



## RESEARCH PAPER

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## Laboratory evaluation of chitin synthesis inhibitor triflumuron and diflubenzuron in mortality of individuals termite *Microcerotermes diversus* (SILV.) (Isoptera: Termitidae)

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### Abstract

The study was conducted to evaluate the direct spraying effects of Triflumuron and Diflubenzuron at 250, 500, 1000 and 2000 ppm on the mortality of alates and workers of termite *Microcerotermes diversus* (Silv.) at  $30 \pm 3^\circ\text{C}$  and  $85 \pm 5\%$  R.H. The results showed that all concentrations of Triflumuron and Diflubenzuron have caused slow and gradual mortality to termite individuals. Hundred percent mortality in termite workers was achieved with all concentrations of both Triflumuron and Diflubenzuron after three weeks of application, except that for the concentration 250 ppm of Diflubenzuron, where the achievement of 100% mortality was taken 2 weeks only. It was found that early mortality in the first week was connected with the concentration so increasing Triflumuron concentration induced increasing in mortality, while increasing Diflubenzuron induced decreasing in mortality. All concentrations of Triflumuron and Diflubenzuron caused deformations in workers, abdomen swollen, abnormal shape, discolored to white lackluster associated with slow movement. High mortality of termite alates have exhibited by the two CSIs reached to 100% after two weeks of application. The termite alates were found to be more sensitive to Triflumuron and Diflubenzuron than workers. All concentration of the two CSIs caused failure eggs hatch laid by termite alates.

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## Introduction

The termites especially species *Microcerotermes diversus* (Silvestri) were considered among the most important economic insects in all regions of Iraq that causing heavy economic losses in old and modern building, fruit trees and field crop plants (Al-Jassany, 1996). It was reported that this species was the most important and wide spread in Iraq, Iran and Oman causing destruction of wooden structures (Edwards, R. A. and E. Mill., 1986). Also the species was reported to spread in Africa and Malezia (Gurbel, 2008) and united Arab Emirates (Kaakah, 2006). The losses and injuries caused by termite in the world were estimated to be about 22 Billion dollars yearly (Su, N. Y. 2003). Termites consumed approximately seven millions tons of biomes yearly mainly wood and waste wood (Horowitz *et al*, 1992). Many termiticides belong to different chemical groups were used to control termites in the worlds, but according to their high toxicity, environmental pollution and long persistence periods of these compounds, efforts of many investigators were oriented for searching of alternative technologies including bait stations systems for termite control through using pathogens, insect growth regulators and some poisons with slow action (Lenz *et al.*, 1996). IGRs were found to be the more suitable chemicals of slow action and preferred to be used in bait stations to control termite, that in addition to its direct effects on termite colony, affect colony dynamic (Perrot, 2003). Because Motivation and aims of the study of the high importance of *M. diversus* (Silv.) in Iraq and the problems recorder by termiticide used in termite control especially in the houses and building, the research aimed to evaluate efficacy of Triflumuron (Alsystin) and Diflubenzuron (Dimilin) against termite individuals under laboratory conditions.

## Materials and methods

### *Source of termite individuals and adaptation to laboratory condition*

Dead stalks of orange tree at high infestations with termites were collected. The stalks containing large numbers of termite individuals (workers and winged) were cut in to small piece.

The pieces were placed in a glass basins (60 x 30 x 40)cm, its four lateral sides were encapsulated with aluminum foil (cellophane), which the tops was covered with metal plate to provide complete darkness for the insects (Yanagawa; F. Yokohari and S. Shimizu, 2009.). Termite individual's activity in the basin, new tunnel constructions, removing dead and abnormal individuals were observed daily. The termites in the basin were moderately sprayed with distilled water as needed for maintaining coverable moisture. The termites were maintained 7-14 days under laboratory conditions for adaptation.

### *Termites media preparation*

Small piece of smooth eucalyptus tree *Eucalyptus camadulensis* (preferred by termites) was cut into small pieces, ground to fine (0.01 mesh size) sawdust particles and transferred in to glass petri plates. The sawdust was oven dried sterilized at 120°C for 48 hours (Su, N. Y. 2003). Fifty grams of sterilized fine sawdust were mixed well with 4% agar solution in petri-plate of 20 cm diameter containing filter paper (Su and Schefferh. 1993)

### *Adaptation and rearing of termite individuals on termite media in laboratory*

After solidification, large numbers of termite (worker and winged) were transferred in to the plate from wooden piece containing termite by shaking or by fine brush. The plates was encapsulated with cellophane and maintained incubator at 30 ± 3°C and 85 ± 5% for a week before the starting of the treatment for adaptation. The activity of termite individuals, removing dead and abnormal individuals, were followed daily.

### *Insect growth regulators used in the study*

Triflumuron (Alsystin 480sc, Bayer crop science) and Diflubenzuron (Dimilin 150sc, Chemtura Company) at 250, 500, 1000 and 2000 ppm, chitin synthesis Inhibitor (CSI) belong to benzoyl phenyl urea (BPU), non-systemic compounds acts through the stomach (Tomlin, C. D. S. 2000).

*Application method and efficacy evaluation*

Thirty six petri-dishes, 9 cm diameter and 1 cm a depth (4 plates for each concentration) were prepared. A mixture of 5 gm of wood sawdust with 10 ml of 4% agar solution was poured in each plat. After solidification, 50 adapted workers and 10 alates (winged) termite were transferred into each plate. The termite in the plates were directly sprayed with IGRs concentration (5ml/plate) and covering the medium surface, using small hand sprayer of 10ml capacity. The plates were then closed, enveloped with cellophane and maintained in an incubator at 30 ± 3°C and 80 ± 5% R.H. For evaluation the efficiency of Triflumuron and Diflubenzuron on individual's mortality, the number of dead and living individuals was recorded every two days until the death of all individuals.

The mortality percentages were corrected on the basis of dead individuals number in control treatment due to Abbott equation (Abbot, W. S. 1925).

*Statically analysis*

Statically program Genstat 5 was used in analysis of results and compared by LSD 0.05.

**Results and discussions**

*Efficiency of Triflumuron (Alsystin) and Diflubenzuron (Dimilin) against termite workers*

Results showed that Alsytin and Dimilin were characterized by high efficiency as proved by gradual mortality of termite workers. The speed of mortality was found depending on IGR concentration and exposure duration. It has been observed that the mortality percentages were low at the beginning of treatment but gradually increased with the time (Table 1).

**Table 1.** Mortality Percentages of termite workers in different treatments at different time after treatment.

Chitin synthecis inhibitor (CSI)	Concentration (ppm)	Mortality percentages during exposure time (week)			Average
		1	2	3	
Alsystin	250	43.7	70.3	100	71.3
	500	45.7	61.7	100	69.1
	1000	66.0	97.0	100	87.7
	2000	83.3	99.0	100	94.1
Dimilin	250	12.3	47.7	100	53.3
	500	19.3	60.3	100	59.9
	1000	24.0	62.0	100	62.0
	2000	40.3	88.7		76.3

LSD 0.05 for exposure time 6.90

LSD 05 for concentration 11.27

LSD 05 for interaction 19.53

The results of the study showed that in the first week of treatment the mortality percentage have increased with increasing Alsystin concentration and decreased with increasing Dimilin concentration. The mortality percentages continued to increase reaching 100% in all Alsystin concentrations (250, 500, 1000 and 2000) ppm after 3 weeks, while in the treatment of Dimilin mortality percentage 100% has been achieved after 2 weeks of application at 250 ppm and after 3 week of application at 500, 1000 and 2000 ppm (Table 1).

Also it was recorded that for the duration of many workers indicated that termites exposed to different concentrations of Alsystin and Dimilin showed abnormalities, swollen abdomen, distorted, workers color turned to white pale associated with slow movement. The increasing of workers mortality percentages at the beginning of treatment with increasing of Alsystin concentration can be attributed to the absence of repellent effects of Alsystin, while the decreasing mortality percentages with increasing

Dimilin concentration may attributed to the repellent effect and prevention of feeding on treated termite media. (Lenz *et al.*, 1996) observed that the influence of Alsystin was increased with increasing of concentration, from 100-5000 ppm when used in the treatment of baits consumed by termite workers of *Coptotermes acihaciforms* and did not have any repellent effect. Su and Schefferhn (N. Y. and Schefferhn, 1993) found that termite *C. formosanus* moved away when feeding on woods treated with Diflubenzuron at 500 and 1000 ppm. The gradual mortality of termite workers can be attributed to the toxic and physiological effects of Alsystin and Dimilin on workers sprayed or feeding on treated media. Horowitz *et al.*, 1992 found that IGR can reach insect body through feeding on food sprayed with IGR solution and the high percentage of mortality can be attributed to the mechanical effect of IGR spray (Lenz *et al.*, 1996).

Found that termite *C. acinciforms* groups were killed through 6 weeks of feeding on wood pieces treated

with Triflumuron at 100-5000 ppm. Rojas and Morales-Ramos (Rojas and Morales-Ramos, 2001) indicated that CSIs Hexaflumuron, Diflubenzuron and Chlorflumuron added to wood bait at 250 ppm causing 100% mortality during 9 weeks in termite *C. form osanus*. Madden (Madden, W. R. 1999) showed the direct dusting of Triflumuron powder at 900-6300 ppm caused elimination 6 of 7 colonies treated.

*Efficiency of Triflumuron (Alsystin) and Diflubenzuron (Dimilin) in mortality of alate (winged) termite*

Results of the study revealed that the direct spraying of all concentrations of Alsystin and Dimilin caused high mortality in termite alate depending on the concentration and exposure time. Alsystin was found to be more efficient than Dimilin in the first week of treatment as shown by the higher mortality percentage in termite individuals but all the concentrations of the two compounds caused 100% mortality after 2 weeks of treatment (Table 2).

**Table 2.** Mortality percentages of termite alate in different treatments at different time after treatment.

Chitin synthesis inhibitor CSI	Concentration (ppm)	Mortality percentage during exposure time (week)		Average
		1	2	
Alsystin	250	33.3	100	66.7
	500	60.0	100	80.0
	1000	60.0	100	80.0
	2000	70.0	100	85.0
Dimilin	250	33.3	100	66.7
	500	36.7	100	68.3
	1000	40.0	100	70.0
	2000	50.0	100	75.0

LSD 0.05 for exposure time 6.52

LSD 0.05 for concentration 13.04

LSD 0.5 for interaction 18.44

It was also observed that all concentrations tested caused failure of egg hatching laid by termite alates. (Lenz *et al.*, 1996) noted that the influence of Triflumuron in termite colony started by elimination of reproductive individuals and small instars followed by elimination of new workers, while the mature workers and soldiers stay alive for longer period. Horowitz *et al.* (Horowitz *et al.*, 1992) pointed out that BPU compounds proved high effectively on

eggs through its effect on chitin in the egg shell of some insect species. Rojas and Morales-Ramos (Rojas and Morales-Ramos, 2001) found that feeding of primary reproductive on food treated with Diflubenzuron at 10 ppm caused failure of all eggs hatching laid by small queens. Romijn and Sissoko (Romijn and Sissoko, 1990) pointed that spraying and baits of Diflubenzuron showed high efficacy against locusts.

It can be concluded that the direct spraying of the Triflumuron (Alsystin) and Diflubenzuron (Dimilin) at 250 ppm on alates and workers of termite *Microcerotermes diversus* (Silv.) in termite media have leading to gradual mortality of termite individual and recommended to be used as safe compounds alternative to chemical pesticides for controlling termite in homes.

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