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EMBRYOTOXIC EFFECTS OF FENVALERATE IN DEVELOPING CHICKS

ASMATULLAH, ZAHRA JABEEN AND SHAGUFTA ANDLEEB

Zoology Department, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

Abstract: A pyrethroids insecticide, Fenvalerate injected into the yolk sac of eggs of *Galus domesliun*. Different dose i.e. 2.5,5.00, 10.00, 15.00 and 20.00 µg/egg were applied under sterilized conditions before incubation. Embryos recovered on day 7 showed significant (P<0.001) reduction in size along with severe structural abnormalities such as anencephaly microcephaly, microphthalmia, ectopia cardis and twisted spinal cord. On day 14, a high tendency of fetal resorption was observed. This study indicates that this insecticide is very dangerous to developing chick.

Key words: Developing chick, malformations, fetal resorption, pyrethroids, Fenvalerate.

INTRODUCTION

In the second half of 20th century a class of pesticides called synthetic pyrethroids was discovered which were expected to replace some of the conventional organochlorine, organophosphate and carbamate insecticides.

They were thought to be less toxic to non target animals including mammals (Akhtar, 1982; Ruscoe, 1977; Chambers, 1980; Malhotra *et al.,* 1981) and highly toxic to wide range of insects (target animals) Elliot *et al.,* 1978) including resistant strains. These were found to be photostable, biogradable and needed to be applied at a minimum dose (Barlow *et al.,* 1971; Hadway, 1972).

Fenvalerate is a synthetic pyrethroids insecticide used against a wide variety of insects, pests in various agricultural, animal husbandry and public health operations (Singh *et al.,* 2001).

Fenvalerate is moderately toxic compound via injection and less toxic via touch or dermal contact. Acute poisoning data in humans come from nearly 600 individual cases of poisoning that were reported between 1982 and 1988. Symptoms of acute poisoning included dizziness and itching (WHO, 1996).

Based on fenvalerate similarities with deltamethrin, toxicity is probably due to effects on both peripheral and CNS caused by interference with Na^+ ion permeability in stimulated nerve membrane. Fenvalerate is classified as type II pyrethroids. (WHO, 1996).

In test animals (rats) high acute exposure to Fenvalerate produced muscle inco-ordination, tremors, convulsions, nerve damage and weight loss. The insecticide may produce nausea, vomiting, headache, temporary nervous system effects such as weakness, tremors and inco-ordination at acute exposure level in humans (WHO, 1996).

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No teratogenic effect was observed in mice and rabbits following oral administration of 50 mg between gestation days 6-15 (WHO, 1996).

In a standard 3-generations reproduction study in rats, a reduced body weight in the F2b generation at 250 mg / kg diet was the only adverse effect observed (WHO, 1996).

Transient hypersensitivity, behavioral changes, hind limb ataxia and deficits in motor performance have been observed in rats, mice and hamster following acute or chronic exposure to fenvalerate. Dose dependent histopathological changes have been found including axonal break, swelling and degeneration of the myelin and increase in lysosomal enzyme activity in the posterior fibular and sciatic nerves (WHO, 1996).

Fenvalerate has very low toxicity to birds when given orally or applied to the diet. LD_{50} values are > 1500gm / kg acute oral dosage and an LD_{50} value for dietary exposure of Bobwhite quail has been reported at > 15000mg / kg diet.

Keeping in view the literature available, the present study was designed to evaluate Fenvalerate embryotoxicity in chick.

MATERIALS AND METHODS

One hundred and eighty fertilized eggs of *Gallus domesticus* were purchased from (Government poultry farm) Veterinary Research Institute, Lahore.

The eggs were divided into 6 groups comprising 30 eggs per group. Five groups were treated with different concentration of Fenvalerate. One group was without any treatment as control group. Fenvalerate was available in yellow liquid form with trade name Forlorn, 20 EC Fenvalerate (Welgreen Chemicals Ltd.)

Various concentrations 20.0, 15.0, 10.0, 5.0, 2.5 μg per egg fenvalerate were prepared. Doses were prepared by dissolving the insecticides in corn oil in such a way that 0.1 ml of solution contains the desired concentration.

Doses were administrated on day-0 of incubation. For administration f insecticide, eggs were randomly selected and cleaned with a piece of cotton soaked in 70% alcohol. A small window was made in egg shell (except control egg) with the help of sterile needle. Using 1ml glass syringe, 0.1ml of solution, containing various concentration of insecticides were injected into the yolk of each egg. After injection the hole in the egg shall was sealed with wax.

Eggs were incubated at standard conditions of hen egg incubation. The eggs were taken out from incubators on day-7 and 15 of incubation and opened at the broader end. The live and dead / resorbed embryos were recorded and fixed in Bouin's fixative for 48 hours. Embryos which were preserved in Bouin's solution were then washed in 70% alcohol.

Morphological and anatomical observations of embryos were made under dissecting stereomicroscope. The macro-photographs of selected embryos were taken with the help of a camera with close up lenses.

RESULTS AND DISCUSSION

Fenvalerate, a pyrethroids insecticide was evaluated for its teratogenicity in developing chicks. The embryos recovered from control group on day 7 of incubation showed normal parameters of development with CR length 18.54 ± 1.28 mm (Table-1, Fig. 1A). In insecticide treated groups, all recovered embryos showed some anomalies, besides a significant decrease in CR length ($P<0.001$) (Table-1). These abnormalities include some severe defects such as anencephaly, ectopia cardis, anophthalmia, microphthalmia and twisting of spinal (Fig. 1 B,C,D,E,F Table-1).

The control fetuses recovered on day 14 of incubation, having CR length 39.93±7.88 mm, were with normal eyes, wings, legs and a very prominent beak (Table-2, Fig.2A). A high percentage of fetal resorption (Table-2) was found in treated groups. Some fetuses from different groups showed degeneration of limbs, eyes and brain parts (Fig.2, B,C,D,E,F).

This is evident from the results of this study that this insecticide, fenvalerate is potentially dangerous to the developing chick. Some studies have reported the toxic effects of this group of insecticides in different animals. Rats fed pyrethrin at high levels for two years showed definite damage to the livers (Hayes, 1982). In another study inhalation of high doses of pyrethrum for 31 days caused lung irritation in rats and dogs (OHS, 1987). Ecobichon (1991) has reported that natural and synthetic pyrethroids can produce skin irritation, itching and local burning sensations. During a study, rats fed very high doses of pyrethrins daily for three weeks before mating had litter size and weanling weight lower than normal (Hayes, 1982). The literature available indicates very little study on pyrethroids as teratogens. Only one study performed on rabbit showed no effects of pyrethrins on development of the offspring (Vettorazzi, 1979).

As the present .study shows adverse effects. of Fenvalerate on chick development, it is pertinent to have more investigations of this group of insecticides to evaluate as teratogenic in birds as well as mammals.

Table I: Effect of Fenvalerate on the Development of Chick Recovered on Day 7 of Incubation.

Dose µg / egg	CR-Length mm ± S.D.	Resorbed / dead (%)	Beak (%)	Brain (%)	Eyes (%)	Hind limb (%)	Forelimb (%)	Cardiac Position (%)	Twisted Spinal Cord (%)
C	18.54 ± 1.28 (n=10)	00	Normal (100)	Normal (100)	Normal closed (100)	Well developed (100)	Well developed (100)	Normal (100)	00
2.5	6±3.08*** (n=10)	20	Not formed (40) Pointed (10)	Abnormal (30) Normal (20)	Not formed (30) Normal (20)	Under devel. (40) Normal (10)	Under dev. (40) Normal (10)	Ectopic 40%	10
5.00	5.85±.89*** (n=10)	50	Not formed (80)	Abnormal (50) Normal (30)	Not dev. (20) Reduced (60)	Not formed (50) Very small (30)	Short (50) Not formed (30)	Ectopic (80)	40
10.00	8.41±.50*** (n=10)	62	Not formed (50)	Abnormal (50) Normal (12.5)	Very small (37.5) Not formed (62.5)	Not formed (62.5) Reduced (37.5)	Reduced (37.5) Not formed (62.5)	Ectopic (37.5)	62.5
15.00	8±3.60*** (n=10)	42	Not formed (28.5) V. Small (28.5)	Abnormal (42.85) Normal (14.2)	At early stage of dev. (57.15)	V. small (42.85) Not formed (14.2)	V. small (57.15)	Ectopic (57.15)	42.85
20.00	11.35±2.40*** (n=10)	56	Not formed (45.45) Normal (18.18)	Abnormal (45.45) Normal (18.18)	V. small (54.54) Normal (9.09)	Short (63.63)	Shortly dev. (63.63)	Ectopic (63.63)	63.63

***** = P<0.001 (Significant against controls)**

Table II: Effect of Fenvalerate on the Development of Chick Recorded on Day 14 of Incubation.

Dose µg / egg	CR-Length mm ± S.D.	Resorbed / dead (%)	Beak (%)	Brain (%)	Eyes (%)	Hind limb (%)	Forelimb (%)	Cardiac Position (%)	Twisted Spinal Cord (%)
C	39.928±7.879 (n=7)	00	Well developed (100)	Normal (100)	Normal closed (100)	Well developed (100)	Well developed (100)	Normal (100)	00
2.5	7.8±2.64*** (n=7)	20.00	Not formed (60) Normal (20)	Abnormal **(30)** Normal **(20)**	V. small (60) Normal (20)	Not devel. (20) V. Small (20) Normal (20)	V. Small (40) Not formed (50) Normal (10)	Ectopic (40) Resorbed (20) Normal (20)	20
5.00	8.87±1.47*** (n=7)	28.00	Not formed (42.8) Normal (20)	Abnormal **(50)** Normal **(28.5)**	Abnormal (42.8) Normal (60)	Not prominent (28.5) Not formed (14.2) Normal (28.5)	Not formed (14.2) Not prominent (28.5) Normal (28.5)	Ectopic (28.5) Not clear (14.2) Resorbed (28.5) Normal (28.5)	(14.2)
10.00	6.16±1.80*** (n=7)	40.00	Not formed (50)	Not formed **(60)**	At early stage & Dev. (6)	Not formed (60)	Not formed (40) V. small bud (20)	Ectopic (60) Resorbed (40)	(60)
15.00	6.75±1339*** (n=7)	53.3	Not formed (66.6)	Abnormal **(50)** Normal **(16.6)**	V. small (66.6)	V. small (33.3) Not formed (33.3)	V. small (33.3) Not formed (33.3)	Ectopic (66.6) Resorbed (33.3)	(50)
20.00	6.45±1.28*** (n=7)	58.5	Not formed (71.42)	Abnormal **(71.42)**	V. small (71.4)	Not formed (71.4)	Not formed (71.4)	66.6	28.5

*** = **P<0.001 (Significant against controls).**

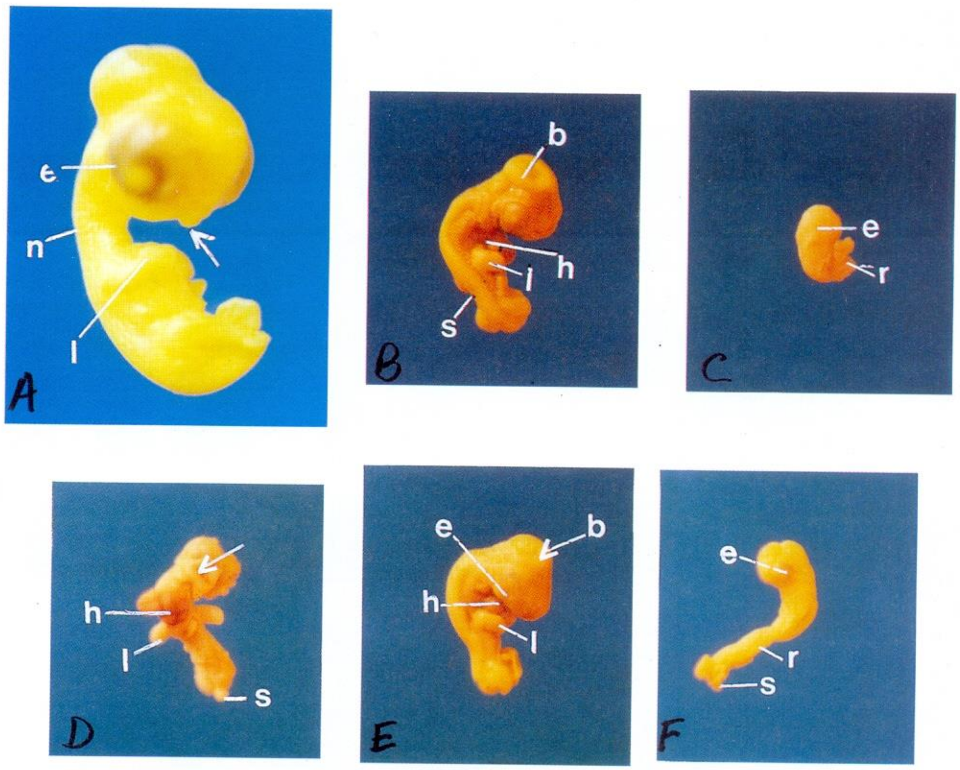

Fig 1: **A:** A macrophotograph of 7 days old chick embryos of control group showing normal development. Note: normal eyes (e), elongated neck (n), normal beak (arrow), normal limbs (1). **B:** Chick embryos treated with 2.5µg dose group showing abnormal development. Note: Abnormal brain bulges (b), ectopic heart (h), twisted spinal cord (s), small limbs (1). **C:** A macrophotograph of 5.00µg group showing abnormal development. Note: Resorption of body parts (r), anophthalmia (e), **D** and **E:** 10.00 and 15.00µg of 5.00µgdose group showing abnormal development. Note: Abnormal brain (arrow), ectopic heart (h), small limb (i), twisted spinal cord (s), small eyes (e). **F:** 2.00µg dose group showing abnormal development. Note: Small eyes (e), twisted spinal cord (s), resorption of body parts (r).

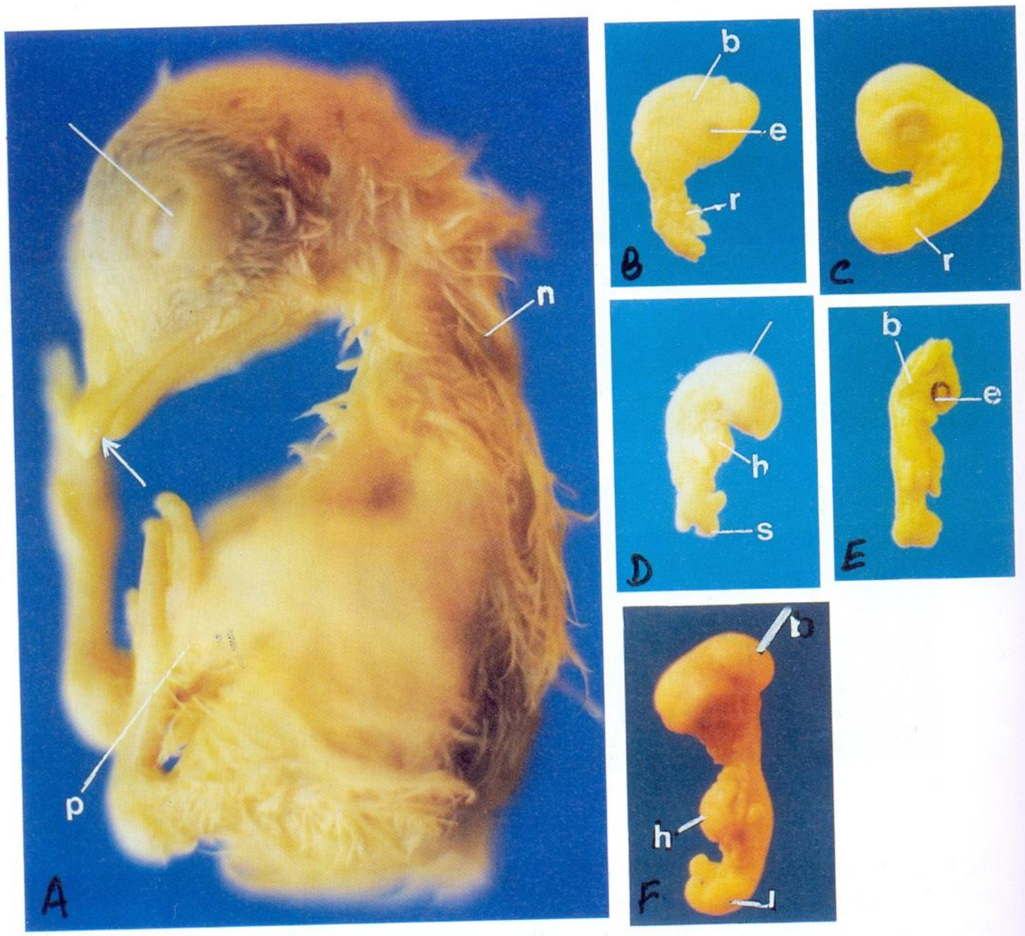

Fig 3: **A:** A macrophotograph of 15 days old chick embryos of control group showing normal development. B, C, D, E and F; Felines from 2.5, 5.00, 10.00, 15.00 and 20 µg/egg dose group, respectively, Note: abnormal brain bulges (b), small eyes (e), ectopic heart (h), small limbs (i).

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TUNNELING ABILITYOF *HETEROTERMES INDICOLA* (WASMANN) TO BIFLEX AND TERMIDOR THROUGH TERMICIDE TREATED SOIL

NADEEM SHEIKH AND M.S. AKHTAR

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore.

Abstract: A test apparatus was designed to facilitate accurate measurement of termite penetration through termiticide treated soil. In this regard two termiticides (Biflex and Termidor) at 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm were evaluated. *Heterotermes indicola* workers tunneled into the soil treated with both termidor and biflex at 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm. Soil treated with 50 and 25 ppm of biflex restricted termite penetration.

Key Words: *Heterotermes indieola,* insecticides, soil penetration

INTRODUCTION

Heterotermes indicola (Wasmann) is widely distributed in different ecological zones of Pakistan (Akhtar, 1974). It damages agricultural crops and wood work in buildings and is the most destructive termite species in Pakistan. Akhtar (1974) also reported that it has a wide range of tolerance to climatic conditions occurring less than 5 inches of rainfall to above 40 inches up to 7000 feet altitude.

For a long time chlorinated insecticides have been used for this purpose but because of their long term residual effects, use of chlorinated insecticides have been strictly restricted and banned by EPA. Other classes of termiticides have shown promising results to control such destructive agents.

The current study was designed to compare the relative tunneling ability of *H. indicola* through soil treated with newly introduced termiticides biflex (Bifenthrin) and Termidor (Fipronil).

MATERIALS AND METHODS

Chemical solutions were prepared in distilled water by mixing a specific amount of the termiticide so as to yield a final concentration of 50 ppm as stock solution. Further concentrations of 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm were prepared by serial dilution. The soil was sandy loam and obtained from a plot near the Zoology department. There have been no known applications of chemicals to this soil and soil was free from insecticidal contamination. The soil was sieved through 10x18 mesh screen and incubated at 100°C for 48 hours.

The test set up is shown in Fig. 1. Each consisted of thin layer of treated soil sandwiched between a glass and plexiglass plate and connected to a container 5 cm diameter and 5 cm height by a 4.5 cm long pipe. An untreated poplar block was placed in

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each container which was filled with a layer of moistened soil and vermiculite. On the opposite end another container was present which was also provided with untreated block and layer of moistened soil and vermiculite. In this way the termites had a choice of foraging, solely in the nest which contained ample food or else of expanding their foraging area through the treated soil to reach the opposite compartment to feed on the block.

Groups of 100 workers were added in one container of each test Set-up and each concentration was replicated thrice. Thus, a total of 24 experimental units per chemical treatment were established.

Test set-ups were examined at 24 hours interval for 7 days. At each examination the number of tunnels and length of each tunnel was measured. After one week the number of surviving termites and their conditions were noted.

Data were analyzed using an Analysis of Variance (ANOVA) according to Steel and Torrie (1981).

RESULTS

As shown in the enclosed Tables (1, 2) *Heterotermes indicola* penetrated into the soil treated with Termidor and Biflex at 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm. Higher concentrations 50 and 25 ppm of biflex restricted termite penetration. Different parameters observed were,

1) Maximum Tunnel Length In case of Termidor tunneling occurred in all the used concentrations 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm. The length of tunnels was 3.6, 14.11, 17.02, 22.38, 26.11, 28.58 and 31.8 mm in 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm, respectively; and 41.5 mm in control. Different concentrations significantly affected the mean length of tunnel made by the termite in treated soil. ($F= 31.21$; $P< 0.05, 0.01$)

In case of Biflex tunneling occurred 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm and not in higher concentration (50 and 25 ppm). The length of tunnels was 13.6, 19.3, 21.9, 25.1 and 28.3 mm in 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm, respectively; and 29.9 mm in control. Different concentrations used significantly affected the mean length of tunnels ($F= 25.91$; $P<0.05, 0.01$)

2) Number of tunnels In case of Termidor average number of tunnels were 0.45, 1.3, 1.5, 1.8, 2.2, 2.6, 3.05 in 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 respectively. The number of tunnels were 3.6 in control. Different concentrations of termidor significantly affected the number of tunnels. ($F=21.48$; $P<0.05, 0.01$)

In case of biflex the average number of tunnels were 1.1, 1.5, 1.6, 1.7 and 1.9 in 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm respectively and 2.3 in control. No tunnel was observed in 50 and 25 ppm. Different concentrations of biflex significantly affected the number of tunnels. ($F=5.24$; $P<0.05, 0.01$)

3) Cumulative tunneling distance: In case of Termidor the cumulative distance was 124.57 mm in control and 10.63, 42.42, 51.14, 67.28, 78.43, 85.85 and 95.71 mm in soil treated with 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm, respectively Different concentrations of Termidor significantly affected the cumulative tunneling distance at each concentration. ($F= 31.15$; $P<0.05, 0.01$)

In case of Biflex the cumulative distance was 89.8 mm in control and 41, 58.2, 65.8, 75.2 and 85.1 in 12.5, 6.25, 3.125, 1.5625 and 0.78125 ppm and no tunneling was observed in soil treated with 50 and 25 ppm. Different concentrations of Biflex significantly affected the cumulative tunneling distance at each concentration. ($F=31.98$; $P<0.05, 0.01$)

4) Survival: The survival of *H. indicola* in the soil treated with different concentrations of termidor was significantly different. ($F= 15.82$; $P<0.05, 0.01$). Similarly biflex in different concentrations also influenced survival of termites. ($F=5.70$; $P<0.05, 0.01$). Soil treated with 50 and 25 ppm of termidor was not repellent whereas the same concentration of biflex did not allow termite to tunnel in the treated soil.

DISCUSSION

Jones (1988) studied tunneling ability of subterranean termites i.e., *Coptotermes formosanus* and *Retieulitermes flavipes* through termiticide treated soil and reported that *C. formosanus* workers tunneled significantly farther in Chlordane treated soil than did *R. flavipes*. Both species did not penetrate soil treated with Permethrin. It indicates that different species réspond differently against the same doses. Kard *et al* (1989) have also reported that organophosphates and pyrethroids provide 100% control for at least five years at same rates.

Beal (1980) tested effectiveness of Chlorpyrifos as a soil treatment for subterranean termites Protection at 6 localities and found that chlorpyrifos gave protection for more than 5 years.

Grace *et al*. (1992) studied the effect of silafluofen against *C. formosanus* Shiraki. They found that tunneling was reduced at 10 ppm but no significant mortality occurred in forced exposure assay. Whereas, 500 ppm concentration of silafluofen is required to prevent complete termite penetration and $\geq$1000 ppm to limit the tunneling distance to 1 cm or less and such concentration would be appropriate for field test.

The present studies with *H. indicola* indicate that 50 ppm of biflex prevent termite penetration, but the same concentration of termidor did not interfere with the foraging activity of this termite and the insecticide can be carried to nest mates.

Table 1: **Tunnel construction and survival of termites (*Heterotermes indicola*) (X±S.D) after exposure to Termidor treated soil for 7 days.**

Concentration (ppm)	Cumulative Tunneling distance (mm)	Max. Tunnel Length (mm)	Number of tunnels	Survival	
				Normal	Affected
Control	124.57±18.64	41.5±6.2	3.6±0. 71	96±1.53	4±1.53
50	10.63±11.34	3.6±4.0	0.45±0.52	76.3±7.7	23. 7±7.7
25	42.42±20.09	14.11±6.6	1.3±0.24	81.0±4.7	19.0±4.7
12.5	51.14±18.45	17.02±6.1	1.5±0.45	83.0±3.6	17.0±3.6
6.25	67.28±16.72	22.38±5.56	1.8±0.53	85.0±3.4	15.0±3.4
3.125	78.43±15.66	26.11±5.22	2.2±0.73	86.5±3.5	13.5±3.5
1.5625	85.85±14.62	28.58±4.88	2.6-+0.71	90.0±3.8	10.0±3.8
0.78125	95.71±14.94	31.8±4.96	3.05±0.61	93. 2±3.2	6.8+-3.2

Table 2: **Tunnel construction and survival of termites (*Heterotermes indicola*) (X±S.D) after exposure to Biflex treated soil for 7 days.**

Concentration (ppm)	Cumulative tunneling distance (mm)	Max. Tunnel Length	Number of tunnels	Survival	
				% Normal	Affected
Control	89.8±1.6.96	29.9±5.64	2.3±0.73	98.1±0.86,	1.9±0.86
50	0	0	0	65.4±16.3	34.5±16.3
25	0	0	0	68.7±15.0	31.2±15.0
12.5	41.0±23.31	13.6±7.76	1.1±0.57	70.9±14.17	29.1±14.17
6.25	58.2±19.81	19.3±6.6	1.5±0.66	73.9±12.85	26.1-+12.85
3.125	65.8±17.28	21.9±5.77	1.6±0.50	77.5±12.42	22.5±12,45
1.5625	75.2±18.09	25.1±6.03	1.7±0.63	81.6±9.3	18.4±93
0.78125	85.1±18.96	28.3±6.3	1.9±0.72	87.1±5.92	12.8±5.92

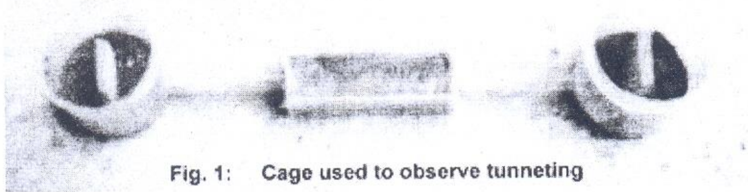

Fig. 1: Cage used to observe tunneting

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