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Host tree species, nest information and the management of an outbreak of medically important *Tetraponera rufonigra* (Jerdon, 1851) (Hymenoptera: Formicidae: Pseudomyrmecinae) using citronella oil or kerosene

Виды заселяемых деревьев, информация о гнездах и методах контроля вспышек численности медицински значимого муравья *Tetraponera rufonigra* (Jerdon, 1851) (Hymenoptera: Formicidae: Pseudomyrmecinae) с использованием масла цитронеллы или керосина

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Key words: Hymenoptera, Formicidae, Pseudomyrmecinae, plant-ant association, nest recognition, medically important formicids.
Ключевые слова: Hymenoptera, Formicidae, Pseudomyrmecinae, связь муравьев и растений, распознавание гнезд, медицински значимые муравьи.

Abstract. Human beings stung instantly by the workers of *Tetraponera rufonigra* need treatments for severe medical conditions so that recognition of nests and host trees and methods for temporary suppression of workers, for the prevention of such health hazards are essentially required. Several urban localities in Sri Lanka were surveyed from March to August, 2015 and nests of the species were observed on Acacia catechu Willd, Casuarina equisetifolia L., Albizia lebbeck (L.) Benth, Cassia fistula L., Adenanthera pavonina L., Filicium decipiens (Wight & Arn.) Thwaites, Pericopsis mooniana Thwaites, Plumeria sp. and Tectona grandis L.f. Galleries in the dead branches of the host trees mainly provided physical space for the nests. Structure of three major types of nests is described with images.

Appropriate toxicity experiments conducted in the laboratory found that LD_{99} of citronella oil was 6.39 mg / mg body weight for the workers. Field simulating experiments conducted with increasing volumes of kerosene or citronella oil showed that 3.0 ml of kerosene or 5.2 ml of citronella oil can be recommended as minimum spray volumes for the temporary suppression of the workers when sudden outbreaks occur due to the felling of host trees. Current findings are valuable for the prevention of such health hazards in the future.

Резюме. Люди, ужаленные рабочими особями муравья *Tetraponera rufonigra*, при тяжелых состояниях нуждаются в лечении. Для профилактики нападения муравьев и предотвращения этой опасности требуется распознавание гнезд этих муравьев и заселяемых ими деревьев, а также разработка методов временного подавления численности рабочих особей. С марта по август 2015 года нами обследовано несколько городских районов в Шри-Ланке, были обнаружены гнезда *Tetraponera rufonigra* на Acacia catechu Willd, Casuarina equisetifolia L., Albizia lebbeck (L.) Benth, Cassia fistula L., Adenanthera pavonina L., Filicium decipiens (Wight & Arn.) Thwaites, Pericopsis mooniana Thwaites, Plumeria sp. и Tectona grandis L.f. Ходы в отмерших ветвях деревьев обеспечивали пространство для гнезд. Даны изображения структуры трех основных типов гнезд.

В ходе лабораторных экспериментов было выявлено, что смертельная концентрация LD_{99} масла цитронеллы составляет 6.39 мг для рабочих особей. Полевые исследования, проведенные с увеличением объемов керосина или масла цитронеллы, показали, что 3 мл керосина или 5.2 мл цитронеллового масла можно рекомендовать как минимальные объемы распыления для временного подавления количества рабочих особей при внезапных вспышках численности, происходящих из-за вырубки населяемых ими деревьев.

Introduction

Members of the subfamily Pseudomyrmecinae (Hymenoptera: Formicidae) are found throughout the Old and New World tropics. The Old World species belonging to the genus *Tetraponera* F. Smith, 1852 contain approximately 110 nominal species or subspecies [Ward, 1990, 2001; Bolton, 1995]. Species of *Tetraponera*

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are known to inhabit various plant species in several countries of the world including Sri Lanka [Bingham, 1903; Buschinger et al., 1994; Djieto-Lordon, Dejean, 1999; Palmer et al., 2000; Ward, 2001; van Borm et al., 2002; Bharti, Aliakbar, 2014]. *Tetraponera rufonigra* (Jerdon, 1851) is widely distributed in the forests and urban parks throughout the Indian subcontinent [Ward, 2001]. Nests of the species in wooden posts or openings in wooden beams with anterior designs [Bingham, 1903] and in the cavities of dead branches or live wood of Xylia dolabriformis and Sonneratia ovata from Singapore, Santalum album, Ficus aculeata and Caesalpinia crista from India have been reported [Bingham, 1903; Ward, 2001; Meshram et al., 2015]. *Tetraponera rufonigra*-induced hypersensitivity after its stings has been reported and may cause severe anaphylaxis in human beings [Wanotayan et al., 2005; Ratnatilake et al., 2011; Potiwat, Sitcharungsi, 2015]. Workers of *T. rufonigra* have been reported from many localities in Sri Lanka [Ward, 2001; Dias, Kosgamage, 2012; Dias, 2014] but its unknown host trees in urban localities, nest appearance and the management of workers in sudden outbreaks are of special interest due to the medical importance of the species. Citronella oil has properties of a biopesticide [Wijesekara, 1973; Kosgamage, 2011] and kerosene is used traditionally to kill nuisance ants in Sri Lanka. This paper reports here the host tree species of *T. rufonigra* and describes main types of its nests observed in several urban localities in Sri Lanka. Also, LD_{99} of citronella oil and minimum volumes of citronella oil and kerosene that should be sprayed to kill a group of workers of the species are reported for the management of its sudden outbreaks that occur due to fallen host trees.

Material and methods

We surveyed the host tree species of *T. rufonigra* throughout the Grounds of Royal Colombo Golf Club, Colombo 8 (6°54′17.45″N / 79°52′55.98″E), along the Tyre Cooperation road, Kelaniya (6°58′26.13″N / 79°54′57.53″E) and Vihara Mahadevi Park, Colombo 07 (6°54′49.15″N / 79°51′40.26″E) during March to August in 2015. Visual search for the presence of *T. rufonigra* workers on the tree trunks was conducted for hours at each locality. A small amount of ground anchovies was placed on the trees and at the tree bases and checked for the presence of workers in 10 minute intervals. Based on the presence of workers on the tree trunk *T. rufonigra* nests on the upper part of each tree were located with the help of a tree climber. Nests of *T. rufonigra* were severed from the host tree, using a knife or a chain-saw. We recorded and photographed the external appearance of each nest and the appearance of a vertical and a cross section of the cut tree branch containing the nest, where possible and measured the diameter and length of each cut branch using a ruler.

Table 1. Frequency of citronella oil spraying using the calibrated sprayer, number of *T. rufonigra* worker ants tested and number of replicates in the field simulating experiment.

Таблица 1. Количество нажатий на распылитель калиброванного опрыскивателя с использованием масла цитронеллы, количество тестируемых рабочих особей *T. rufonigra* и число опрыскиваний в ходе полевых исследований.

Frequency of sprayings / Количество нажатий на распылитель	Approximate spray volume (ml) / Приблизительный объем распыленного масла (мл)	No. of worker ants / Количество рабочих особей	No. of replicates / Число повторов опрыскивания
1	0.64	10	3
2	1.28	10	3
4	2.56	10	3
6	3.84	10	3
8	5.12	10	3

Table 2. Frequency of kerosene spraying with the calibrated sprayer bottle, number of *T. rufonigra* worker ants tested and number of replicates in the field simulating experiment.

Таблица 2. Количество нажатий на распылитель калиброванного опрыскивателя с использованием керосина, количество тестируемых рабочих особей *T. rufonigra* и число опрыскиваний керосином в ходе полевых исследований.

Frequency of sprayings / Количество нажатий на распылитель	Approximate spray volume (ml) / Приблизительный объем распыленного керосина (мл)	No. of workers in a test group / Количество рабочих особей	No. of replicates / Число повторов опрыскивания
1	0.6	10	3
2	1.2	10	3
3	1.8	10	3
4	2.4	10	3
5	3	10	3

Laboratory experiments using citronella oil or kerosene. A range finding test was conducted in three replicates using five workers of *T. rufonigra* collected from a laboratory-maintained colony and acclimated for 1 hour in closed Petri-dishes with a drop of water and a pinch of ground anchovies. Each volume, 5 µl, 10 µl, 15 µl and 20 µl of citronella oil (100%) was applied using a 10–100 µl micro-pipette to the dorsal surface of prothorax of each acclimated worker. A control experiment was also maintained simultaneously without applying citronella oil to the workers. After 10 minutes of citronella oil application, number of dead workers observed out of five workers in each treatment was recorded. Range of volumes of citronella oil for the next experiment, 10–20 µl, was determined from the results of the range finding test.

In the toxicity experiment, each volume, 10 µl, 12 µl, 14 µl, 16 µl, 18 µl and 20 µl, of citronella oil (100%) was applied separately to the dorsal surface of the prothorax of ten acclimated worker ants used in each treatment. Each application was conducted in three replicates with a simultaneous control experiment. After 10 minutes of citronella oil application, number of dead ants observed with each treatment was recorded. Percentage mortality of *T. rufonigra* workers (= (number of dead ants after

Table 3. Number of *T. rufonigra* host tree species observed at each locality.
Таблица 3. Количество деревьев, заселяемых *T. rufonigra*, осмотренных в каждом из районов исследования.

№	Tree species Вид дерева	No. of trees Количество деревьев	Locality Район исследования
1	Acacia catechu Willd	6	Colombo Royal Golf ground
2	Casuarina equisetifolia L.	5	Colombo Royal Golf ground
3	Albizia lebbeck (L.) Benth	5	Colombo Royal Golf ground,
		1	Tyre Cooperation Road, Kelaniya
4	Cassia fistula L.	2	Vihara Mahadevi Park
5	Adenanthera pavonina L.	3	Vihara Mahadevi Park
6	Filicium decipiens (Wight & Arn.) Thwaites	4	Vihara Mahadevi Park
7	Pericopsis mooniana Thwaites	1	Vihara Mahadevi Park
8	Plumeria sp.	3	Vihara Mahadevi Park
9	Tectona grandis L.f.	4	Vihara Mahadevi Park

10 minutes / 10) × 100) in each treatment and the mean value from three replicates of each treatment were calculated. Probit analysis was conducted in Minitab 14 to determine LD_{99} value of citronella oil for *T. rufonigra* workers.

Similarly, a test was conducted to determine the range of volumes of kerosene but workers instantly died at the rate of 1 µl application.

Field simulating experiment with citronella oil. A citronella oil (100%) sprayer bottle purchased from the market was calibrated and used to apply citronella oil to the workers. To determine the volume of liquid released in one spray (calibration), the sprayer bottle was fully-pressed for five times and citronella oil sprayed was collected in to a 5 ml measuring cylinder; volume of citronella oil collected in the measuring cylinder was recorded (V1). From three such measurements (V1, V2 and V3), mean spray volume of the sprayer bottle per a single spray [(V1 + V2 + V3) / (3 × 5)] was calculated.

Table 1 presents the frequency of spraying, volume of citronella oil applied, number of test workers in each trial and the number of replicates conducted with each volume used in the experiment. Ten worker ants were placed in a transparent plastic bottle with sufficient amount of ground anchovies and a piece of cotton wool soaked in water; mouth of the bottle was covered and the group of workers were acclimatized for 30 minutes (room temperature 28 °C; relative humidity 85%). After spraying each volume of citronella oil into the bottle containing 10 workers the mouth of the bottle was covered with a polythene sheet. A control group of 10 workers for each volume of citronella oil was maintained in a separate plastic bottle with the provision of similar conditions.

The survival time (s) of 10 workers in a group since spraying of each volume of citronella oil was recorded and the mean survival time per 10 workers was calculated. One Way ANOVA followed by Tukey's pairwise comparisons were performed using Minitab 14 to compare the mean time period required by each volume of citronella oil to kill ten *T. rufonigra* workers in a test group.

Field simulating experiment with kerosene and a group of workers. A commercially available 1 L sprayer bottle was calibrated using the water. To determine the volume of liquid released in one spray, water was added to the sprayer bottle, fully-pressed for five times and the volume of water sprayed and collected into a 5 ml measuring cylinder was recorded (V1). From three such measurements (V1, V2 and V3), mean spray volume of the sprayer bottle per a single spray [(V1 + V2 + V3) / (3 × 5)] was calculated. The sprayer bottle was filled with kerosene and an experiment similar to that conducted with citronella oil was carried out. Table 2 presents the frequency of spraying, volume of kerosene applied, number of test workers in each trial and the number of replicates conducted with each volume used in the experiment.

The survival time (s) of 10 workers since spraying of each volume of kerosene was recorded and the mean survival time of 10 workers was calculated. One Way ANOVA followed by Tukey's pairwise comparisons were performed using Minitab 14 to compare the mean time period required by each volume of kerosene to kill ten *T. rufonigra* workers in a test group.

Table 4. Main types and the size of *T. rufonigra* nests observed in the dead branches of Acacia catechu, Albizia lebbeck and Plumeria sp.
Таблица 4. Основные типы и размеры гнезд *T. rufonigra*, наблюдаемых в отмерших стволах Acacia catechu, Albizia lebbeck и Plumeria sp.

Nest type Тип гнезда	Tree species Вид дерева	Diameter of the log, cm Диаметр ствола, см	Length of the cut log, cm Длина отрезанного ствола
Type 1 / Тип 1	Acacia catechu Albizia lebbeck	8.4 5.3	25.8 31.9
Type 2 / Тип 2	Acacia catechu Albizia lebbeck	27.5 32.3	36.3 14.7
Type 3 / Тип 3	Plumeria sp.	6.3	40.1

Regression Analysis was also conducted between volume of citronella oil or kerosene sprayed (ml) and survival time of the workers (s) to determine the relationship between two parameters.

Results

Nine species of *T. rufonigra* host trees (Table 3) were recorded from three urban localities. Three major types of nest structures were recognized (Table 4) among the eleven nests collected from the host trees. Type 1: Many longitudinal tunnels with large entry holes in the well-decayed hard wood and peripheral fragile wood (Figs 1–3) were seen in the dead branches of Acacia catechu and Albizia lebbeck; Type 2: Few longitudinal tunnels with small to large entry holes in moderately hard wood or hard wood in the dead branches of Acacia catechu and Albizia lebbeck were observed; many holes were also seen in peripheral soft wood and the bark (Figs 4–6); Type 3: Very small external and internal holes were present throughout the heart wood (Figs 7–8) in the dead, decaying branch of Plumeria sp.

The probability plot from Probit Analysis (Fig. 9) resulted 6.39 mg citronella oil per mg body weight as the LD_{99} of *T. rufonigra* workers. With the increase of citronella

Figs 1–6. *T. rufonigra* nest structures, Types 1 and 2.

1–3 – Type 1: 1 – appearance of vertical section, 2 – cross section, 3 – external appearance of the wood log; 4–6 – Type 2: 4 – external appearance of the wood log, 5 – vertical section of the wood log, 6 – cross section of the wood log showing inner tunnels.

Рис. 1–6. Структура гнезд *T. rufonigra*, типы 1 и 2.

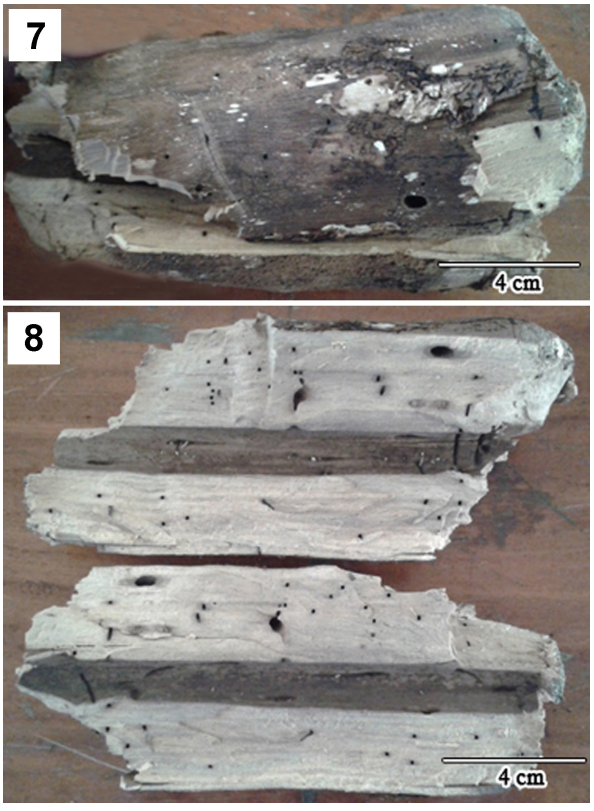
1–3 – тип 1: 1 – продольный разрез ствола, 2 – поперечный разрез ствола, 3 – внешний вид ствола; 4–6 – тип 2: 4 – внешний вид ствола, 5 – продольный разрез ствола, 6 – поперечный разрез ствола с внутренними ходами.

oil or kerosene spray volume the survival time of the group of experimental workers decreased (Figs 10-11). Spraying of eight times released 5.2 ml of citronella oil, which was the least volume that required to kill all the worker ants in five minutes (One Way ANOVA, $p < 0.05$; Tukey's test). The least volume, 3 ml, of kerosene killed all the worker ants in less than 5 minutes (Fig. 11).

Discussion

Sudden outbreaks *T. rufonigra* occurred in several urban localities in Sri Lanka recently [Ratnatilaka et al., 2011; Rodrigo, 2013] after tree felling or due to the fallen host trees that are with its nests. Lack of knowledge on its host trees made it impossible to explain the outbreak directly. Development of symptoms after its stings needed medical treatments and hospitalization of many people. Awareness of host tree species will be very useful to prevent or be cautious in such tree felling and take measures for the temporary control of the situation. Presence of host tree species of the species in an urban park (e.g. Vihara Mahadevi Park) in Sri Lanka was reported for the first time during the current survey. Types of nests of the species were described with colour images for the first time from the urban regions of South Asia.

Host tree species of *T. rufonigra* nests, Xylia dolabriformis, Sonneratia, Santalum album, Ficus aculeata and Caesalpinia crista, were reported from Singapore and India [Bingham, 1903; Ward, 2001; Meshram et al., 2015] but Acacia catechu, Casuarina equisetifolia, Albizia lebbeck, Cassia fistula, Adenanthera pavonina, Filicium decipiens, Pericopsis mooniana, Plumeria sp. and Tectona grandis were reported for the first time as the host trees, only from Sri Lanka showing that the ant species can colonize many tree species. It is recommended that the named host trees should be checked for the presence of nests if tree felling is essential in urban areas to prevent the ant stings.



Figs 7–8. Type 3 of *T. rufonigra* nest structures.

7 – external appearance of the wood log; 8 – vertical section of the wood log showing the holes and tunnels in the nest.

Рис. 7–8. Структура гнезда *T. rufonigra*, тип 3.

7 – внешний вид ствола; 8 – вертикальный разрез ствола с отверстиями и ходами гнезда.

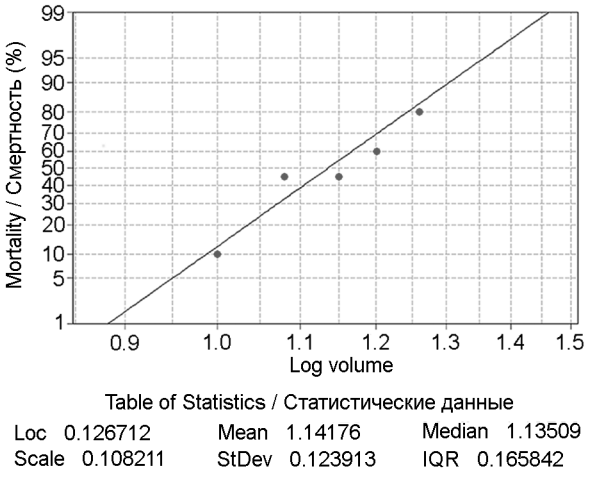


Fig. 9. The probability plot between log volume of citronella oil and percentage mortality of *T. rufonigra* workers.

Рис. 9. График логарифмической зависимости между объемом масла цитронеллы и смертностью рабочих особей *T. rufonigra*.

Although Ward [2001] reported that *T. rufonigra* occupies the cavities made by beetles or beetle larvae in dead branches of trees or live wood the nests reported here were observed in dead, decaying branches that remained on the host trees only. We did not find any other insect body parts or exuviae in the galleries; size and appearance of the holes and tunnels in other tree branches except Plumeria sp. indicated that those were made and abandoned by other insects [Ward, 2001]. Considering the size and appearance of the holes and tunnels in the dead branch of Plumeria sp., we conclude that *T. rufonigra* workers may have made the tiny holes and very narrow cavities in the heartwood of a dead branch of this tree species.

Management of the workers in sudden outbreaks was tested for the first time with kerosene or citronella oil and is essential to prevent the stinging of people because workers appear in huge numbers and crawl everywhere as soon as trees fallen on the ground. Sprayer bottles in the local market are cheaper and available so that such a bottle after calibration was used in the field simulating experiment. Kerosene is cheaper and available in the market and recommended to use with caution, only for temporary suppression of the workers to prevent the ant stings. Although citronella oil, an environmental-friendly, bio-pesticide, can also be recommended in the dosages given here for temporary suppression of the workers to prevent the ant stings, it is sold only in limited places in the local market.

Inspection of trees listed here for the presence of the workers and nests is recommended before essential tree felling in urban localities to avoid sudden outbreaks of the

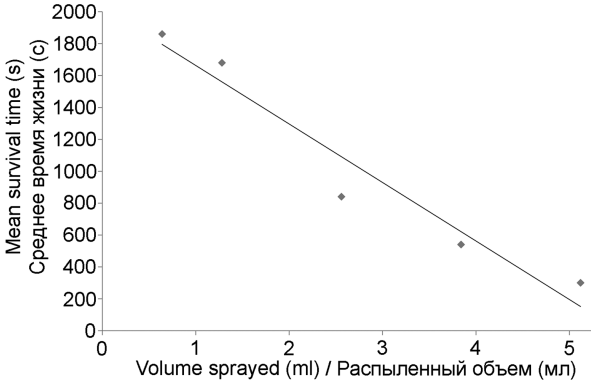


Fig. 10. The negative correlation between volume of citronella oil sprayed and mean survival time of ten workers.

Рис. 10. Отрицательная корреляция между объемом распыленного масла цитронеллы и средним временем жизни 10 рабочих особей после распыления.

species on the ground. Also, the cut ends of the branches on such trees should be covered with a suitable material or a paint to prevent the entry of insects.

Conclusion

Tetraponera rufonigra, a medically important arboreal ant species, forms colonies in the dead branches with three types of insect-made holes and tunnels that are attached to Acacia catechu, Casuarina equisetifolia, Albizia lebbeck, Cassia fistula, Adenanthera pavonina, Filicium decipiens, Pericopsis mooniana and Tectona grandis in the urban localities. The species is also capable of perforating heartwood in the dead branches of Plumeria sp. LD_{99} of citronella oil was 6.39 mg / mg body weight for the workers. Minimum spray volumes, 3 ml of kerosene or 5.2 ml of citronella oil are recommended for the temporary suppression of the workers when sudden outbreaks occur due to the fallen host trees. Current findings are valuable for the prevention of ant stings in the future.

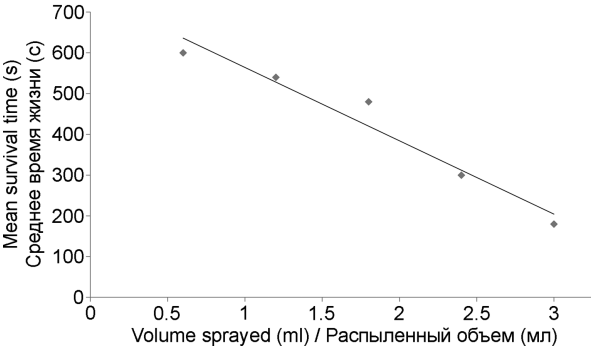


Fig. 11. The mean survival time of the experimental workers decreased with the increase of kerosene spray volume. Spraying of five times using the calibrated sprayer bottle released 3 ml of kerosene, which was the least volume of kerosene required to kill them in 3 minutes (One Way ANOVA, $p < 0.05$; Tukey's test).

Рис. 11. Уменьшение среднего времени жизни рабочих особей с увеличением объема распыленного керосина. Пятикратное нажатие на распылитель высвобождает 3 мл керосина, что является наименьшим объемом, необходимым для уничтожения муравьев за 3 минуты (One Way ANOVA, $p < 0.05$, тест Тьюки).

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