References**

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Fig. 1 Responses of *T. indicum* (TI) (**A**), *M. pharaonis* (MP) (**B**), and *S. geminata* (SG) (**C**) in a Y-tube olfactometer where workers were given a choice between different binary combinations of odor stimuli from different coffee species. Positive bars indicate preference for the first extract. Negative bars indicate preference for the second extract. The asterisk over bars (∗) stands for significant difference ($P < 0.05$) within a dual choice test based on Chi square test.
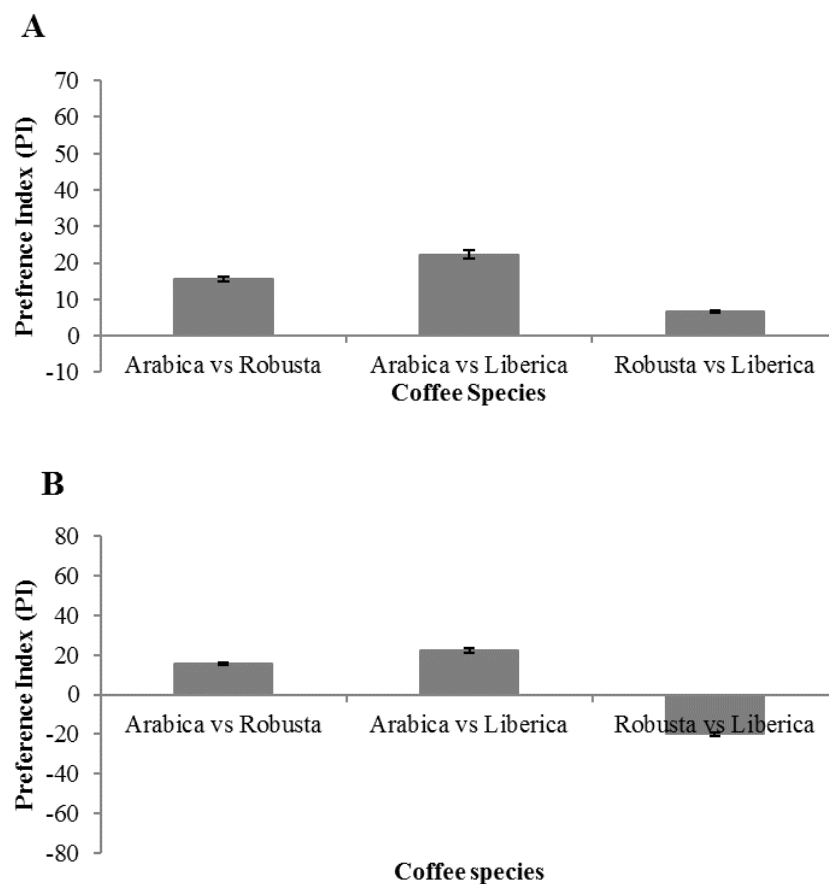
Fig. 2. Responses of *T. indicum* (TI) (**A**), *M. pharaonis* (MP) (**B**), and *S. geminata* (SG) (**C**) in a Y-tube olfactometer where workers were given choices involving different binary combinations differently concentrated Arabica extracts. Positive bars indicate preference for the first extract. Negative bars indicate preference for the second extract. The asterisk over bars (∗) stands for significant difference ($P < 0.05$) within a two-choice test based on Chi square test. Arabica extract ONE (0.01%), Arabica extracts TWO (0.05%) and THREE (0.10%) respectively.
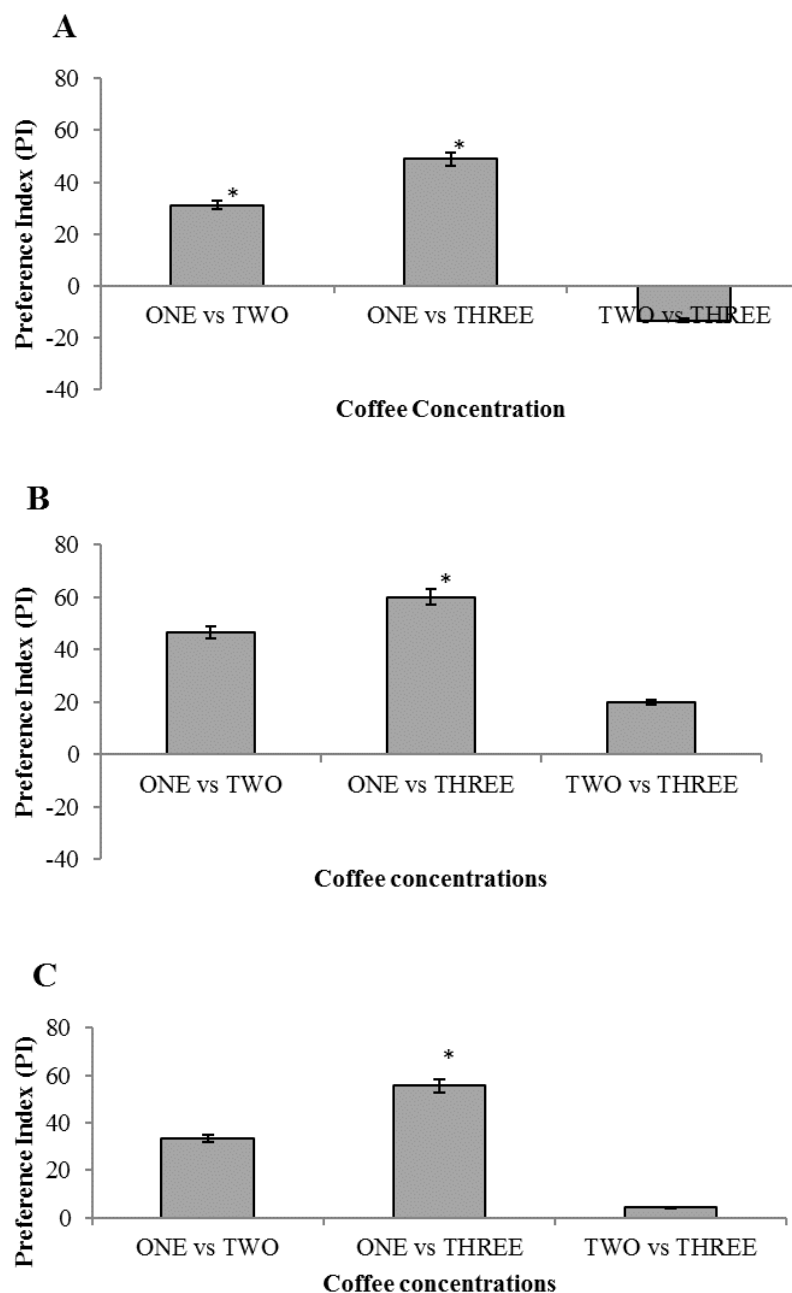
Fig. 3 Responses of *T. indicum* (TI) (**A**), *M. pharaonis* (MP) (**B**), and *S. geminata* (SG) (**C**) in a Y-tube olfactometer where workers were given choices involving different binary combinations Arabica extracts with different sugar contents. Positive bars indicate preference for the first extract. Negative bars indicate preference for the second extract. The asterisk over bars (*) stands for significant difference ($P < 0.05$) within a two-choice test based on Chi square test. S referred Arabica extract with sugar (0.50% of sugar); NS referred to Arabica extract without sugar; C referred to Cool boiled water acted as a control