TOXICITY AND RETENTION OF DYE MARKERS TO *HETEROTERMES INDICOLA*

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Abstract

Different concentrations of Nile blue A, Sudan red 7B, Fuchsin acid and Eosin gelblish were tested as dye markers for *H. indicola.* Nile blue A 0.25% caused a negligible mortality (6%) and was retained in more than 50% of the termites for 4 months. Sudan red 7B was also less toxic at lowers concentrations (≤ 0.25%) but did not remain in the bodies of the termites for more than 2 weeks. At 0.06% concentration, Fuchsin acid and Eosin gelblish caused 16 - 17% mortality. The mortality rate reduced at higher concentrations, probably due to avoidance of feeding by the termites. Fuchsin acid and Eosin gelblish did not remain in the termite for more than 2 - 3 days after the termites fed on these dyes.

Keywords: Termite, dyes, mortality, retention

Introduction

Control of subterranean termites is difficult because their nests are below the ground and it is difficult to delineate their foraging territories. Detection of nesting systems of subterranean termites by excavation methods causes a disturbance in the termite nests making it impossible to continuously monitor the foraging activity of a termite colony. Therefore, there is a need for a dependable and non-destructive method of detection of the foraging territories of subterranean termites. Release and recapture of marked individuals has been used for such studies. Dyes were first used in Hawaii to measure the distance traveled by *Coptotermes formosanus* workers, fed filter paper impregnated with fast green, between interconnected traps (Fujii, 1975).

Termites are among the most sensitive social insects. Whenever they feel any danger in case of mortality or repellency from any direction to their colony, they immediately seal off the channel toward that direction. Therefore, such dyes, which are not toxic against the test termites and have a long retention time in the termites bodies, need to be used for the delineation of foraging territories. Many researchers have studied these characteristics of dye markers on different termite species. Lai (1977) and Lai *et al.* (1983) screened nine histological dyes and identified Sudan red 7B as the most persistent and least toxic dietary dye marker for *C. formosanus*.

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Su *et al*. (1991) found that at proper concentrations Nile blue A did not cause significant mortality in termites and remained visible in the two-week observation period. *H. indicola* is a termite disastrous to crops, fruit trees and buildings throughout Pakistan. The possibility of using different dyes as markers for *H. indicola* has been investigated in these studies.

Materials and Methods

Four dyes, Nile blue A (96%), Sudan red 7B (95%), Fuchsin acid (70%) and Eosin gelblish, at three concentrations (0.06, 0.25, and 0.50%) of each dye, were used in these studies.

Termite Collection

NIFA-TERMAPs, a trapping technique developed by Salihah *et al*. (1993), composed of a PVC pipe (8 mm thick × 15 cm dia. × 20 cm high), having a bundle of 5 poplar wooden slices (1.3 × 8 × 15 cm) wrapped in blotting paper and covered with an earthen lid, was used at a termite-foraging points detected by the stake survey for termite collection (Su and Scheffrahn, 1988) at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar. Each trap was checked fortnightly and the infested bundles of NIFA-TERMAPs were brought to the Entomology laboratory, NIFA Peshawar. Termites were separated from debris using sieves size (40, 50, 60mesh) and were reared under the controlled laboratory conditions (28 ± 2°C and 60 ± 5% RH) in glass petri dishes (15 cm dia. × 2 cm high) with two moistened filter papers (14 cm dia.) for stock culture.

Toxicity Test

Solutions of the required concentrations (0.06, 0.25, and 0.50%) of Fuchsin acid and Eosin gelblish were made in distilled water, while Nile blue A and Sudan red 7B were dissolved in acetone. In order to determine potential toxicity of the dyes, two ml of each dye solution was poured onto two filter papers (Whatman No.I, 5.5 cm dia.) in a petri dish (5.5 cm dia. × 1.5 cm high). Petri dishes were kept open overnight for the evaporation of the solvents. On the next day, 2 ml of distilled water was added to each unit and 300 worker termites (4^{th} - 5^{th} instar) were introduced to each unit for forced-feeding. After 3 days, a group of 100 termites from each unit was transferred to a petri dish (3.2 cm dia.) containing two moistened (with 1 ml water) filter papers. Two soldiers were added to each group. For control, the same food without dye was provided. Mortality was recorded and dead termites removed (based on visual observation) after 3, 6, 9, 12, and 15 days. Percent mortality was corrected using Abbot's formula (1925). All the units were kept under controlled laboratory conditions (28 ± 2°C and 60 ± 5% RH). The experiment was designed as a completely randomized factorial with 4 dyes and 3 concentrations and there were 3 replications. Statistical computing was performed using PROC GLM (SAS Institute, 1990).

Retention Test

Following the same procedure, another set of experiments was conducted on the termites from the same stained culture for retention studies. Data were recorded on the number of stained termites after 3, 6, 9, 12, 15, 30, 60, and 120 days. Statistical computing was performed using PROC GLM (SAS Institute, 1990). The experiment was replicated 5 times.

Results and Discussion

A significant ($p < 0.05$) dye into concentration interaction was found, thus the simple effects of the dyes at each concentration were compared in Table 1. Nile blue A caused a negligible (3%) mortality after 15 days at the lower concentration (0.06%). The stain was retained for 15 days in 75% of the dyed termites. Dye retention was observed in 45% of the dyed termites for 1 month (Table 2). At 0.025% concentration, maximum mortality reached 6% after 16 days. The Nile blue A has retained for 30 days in 70% of the termites, while more than half of the termites retained the stain even after 4 months. At 0.50% concentration, Nile blue A caused 11.33% mortality after 15 days.

Percent retention of dyes in termites at this concentration started decreasing after a week probably due to increasing mortality caused by the dye at this concentration.

Our results showed that Nile blue A caused least mortality. The stain remained visibly persistent in termite bodies at the 3 concentrations throughout the experiment. With the passage of time the termite individuals became more bright and greenish blue in color (Figure 1) and after 114 days 55.51% of the stained termites were still happily living which confirmed that the Nile blue A (0.25%) was non-toxic, persistent and a clearly bright color. Su *et al*. (1991) found that at the proper concentrations (0.05 or 0.25 or 0.50%) and exposure time (3 or 6 days), Nile blue A remained visible in *C. formosanus* and *Reticuletermes flavipes* throughout the 15-day period and did not cause significant mortality.

Sudan red 7B showed a maximum of 7.33% mortality at 0.06% concentration. The mortality was 6.67% and 10% at 0.25% and 0.50% respectively and stayed almost at par with Nile blue A. The dye retention at 0.06% and 0.25% was 93 and 100 respectively for 9 days but abruptly declined afterwards and no dye persisted after 30 or 60 days at 0.06% and 0.25% respectively of Sudan red 7B.

Su *et al*. (1988) found that Sudan red 7B exhibited low toxicity against *C. formosanus* and remained visible in this termite 15 days after exposure when concentrations of 0.25 or 0.50% were used. For *R. flavipes*, however, Sudan red 7B was either too toxic at 0.25 or 0.50%, or did not visibly persist at a concentration of 0.05%. Grace and Abdallay (1989) demonstrated that low concentrations of Sudan red 7B are rapidly excreted by *R. flavipes*, and that extended feeding periods result in high mortality. Moreover, some feeding deterrence was apparent even over short (3 - 5 day) exposure periods. However, they concluded that dye concentrations upto 2% could be used safely to mark *R. flavipes* for short-term studies (ca 15 days), if the exposure period did not exceed 5 days. Su *et al.* (1983) mentioned that the amount of Sudan red 7B in termites decreases sharply immediately after the termites stopped feeding on the stained paper.

Table 1. Percentage of mortality of *H. indicola* after feeding on different concentrations of dyes

Dye (Conc.)	% Mortality after specified days				
	3	6	9	12	15
			0.06%		
Nile blue A	0.33 ± 0.58 c	0.33 ± 0.58 c	1.33 ± 0.58 c	2.00 ± 0.00 c	3.00 ± 1.00 c
Sudan red 7B	0.33 ± 0.58 c	0.33 ± 0.58 c	4.67 ± 1.15 b	4.67 ± 1.15 c	7.33 ± 0.58 b
Fuchsin acid	4.00 ± 0 b	5.00 ± 1.00 b	5.00 ± 1.00 b	10.33 ± 2.08 b	16.00 ± 2.00 a
Eosin gelblish	9.67 ± 1.53 a	10.33 ± 0.58 a	13.33 ± 0.58 a	13.67 ± 0.58 a	17.67 ± 1.53 a
			0.25%		
Nile blue A	0.33 ± 0.58 b	4.33 ± 0.58 b	4.67 ± 0.58 b	5.67 ± 0.58 b	6.00 ± 1.00 b
Sudan red 7B	0.33 ± 0.58 b	0.33 ± 0.58 c	1.33 ± 0.58 c	1.67 ± 0.58 c	6.67 ± 1.53 b
Fuchsin acid	4.00 ± 1.00 a	6.00 ± 1.00 a	12.00 ± 2.65 a	12.00 ± 2.65 a	12.00 ± 2.65 a
Eosin gelblish	5.00 ± 1.00 a	5.67 ± 0.58 a	6.67 ± 0.58 b	10.00 ± 1.00 a	11.33 ± 0.58 a
			0.50%		
Nile blue A	1.33 ± 0.58 ab	7.00 ± 1.00 a	8.00 ± 1.00 ab	11.33 ± 1.53 a	11.33 ± 1.53 ab
Sudan red 7B	0.67 ± 1.15 b	1.00 ± 1.00 c	7.00 ± 1.00 b	8.00 ± 1.00 bc	10.00 ± 0.00 b
Fuchsin acid	1.00 ± 1.00 b	4.00 ± 1.00 b	9.67 ± 0.58 a	9.67 ± 0.58 ab	12.00 ± 1.00 a
Eosin gelblish	3.00 ± 1.00 a	3.00 ± 1.00 b	6.33 ± 1.53 b	6.33 ± 1.53 c	7.33 ± 0.58 c

Figures followed by the same letters are statistically not significantly different from each other (DMR test) within each column.

Table 2. Percentage of termites retaining the dyes after specified number of days, at three concentrations

Dye	Days							
	3	6	9	12	15	30	60	120
0.06%								
Nile blue A	91.60 ± 1.52 a	90.00 ± 2.55 b	85.00 ± 6.04 b	78.00 ± 6.52 a	75.00 ± 3.39 a	45.40 ± 3.05 a	12.40 ± 1.95 a	0.00 ± 0 a
Sudan red 7B	95.00 ± 4.85 a	95.00 ± 4.85 a	93.00 ± 3.87 a	33.00 ± 3.32 b	15.00 ± 2.35 b	0.00 ± 0 b	0.00 ± 0 b	0.00 ± 0 a
Fuchsin acid	0.00 ± b	0.00 ± c	0.00 ± c	0.00 ± c	0.00 ± c	0.00 ± b	0.00 ± b	0.00 ± a
Eosin gelblish	0.00 ± b	0.00 ± c	0.00 ± c	0.00 ± c	0.00 ± c	0.00 ± b	0.00 ± b	0.00 ± a
0.25%								
Nile blue A	93.00 ± 4.74 b	90.00 ± 4.18 b	83.00 ± 1.58 b	73.00 ± 3.16 a	73.00 ± 3.16 a	70.00 ± 1.00 a	61.40 ± 2.70 a	55.40 ± 3.65 a
Sudan red 7B	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a	35.00 ± 2.45 b	26.80 ± 2.39 b	7.60 ± 1.14 b	0.00 ± 0 b	0.00 ± 0 b
Fuchsin acid	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 b	0.00 ± 0 b
Eosin gelblish	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 b	0.00 ± 0 b
0.50%								
Nile blue A	80.00 ± 2.55 b	73.00 ± 3.74 b	46.80 ± 2.17 b	43.00 ± 3.32 a	41.40 ± 3.65 a	20.00 ± 2.00 a	5.40 ± 1.14 a	2.00 ± 1.58 a
Sudan red 7B	95.00 ± 2.92 a	93.00 ± 3.39 a	91.20 ± 3.42 a	26.20 ± 1.30 b	16.80 ± 1.48 b	5.60 ± 0.89 b	0.00 ± 0 b	0.00 ± 0 b
Fuchsin acid	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 b	0.00 ± 0 b
Eosin gelblish	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 b	0.00 ± 0 b

Figures followed by the same letters are statistically not significantly different from each other (DMR test) within each column for each concentration.

Figure 1. Termite individuals stained with Nile blue A (0.25%) became more bright and greenish blue in color

With increasing concentrations of Fuchsin acid and Eosin gelblish, mortality of the termites showed a decreasing trend and minimum mortality was recorded when 0.50% Eosin was used (Table 1). It may be due to avoidance of feeding at higher concentrations, which reduced the mortality rate of termites. Termites are able to utilize their fat reserve in the absence of feeding. According to Ahmad *et al.* (1980), *H. indicola* can survive for about two weeks under starved conditions when kept in groups. This was supported by the fact that the colour did not appear in the termites when observed 3 days after feeding. A similar trend was evident in the later observations. Salih and Logan (1990) tested 30 dyes as markers for *Microtermes lepidus* workers and rejected the dye Eosin for its toxicity.

The above results imply that Fuchsin acid and Eosin gelblish are not desirable to be used against *H. indicola*. Sudan red 7B also caused a lower mortality but was not retained for a long time and thus may be used in short term studies. Nile blue A (0.25%) exhibited a lower mortality (6%) and was retained well for 4 months in more than 50%, and thus it is recommended for use against *H. indicola* for long term mark-release-recapture studies.

Nile blue A has an additional advantage in that it shows a gradient of colour difference over time. After feeding on the dye, the termite's colour is dull blue during the first week. It gradually changes into bright shiny blue within a period of 2 weeks and it stays bright later. It can help differentiate the termites released at different times.

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