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Effects of a gel bait on the suppression of noxious *Crematogaster rogenhoferi* Mayr, 1879 (Formicidae: Myrmicinae) in Sri Lanka

Воздействие гелевой приманки на ядовитых муравьев *Crematogaster rogenhoferi* Mayr, 1879 (Formicidae: Myrmicinae) в Шри-Ланке

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Ключевые слова: опасные муравьи-акробаты, контроль численности, процент смертности, гнездовые деревья.

Abstract. *Crematogaster rogenhoferi* Mayr, 1879 in a home garden in Sri Lanka was reported to cause health hazards and an investigation was conducted from February to November in 2016 to list the tree species bearing its nests and to record the nest structure, nest intensity and colony demography for the evaluation of its level of infestation and to recommend a method for the suppression of its population size. Three types of nests on 19 tree species were recognized. The largest nest had all life stages. The standard laboratory experiment conducted with the provision of 0.25 g of Optigard Ant Gel bait (OAG) showed 68% of mean mortality of workers. A field experiment conducted by applying separate 1 cm band of OAG and honey at 10 cm and 1 cm distance from three initial stage nests and a honey band only at 1 cm distance from three control nests recorded a significantly higher 89.2% of mean mortality of workers. Two mature nests treated with OAG similarly while two other similar nests served as simultaneous control nests resulted significantly higher 97.5% of mean mortality of workers and queens in OAG-treated nests, after 24 hours. Hence, application of 1 cm band of OAG on the tree trunk, if essential, is recommended as a method for suppressing the population size of *C. rogenhoferi* in a home garden.

Резюме. После сообщений о том, что *Crematogaster rogenhoferi* Mayr, 1879 в домашнем саду в Шри-Ланке представляет опасность для здоровья людей, с февраля по ноябрь 2016 года было проведено исследование с целью составления списка видов деревьев, на которых муравьи строят гнезда, изучения плотности гнезд на территории, их структуры, а также демографии колоний для оценки локальной популяции и рекомендации метода подавления численности муравьев. Было выявлено 3 типа гнезд на 19 видах деревьев. В самом большом гнезде присутствовали все жизненные стадии муравьев. Стандартный лабораторный эксперимент с использованием 0,25 г гелевой приманки Optigard Ant Gel

показал 68% средней смертности рабочих особей. При проведении полевого эксперимента с применением полосок геля шириной 1 см на расстоянии 10 см и меда на расстоянии 1 см от трех гнезд начальной стадии и только лишь полосок меда на расстоянии 1 см от трех контрольных гнезд зафиксирована значительно более высокая средняя смертность рабочих особей – 89,2%. В двух полностью сформированных гнездах, обработанных Optigard Ant Gel аналогичным образом, средняя смертность рабочих и королев через 24 часа составила 97,5%. Применение полоски геля Optigard Ant Gel шириной в 1 см на стволе дерева рекомендуется в качестве метода подавления численности *C. rogenhoferi* в домашних садах.

Introduction

The species-rich ant genus *Crematogaster* Lund, 1831 has a global distribution throughout warm-temperate to subtropical and tropical climates. Arboreal species of *Crematogaster* can be dominant elements, with polydomous and strongly territorial colonies that live in carton nests. They possess exceptional dispersal and colonization abilities [Blaimer, 2012a]. Noxious bites followed by spraying of venom of *Crematogaster* workers may cause allergic reactions and are associated with local pathophysiological events, characterized by pain, swelling and redness at the human body part for about 1–2 days [Kang et al., 2004; Hoffman, 2010; Dias, 2011; Rifflet et al., 2011; Chantarasawat et al., 2013]. Their gaster is brought high up over the rest of the mesosoma upon disturbance, either as a defensive or aggressive gesture but the sting is of spatulate form, which is used to spray venom by contact rather than by injection [Blaimer 2012a, b]. Dufour gland secretions (venom) of several *Crematogaster* species consist of long chain conjugated dienones, furan, and trihydroxylated cyclohexane derivatives, as well as diterpenes. In some

Crematogaster species, formation of highly toxic aldehydes during the emission of venom has been reported [Rifflet et al., 2011].

Crematogaster is characterized by the unique post-petiole articulation on the dorsal surface of the gaster, the square-shaped head, 11-segmented antennae, strong, thick mandibles and a very narrow sting at the apex of the gaster [Bingham, 1903; Bolton, 1992; Hosoishi, Ogata, 2009; Blaimer, 2010, 2012c]. Among the 13 species of the genus recorded from Sri Lanka [Dias, 2014] *C. rogenhoferi* Mayr, 1879 is characterized by the presence of angular margins on the broadly flared pronotum, a bilobed post-petiole having a median longitudinal impression and the elongated gaster [Bingham, 1903]. A home garden in Pahala Imbulgoda, Gampaha District in Sri Lanka was heavily infested with *C. rogenhoferi* in 2016 and the workers caused human health problems through their noxious bites and venom [Udeshika, 2016]. We report here the tree species in the home garden that had *C. rogenhoferi* nests and structure and colony demography of differently sized nests of the species. Many insecticide formulations are not suitable for suppressing arboreal ant populations but baits formulated as pastes or gels can be applied to tree trunks, branches and vegetation to control them while minimizing hazards to the environment [Vanderwoude, Nadeau, 2009]. Optigard Ant Gel bait (OAG; active ingredient: Thiamethoxam) is a highly palatable, non-repellent ant bait and transfers within ant colonies through the attending workers, resulting in control of workers, brood and queens (http://www.syngenta-us.com/images/resource_pages/pmp/optigard-ant-gel-tech-bulletin.pdf). However, effects of OAG on the worker and queen of arboreal *Crematogaster* species are not reported. Hence, the current investigation was conducted (a) to describe the structure and colony demography of nests, (b) to record the responses including percentage mortality of *C. rogenhoferi* workers to Optigard Ant Gel bait in the laboratory and (c) to calculate percentage mortality of workers and the queen caused by OAG in the field.

Material and methods

Description of the locality and the field survey on *C. rogenhoferi* population. A home garden of 385 m² consisting of many plant species that formed a canopy (names in results section), was situated at 7°04'18" N / 79°98'75.67" E, Pahala-Imbulgoda of Gampaha District in Sri Lanka. Floor of the land was covered with a thick leaf litter layer. The land was surveyed for the nests of the species while walking and recording preliminary visual observations on the presence of nests and their microhabitats with the naked eye and using a pair of binoculars in February 2016. Scientific names of the plant species that had the nests were listed with reference to The Plant List [2013].

Investigation on the structure and colony demography of nests. Based on the preliminary observations recorded in the field and laboratory, three types of nests were recognized according to the level of completion and the life stages seen in each nest. Initial stage nests (SN) were smaller with loosely woven leaves and the workers as only members whereas intermediate

stage nests (MN) were larger than SN but partly complete with the workers and dealate queens only as colony members. The fully-formed nests (LN) were the largest, globular and complete nests with all life stages. Six SN, two MN and two LN were detached carefully from the host plants, placed in polythene bags of appropriate size and brought to the laboratory. Length and width of each nest was measured and the structure was described. Colony demography of each type of nest was recorded by opening the nest carefully and preserving all life stages quickly in 70% ethanol. Number of individuals of each life stage including eggs seen in each nest was counted with the naked eye or using a low power stereo-microscope. Mean percentage abundance of each life stage in each type of nest was calculated (Mean percentage abundance = (No. of individuals of a particular life stage / total No. of individuals in all nests) × 100) / No. of nests).

Preliminary laboratory experiments for recording responses of workers. A band of vaseline was applied along the open edge of ten, 100 ml Petri dishes and the experimental workers of the species were kept for 7 days in them with the provision of sufficient honey and water for acclimation to the laboratory conditions. On 12th March in 2016, six 100 ml Petri dishes with a band of vaseline applied along its open edge were set as three control and three experimental units. Two pieces of cotton wool each wetted with water or honey and a small piece of original nest were placed in each Petri dish. A 0.25 g drop of OAG from the syringe was added to each experimental unit. Thirty workers acclimated to laboratory conditions were carefully transferred to each of the control and experimental units. After 1 hour, each set of units was observed every 10 minutes and behaviour of the workers was recorded for a half an hour. After 7 hours (at the end of day time), total number of dead workers observed in each control and experimental units was recorded and mean mortality (%) of workers observed in the control and experimental units was calculated (= (No. of dead workers in each category / Total No. of workers (= 90)) × 100) / 3). Any significant difference between mean percentage mortality (Arcsine transformed proportions) of the workers in the control and OAG-treated units was analyzed using two sample t-test in Minitab 14.0.

Field experiment for the calculation of mortality (%) of OAG-fed workers in Initial stage nests. Six nests at reachable heights were located for the field trial in June, 2016. To prevent the movement of workers from any other nest or along other branches of the tree, a thick wax band was applied on all such branches. On the trunk that was without wax application, 1 cm band of honey as a food source was applied at 1 cm distance from all the nests. One cm wide band of OAG was also applied on the same trunk at a 10 cm distance from each of the three experimental nests, external to the honey band. Three nests on the branches without ant gel bait band served as the control nests. Each experimental and control nest was fully-covered with a perforated, very large polythene bag for collecting dead ants if fallen from each nest and to prevent the effects of raining. After 24 hours, each nest was severed carefully, enclosed in a polythene bag of appropriate size while minimizing the escape of nest members and brought to the laboratory. Each nest was placed in a separate basin containing a 0.05 m band of wax



Figs 1–4. Nests of *Crematogaster rogenhoferi*.

1 – small nest (SN); 2 – intermediate nest (MN); 3 – fully-formed whole nest (LN); 4 – the leaf folds inside the nest.

Рис. 1–4. Гнезда *Crematogaster rogenhoferi*.

1 – маленькое гнездо (SN); 2 – среднее гнездо (MN); 3 – полностью сформированное гнездо (LN); 4 – сложенные листья расположены в складчатом виде и образуют внутренние камеры.

along its top, inner edge. From each nest, live workers were collected immediately into a bottle containing 70% ethanol while dead workers were preserved next in 70% ethanol in a separate bottle with appropriate labels. Number of live and dead workers was recorded and percentage mortality of workers observed in each nest was calculated ((No. of dead workers/ Total No. of workers) \times 100) and the values of mean mortality (%) of workers in the control and treated nests were estimated ($= (\Sigma \text{percentage mortality observed in each control or OAG treated nest}) / 3$). Any significant difference between the mean mortality (%) (Arcsine transformed proportions) observed in the control and OAG-treated nests was analyzed using two sample t-test in Minitab 14.0.

Field experiment for the calculation of mortality (%) of OAG-fed life stages in mature nests. Nests with the workers and dealate queens were considered as mature nests in this experiment. Four mature nests at reachable heights could only be located; two nests served as the control and the other two nests were treated with OAG as described in the previous section. Mortality (%) of worker and queen in each control and OAG-treated nest was calculated according to the procedure described previously under the initial stage nests. Any significant difference between the mean percentage mortality (Arcsine transformed proportions) observed in the two control and two OAG-treated mature nests was analyzed using two sample t-test in Minitab 14.0.

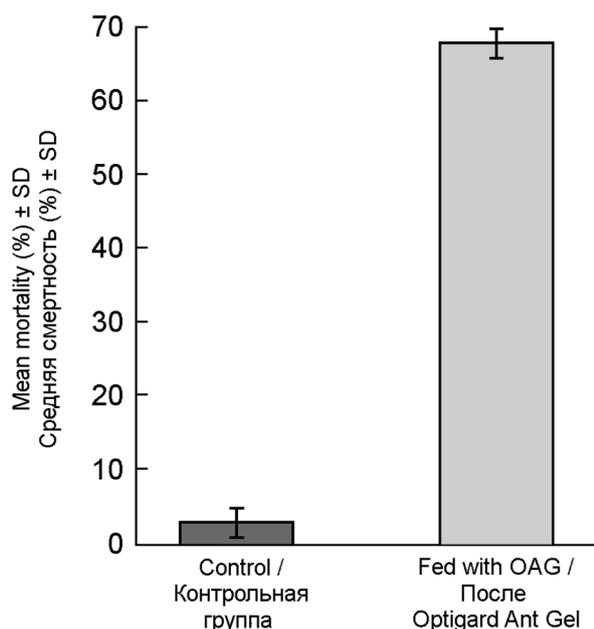


Fig. 5. Mean percentage mortality of workers observed in Optigard Ant Gel bait-treated units ($n = 30$) and the control units ($n = 30$) at the end of preliminary laboratory experiment.

Рис. 5. Средняя смертность (%) рабочих особей после воздействия Optigard Ant Gel ($n = 30$) и контрольной группы ($n = 30$) в конце предварительного лабораторного эксперимента.

Results

Preliminary observations on the nest occurrence and medical importance of *C. rogenhoferi*.

Crematogaster rogenhoferi nests hanged from forks of 19 tree species, *Artocarpus heterophyllus* Lam. (jackfruit), *Averrhoa bilimbi* L. (bilimbi), *A. carambola* L. (star fruit), *Azadirachta indica* A. Juss. (neem), *Cinnamomum verum* J. Presl. (cinnamon), *Citrus aurantiifolia* (Christm.) Swingle (lime), *C. sinensis* (L.) Osbeck (orange), *Cocos nucifera* L. (coconut), *Elaeocarpus serratus* L.

Table 1. Percentage mortality of *C. rogenhoferi* workers observed in the control initial stage nests and those treated with OAG in the field experiment.

Таблица 1. Процент смертности рабочих особей *C. rogenhoferi*, наблюдаемых в контрольных и обработанных Optigard Ant Gel гнездах начальной стадии (SN) в полевом эксперименте.

Nest Гнездо	Mortality, % Смертность, %	Mean mortality, % / Средняя смертность, %	N
Control 1	3.9	4.67 ^a	375
Control 2	2.0		298
Control 3	8.0		115
Experimental 1	85.5	89.27 ^b	265
Experimental 2	90.2		221
Experimental 3	92.1		128

Note. N – total number of workers observed in each nest. Superscripts indicate significantly different values ($p < 0.05$).

Примечание. N – общее количество рабочих в каждом гнезде. Верхние индексы указывают достоверное различие ($p < 0.05$).

(Ceylon olive), *Lansium parasiticum* (Osbeck) K.C. Sahni et Bennet (langsat), *Limonia acidissima* Groff (wood-apple), *Mangifera indica* L. (mango), *Musa balbisiana* Colla (banana), *Myristica fragrans* Houtt (nutmeg), *Persea americana* Mill (avocado), *Phyllanthus acidus* (L.) Skeels (gooseberry), *Ph. emblica* L. (Indian gooseberry), *Swietenia mahagoni* (L.) Jacq. (mahogany) and *Tectona grandis* L. f. (teak). Many workers occupied small cavities and crevices in the tree trunks and spaces in the thick leaf litter layer on the ground. They invaded the home while foraging and sometimes bit the occupants and visitors aggressively causing allergic reactions. Plucking of flowers and fruits in the garden was impossible due to the biting habit of workers. People who entered the premises or tried to pluck fruits or flowers developed health hazards such as painful itching and related symptoms.

Structure of each nest type and colony demography.

The SN nests (Fig. 1) were observed among the tree forks and mean length and mean width of SN ranged from 5–9 cm and 4.7–9 cm, respectively. Green leaves had been bent occasionally to form the nest and a black, hard paste and a clay-like material covered the green leaves. Number of workers being the only members (100%) ranged from 115 to 375. MN nests (Fig. 2) ranged from 6–11 cm in mean length and 8–15 cm in mean width. A black hard paste and clay well-covered the nest externally. Inside the nest, brownish leaves had a pleated arrangement. Those had more than 2000 workers (98.5%) and several dealate queens (1.5%). The LN nests (Fig. 3) were of elongated globular shape with 36–45 cm mean length and 20–24 cm mean width. Many brownish leaves covered with hard black paste were seen outside and inside of LN and the folded leaves arranged in pleated manner formed inner chambers (Fig. 4). Colonies in LN nests consisted of the workers (90.7%), dealate queens (0.07%), eggs (1.4%), larvae (3.1%) and pupae (4.7%) while total number of life stages observed in each LN ranged from 13,385 to 18,134.

Mean mortality (%) of workers caused by OAG bait.

Each worker that reached the OAG carried a small amount of the gel to the piece of nest. In the laboratory experiment, workers provided with OAG showed a higher significant ($p < 0.05$) mean percentage mortality than that observed in the control group (Fig. 5). In the field experiment, initial stage nests provided with OAG resulted a significantly higher ($p < 0.05$) percentage mortality of the workers (Table 1) than that observed in the control nests. Also, mature nests treated with OAG showed significantly higher mortality ($p < 0.05$) of the adult life stages, the workers and dealate queens (Table 2), than that observed in each corresponding control nest.

Discussion

Arboreal nests of *C. rogenhoferi* have been reported from the forest trees in other countries [Watanasit, Jantarit, 2006; Chavhan, Pawar, 2011; Langshiang, Hajong, 2018]. The presence of its nests on *Artocarpus heterophyllus*, *Averrhoa bilimbi*, *A. carambola*, *Azadirachta indica*, *Cinnamomum verum*, *Citrus aurantiifolia*, *C. sinensis*, *Cocos nucifera*, *Elaeocarpus serratus*, *Lansium parasiticum*, *Limonia acidissima*, *Mangifera indica*,

Musa balbisiana, *Myristica fragrans*, *Persea americana*, *Phyllanthus acidus*, *P. emblica*, *Swietenia mahagoni* and *Tectona grandis* trees was recorded for the first time in Sri Lanka. Variations in size, structure and colony demography of initial stage nests, intermediate stage nests and fully-formed nests of the species was described for the first time and is very useful in locating its nests in future studies. Colour images of the initial, intermediate and large nests of the species provided here will be very useful as the clues for the quick identification of the presence of the species in any habitat, even without any knowledge on the identification of the ant species. Findings also revealed that SN built by workers were occupied later by dealate queens (polygynous colony) for the oviposition because workers and dealate queens were only observed in intermediate nests. It seemed that an intermediate stage nest was expanded gradually to a large nest because oviposition by queens produced progeny continuously, which demanded more chambers for the new members of the colony. Nest materials included leaves covered with a black paste, plant fibre and clay although Langshiang and Hajong [2018] revealed the presence of an additional material, the fungal hyphae. Watanasit and Jantarit [2006] reported that a large number of winged females occupied the nests of the species and total population of each nest varied from 1520 to 67755 individuals in a National park in Thailand. Few queens and many workers but winged females were observed in a mature nest and total population size (115 to 18134 individuals) was smaller in the nests collected from the current home garden.

Laboratory experiment was conducted to record the preliminary observations so that it ended after 7 hours, at the end of the day time and showed that workers carried the gel to the nest and a higher percentage died only in the treated units. Significantly higher proportions of experimental workers in the laboratory experiment and the colony members of the species died after consumption of OAG in the field experiments, most probably, due to the action of thiamethoxam on nicotinic acetylcholine receptors in their nervous system (http://www.syngenta-us.com/images/resource_pages/pmp/optigard-ant-gel-tech-bulletin.pdf). Very small amount of gel bait carried to the nest by workers should have been fed to the queens; perhaps the trophallactic activity also contributed to the death of the queens. Perforated polythene bags used to cover the nests were large enough to provide ventilation and a very small percentage died (perhaps due to natural reasons) in the control nests although covered with such bags. Optigard Ant Gel bait was tested for *C. rogenhoferi* for the first time in Sri Lanka and a considerable percentage mortality of both workers and queens observed in the field clearly indicated that it can be used in the suppression of the population size. Dealate fertile queens lay eggs continuously and the colony size is expanded so that killing of the queens is essentially required for the suppression of the population size. In addition, removal of leaf litter from the ground and reduction of tree canopies to allow more sunshine to the home garden that confine workers to the nests (personal observation) are also essential to remove temporary microhabitats of the species. Those practices together with repeated Optigard Ant Gel bait application are recommended for the elimination of *C. rogenhoferi* from the home garden.

Table 2. Percentage mortality of the adult caste, both workers and queen, observed in the control and OAG treated mature nests of *C. rogenhoferi* in the field experiment.

Таблица 2. Процент смертности имаго (как рабочих особей, так и королевы) наблюдаемой в контрольных и обработанных Optigard Ant Gel полностью сформированных гнездах *C. rogenhoferi* в полевом эксперименте.

Nest Гнездо	Mortality, % Смертность, %	Mean mortality, % / Средняя смертность, %	N
Control 1	1.7	2.3 ^a	2049
Control 2	2.9		11857
Experimental 1	96.1	97.5 ^b	2134
Experimental 2	98.9		16822

Note. N – total number of workers observed in each nest. Superscripts indicate significantly different values ($p < 0.05$).

Примечание. N – общее количество рабочих в каждом гнезде. Верхние индексы указывают достоверное различие ($p < 0.05$).

Conclusion

Arboreal acrobat-ant, *C. rogenhoferi*, built smaller (initial stage nests) to larger nests (fully-formed nest) on 19 tree species. Nests were of different levels of completion and had workers only or workers and queens or all life stages except the winged forms. Optigard Ant Gel bait tested first time for the species caused 68% of mean mortality of the workers in the laboratory and 97.5% mean mortality of workers and queens in the field can be recommended for the suppression of its population size.

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