Assessment of toxic baits for the control of the samsum ant, *Pachycondyla sennaarensis* (Formicidae: Ponerinae)

A.M.A. Mashaly[1,2*], M. Farooq[1], W. Hozzein[1,3], A.M. Al-Qahtani[1] & F.A. Al-Mekhlafi[1,4]

[1]*Department of Zoology, College of Science, P.O. Box 2455, King Saud University, Riyadh 11451, Saudi Arabia*
[2]*Department of Zoology, Faculty of Science, Minia University, El-Minia, Egypt*
[3]*Department of Botany, Faculty of Science, Beni Suef University, Beni Suef, Egypt*
[4]*Department of Agricultural Production, College of Agriculture and Veterinary Medicine, Thamar University, Yemen*

The use of synthetic organic pesticides has serious economic, social and environmental ramifications. Thus, this study describes the experiments using botanical and bacterial extracts to control the samsum ant (*Pachycondyla sennaarensis*). This ant is widely distributed in many parts of southern Saudi Arabia, and has been established as a household pest ant. Three Saudi plants, harmal (*Rhaza stricta*), boxthorn (*Lycium shawii*) and artemisia (*Artemisia inculta*) and two bacterial extracts, *Pseudomonas frederiksbergensis* and *Streptomyces* sp. were tested in a minced meat bait against the workers of samsum ant. Among the plant extracts tested, at a concentration of 0.3 mg of the plant extract of boxthorn per gram of food exhibited the highest toxicity to samsum ants, causing 20.30 % mortality per day and 100 % average death rate of all the ants in 4.9 days. Furthermore, the results demonstrate that the bacterial extract of *Streptomyces* sp. was the most effective agent to control these ants, with the average death rate at 30 ants per day at a concentration of 0.3 mg/g food.

Key words: ant control, artemisia, bacteria, boxthorn, harmal, samsum ant, *Pachycondyla sennaarensis*.

INTRODUCTION

The samsum ant, *Pachycondyla sennaarensis* (Mayr, 1862), has been found in Saudi Arabia since 1862 (Collingwood 1985) and is also widely distributed in the Afrotropical Region. It has been monitored only in the urban and the rural areas, closely tied to human residences, and described as unique among ponerines in its seed-eating habits (Lachaud & Dejean 1994). This ant became a recognized public health hazard as its sting has resulted in few cases of fatal anaphylactic shock (Al-Anazi *et al.* 2009). This venomous ant was abundantly found in the southern, eastern and middle regions of Saudi Arabia (Al-Khalifa *et al.* 2010).

Chemical insecticides are often incorrectly applied as ground sprays or trunk bands for killing foragers through contact (Addison 2002). With the chemical stem barriers, only limited control can be achieved because the queen or queens and the vast majority of the workers in the nest are not affected (Baker *et al.* 1985; Knight & Rust 1990; Nelson & Daane 2007; Styrsky & Eubanks 2007). On the other hand, baits are becoming an indispensable tool in urban and agricultural pest control. For bait toxicants to be successful in controlling the target pests (ants), it is imperative that the bait must not be a repellent to the ant foragers (Stevens *et al*. 2002) and it must be preferred over the competing natural food sources (Nelson & Daane 2007). Furthermore, bait should have an optimized attractant and toxicant that does not deter feeding, mass recruitment and trophallaxis (Goss *et al.* 1990). Before using toxic baits, the biology of ants and their foraging behaviour must be well understood. This will give an insight into the type and volume of bait that a station should contain, and the number of bait stations needed per unit area. From the foraging activity point of view and the food preferences of the samsum ant, Mashaly *et al*. (2013) reported that there were no seasonal shifts in the food preference and the foraging activity of *P. sennaarensis* which consistently prefers protein and carbohydrate rewards to lipids. However, the results indicated that minced meat was consumed significantly more than other proteinaceous foods. Lipids were generally ignored by *P. sennaarensis*. The foraging activity of the samsum ant may be influenced by time of day, temperature and relative humidity. During the spring, the activity of workers in the daytime was at its maximum level at 12:00, the temperature at 16–21 °C and with a relative humidity of 52–58 %. However, in the

*Author for correspondence. E-mail: mmashely@ksu.edu.sa

summer, when the temperature was generally higher, foraging activity was greater during cooler times of the day and night. Foraging activity decreased during the autumn and winter.

Pest management is facing an economic and ecological challenge worldwide due to human and environmental hazards caused mainly by synthetic chemical pesticides. Identification of novel effective natural insecticidal compounds is essential to combat increasing resistance rates and ensuring environmental safety. In this context, botanical pesticides have long been touted as attractive eco-friendly alternatives to synthetic toxic chemical pesticides for pest management as they reputedly pose little threat to the environment or to human health (El-Wakeil 2013). An additional alternative is the use of bacterial insecticides that contain bacteria or their toxic by-products. Bacterial insecticides are valuable due to their extremely low toxicity to non-target animals and humans (McDonald & Linde 2002). It has been shown that botanical insecticides and microbial pesticides are highly effective, safe and ecologically acceptable (Subramaniam *et al.* 2012).

To the best of our knowledge, no previous research work has examined the control of the samsum ant. Thus, the current study was conducted to test some botanical and bacterial extracts as toxic baits, which can be practically utilized as insecticides in the battle against samsum ant in Saudi Arabia.

MATERIAL AND METHODS

Insects

Colonies of *P. sennaarensis* (containing 2000–2500 workers, with brood of all stages and multiple queens (3–8), were collected from Al Ehsaa Governorate, East Riyadh, and the Kingdom of Saudi Arabia. Collected nests were moved to the ant insectary in the Zoology Department, College of Sciences, King Saud University. Ants were housed in plastic nest-bottles within a large plastic box (45 × 30 × 18 cm), with Fluon®-coated walls to prevent ant escape, that was used as a foraging area. The insectary was maintained at 28 ± 1 °C, ~30 % RH, and 12L:12D photoperiod. Ants were allowed to access fresh water in glass tubes blocked with cotton wool and fed daily with mealworm larvae and sesame seeds. Ten days prior to the start of the experiment and during the experiment, ants were not fed to ensure that they would readily form foraging trails to the feeder. To prevent desiccation, nests were moistened by adding a few drops of water when needed.

Plant extracts

Three plants were targeted for extraction in this study namely, harmal (*Rhaza stricta* Decne.), boxthorn (*Lycium shawii* Roem. & Schult.) and artemisia (*Artemisia inculta* Delile) (Gupta *et al.* 2012). Plants were washed with tap water and shade-dried at room temperature. An electrical blender was used to powder the dried plant materials (leaves). The powder (500 g) of the leaf was extracted with 1.5 l of organic solvents of ethanol using a Soxhlet apparatus at 60–80 °C for 8 h (Bassett *et al.* 1978). The extract was concentrated under reduced pressure 22–26 mm Hg at 45 °C and the residue obtained was stored at 4 °C. The extracts were filtered through a Buchner funnel with Whatman No. 1 filter paper. The crude plant extracts were evaporated to dryness in a rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 ml acetone (stock solution) and considered as 1 % stock solution. From this stock solution, different concentrations were prepared ranging from 0.1 mg/g food (minced meat) to 0.3 mg/g food.

Bacterial extracts

Two plant growth promoting bacterial strains namely, *Pseudomonas frederiksbergensis* and *Streptomyces* sp., were obtained from the Department of Botany and Microbiology and Bioproducts chair, College of Science, KSU, Riyadh, Saudi Arabia, respectively. Culture filtrates (1 l) of the aforementioned five bacterial cultures were prepared by centrifuging 24-h-old cultures grown in Luria broth at 10 000 *g* for 20 min (Abdel-Megeed & Majhdi 2009). The supernatants were then collected and further evaluated for their efficacy against the samsum ants. The required quantity of bacteria was thoroughly mixed with distilled water and various concentrations were prepared, ranging from 0.1–0.3 mg/g food (minced meat).

Bioassays

Each treatment, 100 adult workers with three replicates were used with all extracts. The impregnated filter paper method modified from Visetson *et al.* (2003) was used to evaluate their efficacy in terms of LC_{50} from placing *P. sennaarensis* on impregnated filter paper with each extract. The LC_{50} at 24 h was derived from simple linear regressions

Table 1. Mortality prcentage of *Pachycondyla sennaarensis* against crude plant extracts under laboratory conditions.

Plant	Extract concentration (mg/g)	Total treated	Average deaths of all ants (days)	% Mortality of ants per day (mean ± S.E.)
Harmal (*Rhaza stricta*)	0.1	100	21.0	4.76 ± 1.322 c
	0.2	100	16.3	6.12 ± 1.693 c
	0.3	100	10.4	9.55 ± 3.609 b
	Control	100	34.7	2.88 ± 0.396 c
Boxthorn (*Lycium shawii*)	0.1	100	13.6	7.33 ± 1.859 bc
	0.2	100	10.6	9.43 ± 2.569 b
	0.3	100	4.9	20.30 ± 7.282 a
	Control	100	26.0	3.84 ± 0.448 c
Artemisia (*Artemisia inculta*)	0.1	100	17.3	7.50 ± 1.407 b
	0.2	100	8.8	11.33 ± 2.659 ab
	0.3	100	7.0	14.19 ± 3.731 ab
	Control	100	27.4	3.65 ± 0.308 c

Numbers followed by different letters are significantly different ($P < 0.05$).
S.E. = standard error of mean.

between mortality percentage and various concentrations. Each control group of ants was contacted in dissolved solvent. The mortality of treated ants was observed at 24 h after contact.

Commercial bait

Commercial bait (ARS Chemical Co., Thailand) was used to compare with plant and bacterial extracts in order to determine which of them had the superior impact on the samsum ant control.

Statistical analysis

Average death of all ants per days was counted. Statistical Package for the Social Sciences (SPSS) was used to analyse the mortality of ants. A comparison between plants, bacterial extracts and commercial bait (ARS Chemical Co., Thailand) was done to select the best bait.

RESULTS

Plant extracts

Plant extracts of harmal (*R. stricta*), boxthorn (*L. shawii*) and artemisia (*A. inculta*) showed insecticidal effects against the samsum ant workers, since considerable insecticidal effect of treated ants occurred when compared to that of the control group (Table 1). Among the plant extracts tested, the extract of boxthorn at the concentration of 0.3 mg/g exhibited the highest toxicity to samsum ants, causing 20.30 % mortality per day and 100 % mortality average of all ants in 4.9 days. For the control ants in the same experiment, the results indicate that the average days to death of all the ants was 26 days.

Bacterial extracts

Generally, bacterial extracts *P. frederiksbergensis* and *Streptomyces* sp., showed high significant mortality rates. The *Streptomyces* sp. extracts showed a significantly higher mortality rate than the bacterial extract of *P. frederiksbergensis* and the control group, whereas, the *Streptomyces* sp. extracts caused 30 % mortality per day and 100 % average death rate of all ants in 3.3 days compared to the control group in the same experiment; this indicates that the average death of all ants was 26.9 days (Table 2).

The commercial bait (ARS) showed insecticidal effects, as the mortality increased to 11 % per day after treatment and caused 100 % mortality at the average deaths of all ants in 9 days. Compared to the control in the same experiment, this indicates that the average time to death of all ants was 38 days (Table 3).

Commercial bait

The mortality percentage of *P. sennaarensis* due to different extracts from the three sources, boxthorn, *Streptomyces* sp. and the commercial bait was compared in order to determine which of them has the superior impact on control of this ant.

Table 2. Mortality percentage of *Pachycondyla sennaarensis* against bacterial extracts under laboratory conditions.

Plant	Extract concentration (mg/g)	Total treated	Average deaths of all ants (days)	% Mortality of ants per day (mean ± S.E.)
P. frederiksbergensis	0.1	100	17.3	5.76 ± 0.863 c
	0.2	100	12.1	8.25 ± 1.735 bc
	0.3	100	10.1	9.90 ± 2.159 b
	Control	100	31.0	3.22 ± 0.309 c
Streptomyces sp.	0.1	100	12.9	7.72 ± 2.358 bc
	0.2	100	7.5	13.22 ± 4.449 b
	0.3	100	3.3	30.00 ± 12.688 a
	Control	100	26.9	3.71 ± 0.345 c

Numbers followed by different letters are significantly different ($P < 0.05$).
S.E. = standard error of mean.

Table 3. Mortality percentage of *Pachycondyla sennaarensis* against commercial bait under laboratory conditions.

Commercial bait	Total treated	Average deaths of all ants (days)	% Mortality of ants per day (mean ± S.E.)
ARS	100	9	11.00 ± 1.724 a
Control	100	38	2.63 ± 0.323 b

Numbers followed by different letters are significantly different ($P < 0.05$).
S.E. = standard error of mean.

From observation, *Streptomyces* sp. extract has the most potential, which caused about 34.09 % mortality soon after application and 100 % mortality 3.1 days after application. Boxthorn extract was found to cause 19.67 % mortality soon after application and 100 % mortality 5.2 days after application. Unlike the control, boxthorn extract was found to cause 100 % average deaths of all ants in 29 days (Table 4). This indicates that the extract of *Streptomyces* sp. had significantly higher mortality and can also be used to control samsum ants.

DISCUSSION

Biological control of insect pests on economically important plants has been stimulated in recent years by trends in agriculture towards greater sustainability and public concern about the use of hazardous pesticides. Botanicals and microorganisms have the capability to synthesize biologically active secondary metabolites such as antibiotics, herbicides and pesticides (Gopalakrishnan *et al.* 2011). Plant-derived products can be used to control ant populations through several mechanisms.

Table 4. Comparison of the mortality percentage of *Pachycondyla sennaarensis* against the best from the three extracts under laboratory conditions.

Extract	Total treated	Average deaths of all ants (days)	% Mortality of ants per day (mean ± S.E.)
Boxthorn (0.3 conc.)	100	5.2	19.67 ± 6.391 b
Streptomyces sp. (0.3 conc.)	100	3.1	34.09 ± 11.943 a
Commercial bait	100	9.0	9.21 ± 0.84 c
Control	100	29.0	3.67 ± 0.62 c

Numbers followed by different letters are significantly different ($P < 0.05$).
S.E. = standard error of mean.

Some of these substances, such as citrus seed oils obtained from *Citrus sinensis* (L.) Osbeck, *Citrus limon* (L.) Burm. f. or *Citrus reticulata* Blanco (Rutaceae) (Fernandes *et al.* 2002) and the extracts from the castor bean (*Ricinus communis* L.) (Euphorbiaceae) (Bigi *et al.* 2004) and timbo (*Ateleia glazioviana* Baill.) (Leguminosae) (Cantarelli *et al.* 2005) can act directly against the ant, leading to its death. Generally, botanical insecticides and microbial pesticides are highly effective, safe and ecologically acceptable.

Results of this the current study suggest that the bait mixture **(***Streptomyces* sp. extract with minced meat**)** shows potential in the future options for samsum ant control. Tangchitphinitkan *et al.* (2007) evaluated the efficiency of three Thai herbs, *i.e*. tuba root (*Derris elliptica* Benth.), yam bean seeds (*Pachyrhizus erosus* L.) and tea seed cake (*Camellia* sp.) against the adult worker of the pharaoh ant (*Monomorium pharaonis* L.). Their results showed that the tuba root extracts had a LC_{50} against the adult worker *c.* 0.22 % w/v whereas yam bean seed extracts had a LC_{50} against adult worker *c.* 0.35 % w/v and tea seed cake extracts had a LC_{50} against the adult worker *c.* 0.55 % w/v after 24 h exposure, respectively.

Ribeiro *et al.* (2008) evaluated the toxic effects of four hexane extracts (*Ruta graveolens, Cordia verbenaceae, Mentha piperita* and *Ageratum conyzoides*) applied on the workers of *A. sexdens rubropilosa* and *A. subterraneus molestans*; they obtained lower mortalities (<40 %) at the same concentration. In current study, mortality reached 100 % only for concentrations of 50 and 100 mg/ml, depending on the plant extract. In a short period of time, the mortality of workers caused by the extracts, such as *Streptomyces* sp. is more appropriate as an ant control method using direct application (such as thermal fogging). On the other hand, if the purpose is to utilize these extracts for bait, then the ideal extract is one that slowly causes ant death. In this way, worker ants live long enough to take the baits back to the nest and feed it to the colony and the queen (Della Lucia & Arújo 2000).

CONCLUSION

This study therefore concluded that the use of minced meat with the bacterial extract *Streptomyces* sp. at a concentration of 0.3 mg/g food could be a suitable bait for use in controlling the venomous samsum ant.

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