

## DETERMINING DIFFERENT PARAMETERS OF CO-60 BAIT AS A RADIOTRACER FOR THREE SPECIES OF SUBTERRANEAN TERMITES

\*A. SATTAR, J.F. A. TRANIELLO<sup>1</sup>, Z. SALIHAN and A. FARID

Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan

<sup>1</sup> Biology Department, Boston University, Massachusetts, USA

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Laboratory studies were conducted to test the feasibility of using Cobalt-60 for tracing the nesting system of subterranean termites. Over a period of four weeks, mortality caused by feeding on bait containing Cobalt-60 (30 microcurie) did not exceed 6% in *Heterotermes indicola*, *Microtermes unicolor* or *Reticulitermes*. The data on radiation distribution in different body parts of *H. indicola* after feeding on radioactive bait showed maximum radioactivity in gut (64.63%) followed by external contamination (26.81%), appendages (5.87%) and exoskeleton (2.92%). It was concluded that Co-60 label bait could safely be used as a radiotracer to study the nesting system of subterranean termites.

**Keywords:** Radiotracer Co-60, *Heterotermes indicola*, *Microtermes unicolor*, *Reticulitermes flavipes*, Toxicity, Radiation distribution.

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### 1. Introduction

Determination of the composition, size and foraging behaviour of subterranean termites is important for formulating control strategies. The conventional excavation methods for population estimation and detection of nesting system of subterranean termites destroy the living trees/wood and termite nests, making it impossible to continuously monitor the population dynamics of the colony. Safe and non-destructive excavation methods are thus required for such studies.

The radioisotope-tagging is an efficient technique for locating and rapidly identifying large numbers of underground, in and under debris, and under bark and within trees termites. The gamma-emitters i.e. Sc-46, Co-60, Zn-65, I-131, Ir-192, Ta-182, Au-198, Ra-226, and Th- can successfully be used for tagging insects [1]. The chief advantage of gamma-emitters lies in the fact that intact insects can be measured for radioactivity without the necessity of data correction and absorption of radiation by the insect's body [2].

For tracing the termites in their natural environment, it is necessary to develop a bait containing a radioisotope that is non toxic, has a short biological half-life, a longer physical half-life,

energetic gamma-rays to allow monitoring through several centimeters of soil, and would pass through the colony by trophallaxis [3,4].

Gamma-emitting radioisotope, Cobalt-60 has been reported to be a good radiolabel because it produces characteristic emission spectrum. Moreover, the physical half-life of Cobalt-60 is 5.27 years and biological half-life is 10.1 days [5]. According to [6], Co-60 present in a food source was rapidly incorporated into worker of *Reticulitermes flavipes* (Koller) and reached detection limits within one to three days. Isotopic labels were transferable between colony members and did not produce significant mortality.

The reported studies were conducted to test the feasibility of using Co-60 for tracing the nesting system of *Heterotermes indicola*, *Microtermes unicolor* and *flavipes* and to determine its distribution in different parts of *H. indicola* and exchange through trophallaxis.

### 2. Materials and Methods

The experiments were conducted in the laboratory of Biology Department, Boston University, Massachusetts (USA). Termite species, *H. indicola* and *M. unicolor* from Pakistan and

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\* Corresponding author : nifa@psh.paknet.com.pk

*R. flavipes* from USA were included in these studies.

#### 2.1. Collection and maintenance of termite colonies

Colonies of *H. indicola* and *M. unicolor* were collected from different ecological sites of Peshawar valley (NWFP) in Pakistan using NIFA-TERMAP, developed by [7], and composed of a PVC pipe (8 mm thick x 15 cm dia. x 20 cm high), having a bundle of 5 poplar wooden slices (1.3 x 8 x 15 cm) wrapped in blotting paper and covered with earthen lid.

These termites were transported to USA after obtaining permission from the State Department of USA. The colonies were maintained according to the method used by [6], at 24 °C in plastic boxes (24 x 18 x 8 cm) in the Biology Department at Boston University, Boston, Massachusetts (USA). The food source composed of poplar wood slices and moist paper towels. From these colonies, sub-nests housing 600-700 workers were established in plastic petri dishes (dia 15x2 cm), which contained 27 grams of sand that permitted the termites to burrow, and kept moist. The food source for each sub-nest was pure cellulose. Termites, *R. flavipes* were collected from the laboratory reared stock culture in the laboratory. Colonies of *R. flavipes*, were maintained at 24 °C in plastic boxes (24x18x8 cm) with decayed birch wood and moist paper towels.

#### 2.2. Preparation of radioactive bait

Gamma-emitting Co-60 was used as radiotracer owing to its shorter biological half-life (10.1 day) and higher physical half-life (5.27 years). The radioactive bait was prepared by mixing two gram cellulose with 1.5ml aqueous solution of Co-60 (30 micro-curie).

#### 2.3. Toxicity of Cobalt-60 to *H. indicola*, *M. unicolor* and *R. flavipes*

A group of 100 termite workers were confined in a petri dish (dia. 8.5 x 1.5 cm high) and provided with radioactive bait for four weeks. A paste of 2 grams of pure cellulose and 1.5ml distilled water was used as control. Mortality data were recorded after 1, 2, 3 and 4 weeks and dead and moribund termites were removed. Experimental units were kept at controlled laboratory conditions (24±1 °C and 60±2% R.H.). Mortality data of each termite species were corrected with their respective control

according to Abbott's formula [8]. Each experiment was replicated 3 times.

#### 2.4. Detection and measurement of radioactivity in different body parts of *H. indicola*.

One hundred *H. indicola* were reared on radioactive bait at controlled laboratory condition (24±1 °C and 60±2% R.H.). After three weeks, radioactivity in 10 randomly selected termites was detected following [6]. A high-resolution whole-body gamma spectroscopy was used to detect and measure isotope incorporation. A heavily shielded whole body counter consisting of a lead-enclosed chamber with 2 large NaI well scintillation detectors connected to a 1024 channel pulse height analyzer (Tracer Northern Model TN-7200) at a high coarse voltage of 1 kV was used. An automatic timer was used for presetting counting intervals. A beam capsule (Ladd Research Industries, size # 3) was used for each individual. The background data of each beam capsule was recorded. The radioactive termites were picked up with forceps, placed in the beam capsule, and then placed in the well of detector. The detection sensitivity of the system was less than one picocurie per gram and counting time was 120 second. The radioactivity levels were obtained by subtracting the background radioactivity from the total radiation counts. Each specimen was then washed with ethanol and external contamination was recorded by subtracting the counts after washing from the first total counts. Thereafter antennae/legs, gut and exoskeleton of each of the specimen were removed and their radioactivity was recorded separately in a new beam capsule for each body part. The radioactivity levels were obtained by subtracting the background radioactivity from the total radiation counts. The experiment was replicated ten times.

#### 2.5. Social food flow (trophallaxis) in *H. indicola*, *R. flavipes* and *M. unicolor*

From the laboratory reared stock culture of *H. indicola*, *M. unicolor* and *R. flavipes*, two hundred worker termites (4<sup>th</sup>-5<sup>th</sup> instar) of each species were transferred to plastic petri dishes (dia. 8.5x1.5 cm high) having radioactive bait. The paste of radioactive bait was composed of cellulose (2 gram), 1.5ml Nile blue (1%) solution in distilled water with 0.3ml (30 microcurie) aqueous solution of Cobalt-60. Nile blue was used to differentiate recipient, donor and moulting termites. Termites were allowed to feed on the radioactive bait for a period of two weeks. Ten radiolabeled (well-stained and active) termites were collected from

Table-1. Percent mortality in radiolabeled foragers of *H. indicola*, *M. unicolor* and *R. flavipes*.

Weeks	Termite species		
	<i>H. indicola</i>	<i>M. unicolor</i>	<i>R. flavipes</i>
1	0.00 b	0.00 b	0.00 b
2	1.33 ab	0.00 b	0.00 b
3	1.33 ab	3.50 a	0.00 b
4	2.33 a	5.10 a	1.00 a

Figures within each column followed by the same letter are not statistically different from each other (DMR test,  $p=0.05$ ) within each column (figures corrected with their respective control according to Abbott's formula).

the sub-nests and were mixed with 36 active, non-labeled workers with 4 soldiers of the same age and colony starved for 24 hours in a petri dish (dia. 8.5x1.5 cm high). The petri dish contained 27 grams of moist sand that permitted the termites to burrow and keep moist. The food source was 2 gram cellulose mixed with 1.5ml distilled water. The donors and recipients were allowed to stay together until the end of experiment. Radioactivity in 10 randomly selected recipients was measured after 4, 5, 7 and 10 days after the exposure, following [6]. The tested termites were again mixed with their original lot after testing. The experiment was replicated 3 times. The behaviour of termites was observed for one hour after mixing the termites.

### 3. Results and Discussion

#### 3.1. Toxicity of Cobalt-60 to *H. indicola*, *M. unicolor* and *R. flavipes*

The results on the mean percent mortality after feeding on radioactive bait for 1,2, 3 and 4 weeks are presented in Table 1. Over a period of four weeks, mortality caused by feeding on the radioactive bait did not exceed 6% in any of these species. Although mortality difference over time shows some statistical differences, they do not seem to have much biological significance as the mortality ranged between 1-5.10% after four weeks. Radioisotopes have been reported to cause mortality by interference with osmotic regulation on excretion via concentration in the malpighian tubules [9] or interference with digestive functions of the midgut epithelium [10]. Co-60 does not seem to interfere with the normal functioning of tested termite species at the concentration used. Similar results where Co-60 did not cause a significant mortality in *R. flavipes* have been reported [6]. This indicates that Co-60

can safely be used in radiolabelling studies in these termite species.

#### 3.2. Detection and measurement of radioactivity in different body parts of *H. indicola*.

Maximum radioactivity was found in the gut (64.63%) followed by external contamination (26.81%), appendages (5.87%) and exoskeleton (2.92%) (Fig. 1). Most of the activity was observed in the gut. Since the radiation counts were recorded immediately after removing the termites from radioactive bait, major cause of the radioactivity would be the radioactive food that was under passage in different parts of alimentary canal. Symbiotic protozoan in the hindgut are reported to concentrate cobalt thus retaining radiation for a long time [9]. We were unable to confirm it because radiation counts were taken on whole gut only. The second major destination of radioactivity was external contamination. External contamination can help transfer the radioactivity to other nestmates through grooming that can increase the tracing efficiency.

#### 3.3. Social food flow (trophallaxis) in *H. indicola*, *R. flavipes* and *M. unicolor*

When the starved, non-labeled recipient worker termite were shifted to the petri dishes having cellulose bait and donor worker termites (labeled with Cobalt-60), the following observations were made in the first hour: In all the three species, workers engaged in allo-grooming immediately after placement in the petri dishes. Starved workers started feeding on cellulose bait, but previously fed workers (donors) only rarely fed. Immediate attempt was made to present food to recipient. There was a brief antennal contact between donor and recipient prior to food exchange. Grooming behaviour was also observed

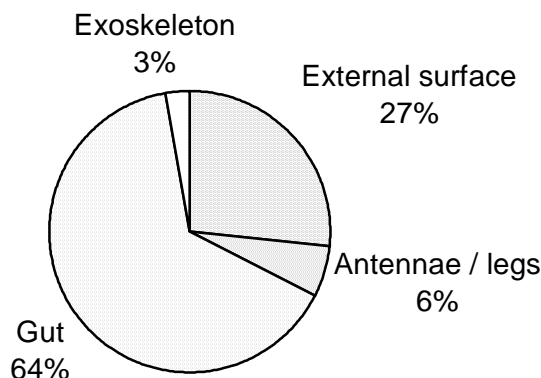


Figure 1. Percent distribution of radioactivity in different body parts of *H. indicola* fed on the radioactive bait of Cobalt-60.

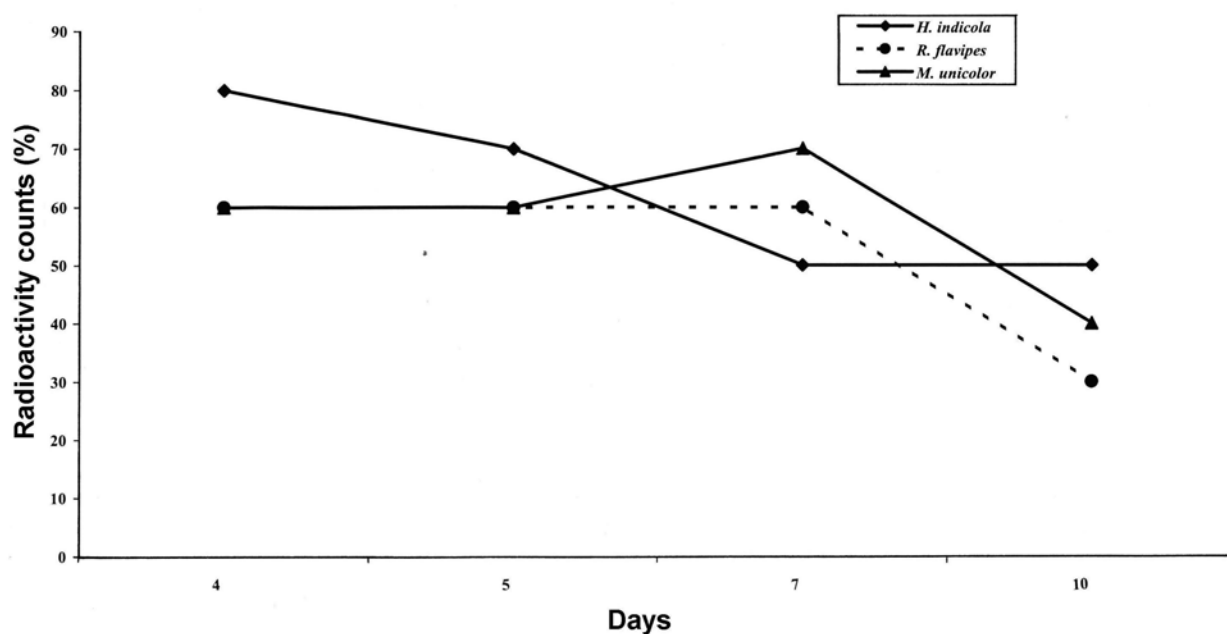


Figure 2. Percent receipt of untreated *H. indicola*, *R. flavipes* and *M. unicolor* of termites after interacting radiolabelled ones.

in groups of all the three termite species containing radiolabeled workers and starved soldiers.

The radiation count showed incorporation and accumulation of the radioisotope (Cobalt-60) in recipients of all the three species (Fig. 2). The data on radiation counts (at least 25 counts above the background) indicated that 80, 70, 50 and 50% of *H. indicola*; 60, 60, 60 and 30% of *R. flavipes*; and 60, 60, 70 and 40% of *M. unicolor* recipient termites became radio-labeled after 4, 5, 7 and 10 days respectively. Maximum recipient termites i.e. 80, 60 and 70% were found radiolabeled in *H. indicola*, *R. flavipes* and *M. unicolor* after 4, 4 and 7 days, respectively. Transfer of radioactivity may be due to trophallaxis (stomodaeal and proctodaeal)

as well as grooming. As major portion of radioactivity in the donors was found as external contamination, grooming would have been a major source of transfer of radioactivity.

Initial observations indicated a higher percentage of radioactivities in all the recipients but later the percentage dropped speedily after 7-10 days. It might have been due to the dilution of Co-60 through the transfer from recipient and donor to other worker leading to a radioactivity level below detectable limits. Removal of Co-60 through excretion may be another factor responsible for a reduction in the radioactivity in the sample collected later during the observation.

#### 4. Conclusions

It is concluded that Co-60 (30 micro-curie) does not seem to interfere with the normal functioning of termite species, *H. indicola*, *R. flavipes* and *M. unicolor*. External contamination can help transfer the radioactivity to other nestmates through grooming that can increase the tracing efficiency.

Transfer of radioactivity may be due to trophallaxis (stomodeal and proctodial) as well as grooming. As major portion of radioactivity in the donors was found as external contamination, grooming would have been a major source of transfer of radioactivity. This indicates that Co-60 can safely be used in radiolabelling studies without having a direct adverse effect on these termite species. However, effect of gamma radiation in the non target species specially the soil inhabiting microorganisms may warrant further investigations.

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