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Toxicity and Field Efficiency of Certain Insecticides Against the Subterranean Termite, *Psammotermes hypostoma* Desneux (Isoptera: Rhinotermitidae)

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ABSTRACT

The subterranean termite, *Psammotermes hypostoma* Desneux (Isoptera: Rhinotermitidae) is considered one of the most dangerous termites in urban, rural and agricultural areas in the desert belt of Assiut governorate, Egypt. Organophosphate insecticides such as chlorpyrifos have been recommended to control subterranean termites in Egypt. The present research was conducted to evaluate the toxicity, persistency and effectiveness of chlorpyrifos (chlorzan 48% EC), imidacloprid (imidammex 70% WG), chlorantraniliprole (coragen 20% SC), indoxacarb (avaunt 15% EC) and spinosad (tracer 24% SC) against subterranean termites. The toxicity of these insecticides was tested against field strain of the subterranean termites using the cardboard dip-bioassay technique under laboratory conditions. The toxicity index after 12 and 24 hr exposure showed that chlorpyrifos has the highest termiticidal activity, with LC_{50s} 1.19 and 0.13 ppm, followed by imidacloprid with LC_{50s} 3.24 and 0.61 ppm, while spinosad was the least toxic one with LC_{50s}.9.96 and 4.95 ppm. Furthermore, chlorpyrifos, imidacloprid and chlorantraniliprole registered significantly high percent reduction of the infested rural homes by subterranean termite, *P. hypostoma*, while indoxacarb and spinosad recorded the lowest reduction percentage after 30, 60, 90 and 120 days of treatment. In addition, the efficiency and residual effects of chlorpyrifos persisted up to 120 days followed by imidacloprid and chlorantraniliprole after treatment. Results of this study indicated that, chlorpyrifos exhibited the most powerful pesticide among all the insecticides tested, and imidacloprid and chlorantraniliprole were highly effective and it is given as unique and hopeful trends against subterranean termites under field conditions.

INTRODUCTION

The subterranean termite, *Psammotermes hypostoma* Desneux (Isoptera: Rhinotermitidae) is classified as one of the most destructive termites in urban, rural and agricultural areas not only in Egypt but also extended to all over the world. In Egypt, It causes great damage to both villagers' dwellings and agriculture especially in the cities and villages near the desert belt (Rizk *et al.*, 1982; Abdel Galil, 1986; Ahmed *et al.*, 2014 and 2015). Annually, to control this pest some countries cost millions of dollars owing to it causes economic effects in both rural and urban homes and agricultural crops (Su, 1994; Su and Scheffrahn, 1998; Ahmed *et al*., 2014; 2015). In the last twenty years, the subterranean termites caused great damage to many houses and buildings in several Egyptian regions like Cairo, Alexandria, Port-Said, Damanhour, New Valey, Assiut, Quna, Luxor, Asswan, and Sinai, (Ahmed and Mohany, 2008; El-Bassiouny *et al*., 2011; Ahmed *et al.*, 2014; 2015; Ghesini and Marini, 2017). Organophosphorous pesticides are the most toxic to vertebrate animals (Malhat and Nasr, 2013). Chlorpyrifos is one of these pesticides which are highly toxic (WHO, 2020) and recommended to control subterranean termites in Egypt. Chlorpyrifos is an insecticide that is a (1B) cholinesterase inhibitor with direct contact, stomach or respiratory action (IRAC, 2020). The heavy use of chlorpyrifos leads to different damage, including environmental pollution, the development of resistance of pest to insecticides, pest resurgence, and direct hazard to users. The use of the new different insecticide groups, such as neonicotinoids, is deemed the right path in respect of developing a new strategy to avoid the development of pest resistance (Rust and Saran, 2008; Smith *et al*., 2008; Ahmed *et al*., 2014; 2015; Hassan *et al*., 2018).

Imidacloprid is a wide neonicotinoid insecticide used all over the world, based on a unique combination of characteristics (Elbert *et al*., 1991; Cox *et al*., 1998) which has a novel mode of action nAChR competitive modulators (4A, IRAC, 2020), excellent systemic and contact activity, low application rates, long residual control, strong binding to soil organic matter and favorable toxicological and environmental profiles. Imidacloprid recommended for controlling the termites began in the late 1980s (Zeck, 1992), in Japan in 1993, and it was registered in many other countries such as United States, Australia. The soil treatment by imidacloprid has been evaluated against more than 20 species of termites (Reid *et al*., 2002; Ahmed *et al*., 2014; 2015; Hassan *et al.*, 2018).

Chlorantraniliprole is from the diamide group of insecticides with a novel mode of action that targets and ryanodine receptor modulators (28, IRAC, 2020), causing the release of internally stored calcium. The release of stored calcium causes loss of muscle control leading to rapid feeding cessation, lethargy, partial paralysis, cardiac muscle failure, and regurgitation (Cordova *et al*., 2006). Chlorantraniliprole has been demonstrated no repellency and delayed action in laboratory studies against subterranean termites (Quarcoo *et al*., 2010), and the toxicity and horizontal transfer (Buczkowski *et al.*, 2012). The bioavailability in various soil has been examined (Spomer *et al*., 2009), as well as the potential of toxic transfer has been investigated (Gautam and Henderson, 2011).

Indoxacarb belongs to the oxadiazine chemical family, which has low ecotoxicological risks. The mode of action of indoxacarb is voltage-dependent sodium channel blockers (28A, IRAC, 2020). Indoxacarb action selectively towards insects and high quickly degrade in animals to inactive metabolites. This rapid metabolic degradation is a pivotal factor for the safety of higher non-target organisms including humans (Wing *et al.,* 2000). Indoxacarb has been evaluated against different subterranean termites for the last few years. The efficiency and non-repellency of this insecticide treated soil against, *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar) (Hu, 2005; Misbah-Ul-Haq *et al*., 2016, Quarcoo *et al*., 2019). Also, Hu *et al*. (2005) stated that, indoxacarb transfer among the subterranean termite workers.

Spinosad is a fermentation metabolite of the soil-inhabiting actinomycete, *Sacharopolyspora spinosa* and acts as a contact and stomach poison. It has a novel molecular structure and mode of action that nAChR allosteric modulators (5, IRAC, 2020) and provides excellent crop protection typically associated with synthetic insecticides. In addition, a few studies were done to determine the efficiency of spinosad on subterranean termites (eg. Scheffrahn *et al*., 1997; Scheffrahn and Thoms 1999; Iqbal and Saeed 2013; Bhatta *et al*., 2016).

The main aim of the present studies to compare the toxicity, persistency and effectiveness of chlorpyrifos (chlorzan 48% EC), imidacloprid (imidammex 70% WG), chlorantraniliprole (coragen 20% SC), indoxacarb (avaunt 15% EC), and spinosad (tracer 24% SC) against the subterranean termites, *P. hypostoma* and find alternatives to the broadly used insecticides.

MATERIALS AND METHODS

Study Site:

The field experiments were conducted in Almatmar village, Sahel Selim city at Assiut governorate (24 km east south from Assiut City), and the toxicity experiment was conducted in the laboratory of the Plant Protection Department, Faculty of Agriculture, Assiut University.

Termite Collections:

Field strain of subterranean termite, *P. hypostoma* were collected by El-Sebay trap, (El-Sebay, 1991) (Fig. 1) from the infested home of Almatmar village, Sahel Selim city at Assiut governorate, during March 2019. Polythene bags were used to transfer infested cardboard rolls (El-Sebay trap) with termites to the laboratory of the Plant Protection Department, Faculty of Agriculture, Assiut University. Termites were separated by small brush from the cardboard rolls and kept in Petri-dishes (9 cm diameter) provided with moistened corrugated cardboards as a source of cellulose and moisture, then, the health workers were used in the bioassay.

Fig. 1. El-Sebay traps were used for collecting the field strain of subterranean termite**,** *P. hypostoma* from an infested home of Almatmar village, Sahel Selim city at Assiut governorate, Egypt during May 2019.

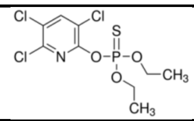
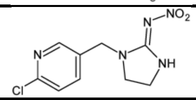
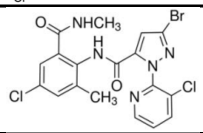
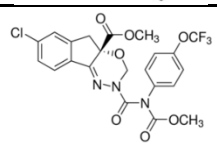
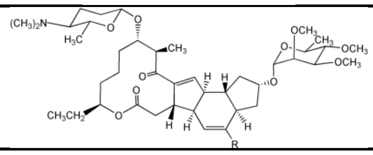
Insecticides:

For the bioassay and the field experiments, commercial formulations of chlorpyrifos (Chlorzan 48% EC, Coromandel International Limited, Kafr Elzayat Com.), imidacloprid (Imidammex 70% WG, MAC-GmbH) chlorantraniliprole (Coragen 20% SC, DuPont de Nemours, France), indoxacarb (Avaunt 15% EC; FMC Corporation, USA) and spinosad (Tracer 24% SC, Dow AgroSciences, UK) were used (Table 1).

Laboratory Bioassay:

The cardboard dip-bioassay technique was used to evaluate the efficacy of insecticides in the field against subterranean termite, *P. hypostoma* the results are reported separately. Reported here are the results of laboratory tests to determine the concentration of these insecticides that are required to kill 50% (LC_{50}) and 90% (LC_{90}) of subterranean termite, *P. hypostoma* worker with a modification in the toxicity tests. Six concentrations of aqueous solution of each insecticide (0.01, 0.1, 1, 10, 100, 1000 mgL^{-1}) were used. For each concentration of pesticides used, cardboards (5 cm x 5 cm) were dipped for 10 seconds in the concentration and left to dry under laboratory conditions for about half an hour, and then placed in plastic cans covered with a sieved lid. Fifty healthy termite workers (same size and shape) with 3 replicates of the same termite colony were transferred to these plastic cans and held for 24 hr at 22 + 2 °C, 60 + 5 RH% and photoperiod 12:12 (L:D). Control cardboards were similarly dipped in a solution of distilled water. The toxicity experiment of each insecticide was repeated twice. The termite mortality was recorded after 12 and 24 hr of treatment. A termite worker was considered dead if it was incapable of coordinated forward movement. The results were corrected by Abbott's formula (Abbott, 1925). Median lethal concentrations (LC_{50}) and slope values of these insecticides were determined by the Probit regression analysis using the software SPSS (2017) (Version 16.0 for windows, SPSS Inc., Chicago, the USA) and expressed as parts per million (ppm) (Fenney, 1971). The toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) 100] (Sun, 1950).

Table 1. Common name, group, mode of action and chemical structure of insecticides used against subterranean termite**,** *P. hypostoma***.**

Common name	Group	Mode of Action*	Chemical Structure
Chlorpyrifos	Organophosphates	**(1B) AChE inhibitors**	
Imidacloprid	Neonicotinoids	**(4A) nAChR competitive modulators**	
Chlorantraniliprole	**Diamides**	**(28) Ryanodine Receptor modulators**	
Indoxacarb	**Oxadiazines**	**(22A) Voltage-dependent sodium channel blockers**	
Spinosad	Spinosyns	**(5) nAChR allosteric modulators Site I**	

* IRAC Mode of Action Classification Scheme, Version 9.4, Issued, March 2020, AChE: Acetylcholinesterase, nAChR: Nicotinic acetylcholine receptor

Field Treatments:

The field experiments were conducted in Almatmar village (24 km east south from Assiut City) Sahel Selim city, at Assiut governorate, Egypt during 2019 for the infested rural homes by subterranean termite, *P. hypostoma*.

Examining and Exploring the Area Affected by Termites:

An old building was tested in the village of Almatmar village, Sahel Selim city at Assiut Governorate during May 2019, and the area was explored by planting exploratory fisheries (the cartoon fish trap) in a chess method at dimensions (1 meter) between the trap and the other for a month before the experiment began to ensure that the area was infected and to locate colonies under the soil. The exploration revealed that the area is highly infested with underground termites (Fig. 1).

Field Trials, Experimental Design and Termite Inspection:

All effective method of control is by making a ditch 30 cm width X 30 cm depth surrounding the home. Each insecticide has one concentration (2%) and five replicates (5 meters) for each one and control treatment. Each one longitudinal meter was treated with 4 liters of insecticide solution. Five corrugated board traps (El- Sebay, 1991) for five meters were used for each insecticide as bait traps hence, soil returned back after treatment. Corrugated cardboard was prepared where wrapped in a roll shape (10 cm high and 7 cm in diameter). Corrugated card board was wetted with water and closed with a plastic page before buried in a ditch (12 cm depth in the soil, the plastic cover of traps was appeared at the same level of the soil surface and numbered for a marking sign of traps. Traps were served as a food source of cellulose material and humidity which attracted the subterranean termite to the soil surface. Treatment was conducted on 15 June 2019 and inspections were made after 30, 60, 90- and 120-days intervals. The percentages of infestation reduction were calculated according to Abbott's equation (1925).

$$\text{Reduction } (\%) = \left(\frac{\text{Total No. of traps} - \text{No. of infected traps}}{\text{Total No. of traps}}\right) \text{x } 100$$

Statistical Analysis:

Data were analyzed using one-way ANOVA and presented as mean ± SEM (standard error mean). Means were separated by Duncan's multiple range test (DMRT). Figures and statistical analysis were done using Graph Pad Prism 5TM software (San Diego, CA)

RESULTS AND DISCUSSION

Comparative Toxicity of Certain Insecticides Against *P. hypostoma*:

Results in Table 2 represent the toxicity of imidacloprid, chlorantraniliprole, indoxacarb and spinosad compared with chlorpyrifos against subterranean termite after 12 and 24 hr using a cardboard-dip bioassay. The LC_{50} values after 12 and 24 hr for the tested insecticides, showed that, the most toxic insecticide was chlorpyrifos (1.19 ppm and 0.13 ppm) followed by imidacloprid (3.24 ppm and 0.61 ppm), chlorantraniliprole (6.35 ppm and 1.41 ppm), indoxacarb (7.10 ppm and 2.17 ppm) and spinosad (9.96 ppm and 4.95 ppm). The LC_{50s} for and chlorantraniliprole are not significantly different (95% FL overlap), while the LC_{50s} for chlorpyrifos, imidacloprid insecticides are significantly different from indoxacarb and spinosad after 12 h (95% FL did not overlap). In addition, after 24 hr the LC_{50s} for chlorpyrifos and imidacloprid are not significantly different, whereas the LC_{50s} for chlorpyrifos are significantly different from chlorantraniliprole, indoxacarb and spinosad. In general, chlorpyrifos, imidacloprid and chlorantraniliprole showed the most potent insecticides while, indoxacarb and spinosad were the lowest toxic insecticide among all tested pesticides. Based on the toxicity index, chlorpyrifos was more toxic than imidacloprid, chlorantraniliprole, indoxacarb and spinosad by 2.72, 5.34, 5.96 and 8.37 folds after 12 hr, respectively and by 4.69, 10.85, 16.69 and 38.02 folds after 24 hr, respectively (Table 2). These results indicated that chlorpyrifos was the most potent insecticides against subterranean termite, *P. hypostoma* with potencies 8.37 and 38.02-fold higher than spinosad after 12 and 24 hr followed by imidacloprid, chlorantraniliprole.

Table 2. Toxicity of certain insecticides against the subterranean termite, *P. hypostoma*, using cardboard-dip bioassay after 12 and 24 h exposure.

Insecticides	After 12 hr			After 24 hr		
	Slope ± SE	LC_{50} (ppm) (95% FL)	Toxicity index	Slope ± SE	LC_{50} (ppm) (95% FL)	Toxicity index
Chlorpyrifos 48% EC	1.88 ± 0.04	1.19 (0.35-3.10) a	100.00	1.76± 0.05	0.13 (0.07-0.33) a	100.00
Imidacloprid 70% WG	1.50 ± 0.03	3.24(1.01-7.05) a	36.73	1.35± 0.04	0.61(0.36-0.98) b	21.31
Chlorantraniliprole 20% SC	0.88 ± 0.04	6.35(3.01-9.95) ab	18.74	0.81± 0.06	1.41(1.01-2.33) c	9.22
Indoxacarb 15% EC	0.98 ± 0.05	7.10(4.35-10.18) ab	16.76	0.95± 0.04	2.17(1.67-3.10)c d	5.99
Spinosad 24% SC	1.25 ± 0.06	9.96(7.35-12.56) bc	11.95	1.11± 0.04	4.95(2.85-7.65) d	2.63

Notes: FL: fiducial limits, toxicity index = [(LC_{50} of the most toxic tested insecticide/LC_{50} of the tested insecticide) X 100]. LC_{50} values in each column having different letters are significantly different (95% FL did not overlap)

Our results are in the same line with the results reported by Ahmed *et al.* (2014), who studied the toxicity of chlorpyrifos 48% EC and imidacloprid 20% SL on the subterranean termite, *P. hypostoma*, after 3, 6, 12 and 24 hr using the cardboard-dip technique. The LC_{50} value of chlorpyrifos was 28.29 ppm and 0.36 ppm at 3 and 24 hr, and imidacloprid showed the most toxic neonicotinoid pesticide with LC_{50} value 50.95 ppm after 3 hr and decreased to 0.82 ppm after 24 h of treatment. Chlorpyrifos (48% EC) exhibited the most potent pesticide among the pesticides used, whereas imidacloprid (20% SL) was the most potent among the neonicotinoid pesticides against *P. hypostoma.* Whereas Iqbal and Saeed (2013) showed that, the LC_{50} values of imidacloprid against *Microtermes mucophagus* Desneux workers were in the range of 12.59-24.06 ppm. Gautam and Henderson (2011) reported that, chlorantraniliprole exhibited a more delayed mortality on termites than fipronil in the sand. Buczkowski *et al.* (2012) showed that, chlorantraniliprole exhibited a highly toxic to workers of termites in short and continual exposure assays towards a range of doses from 5 to 100 ppm. They stated that, chlorantraniliprole was highly active by contact and feeding. In addition, a few studies were done to test the effect of indoxacarb on a subterranean termite. Iqbal and Saeed (2013) showed that, the LC_{50} values of indoxacarb ranged from 8.72-9.57 ppm. In a comparison of the different termiticides, on *M. mucophagus*, the LC_{50} of spinosad was low, ranging from 3.24 - 3.72 ppm (Iqbal and Saeed, 2013). Scheffrahn and Thoms (1999) showed that, spinosad applied in wood galleries in lab bioassays at 0.23% and 0.5% resulted in 98-100% mortality for dry wood termites, *C. brevis* and *I. synderi*. Interestingly, at present, there is few published studies available on the effects of chlorantraniliprole, indoxacarb and spinosad on a subterranean termite, *P. hypostoma*. The difference in toxicity may be due to the difference in concentration or species of termite or the formulation types or the mode of action of insecticides.

Efficiency and Persistency of Certain Insecticides against *P. hypostoma*:

The present studies were conducted to evaluate the efficiency and persistency of imidacloprid (70% WG), chlorantraniliprole (20% SC), indoxacarb (15% EC) and spinosad (24% SC) compared with chlorpyrifos (48% EC) against subterranean termites *P. hypostoma*, when treatment in the soil at a different time interval. Data represented in Figure (2) showed that all treatments caused a significant reduction in termite after 30, 60, 90 and 120 days after treatment (DAT). At 30 DAT all insecticides caused 100 % reduction in a subterranean termite. After 60 days of treatment, chlorpyrifos (48% EC), imidacloprid (70% WG) chlorantraniliprole (20% SC), recorded 100% of reduction, whereas indoxacarb (15% EC) and spinosad (24% SC) recorded 80 and 80 % of reduction. After 90 days of treatment, chlorpyrifos and imidacloprid recorded 100% of reduction, whereas chlorantraniliprole, indoxacarb and spinosad recorded 80, 60 and 60 % of reduction% respectively. Furthermore, after 120 days of treatment, chlorpyrifos recorded 100% reduction, by contrast, imidacloprid, chlorantraniliprole, indoxacarb and spinosad recorded

80, 60, 40 and 40% of reduction %, respectively. These results indicated that, chlorpyrifos, imidacloprid and chlorantraniliprole registered significantly high percent reduction of the infested rural homes by subterranean termite, *P. hypostoma*, while indoxacarb and spinosad recorded the lowest reduction percentage after 30, 60, 90 and 120 days of treatment. In addition, the efficiency of chlorpyrifos persisted up to 120 days, followed by imidacloprid and chlorantraniliprole.

The present studies revealed that imidacloprid and chlorantraniliprole are an effective termiticide and can be comparable with popular and commonly available chlorpyrifos 48 EC which has been used generally in the past until recently. Chlorpyrifos 48 EC at 4 Liter per 1 meter 30 cm width X 30 cm depth is recommended by household subterranean termite's operator in Egypt. Chlorpyrifos is also a reference insecticide and is usually included in bioassay and field treatments for comparison with another intended termiticide (Ahmed *et al*., 2014; Manzoor and Pervez, 2014). Chlorpyrifos is repellent termiticide and prevent termites' movement in soil and thus limit access to food which causes mortality (Ahmed *et al*., 2015; Ahmed *et al*., 2017). In agreement with the present study Hassan *et al.* (2018) stated that the efficiency of imidacloprid and chlorpyrifos in three different types of soils (sand, loam and clay) was determined after 2, 4, 6, 8, 10 and 12 weeks after exposure of termites. They showed that, at each time interval, the interaction of concentrations in three different soils in terms of mortality of termites was found to be non-significant at different time intervals. Gautam and Henderson (2011) reported that, chlorantraniliprole caused a more delayed mortality than fipronil in sand termites. They also stated that chlorantraniliprole in soil did not purpose higher mortality at any of the examined concentrations through a 21-day test period when compared with controls. Buczkowski *et al.* (2012) showed that, on the untreated soil, termites consumed 79% of the available paper cellulose in 3 days; whereas termites on chlorantraniliprole treated did not feed any paper before they became accidental and die. Jones *et al.* (2017) showed that, the efficacy of chlorantraniliprole, in prevailing constructional damage of *R. flavipes* and determined the post-treatment fate of termite colonies in and around the structures provided effective structural protection as there was no further evidence of termite activity in and on the majority of structures from approximately 1 month to 2 years post-treatment.

Our results indicated that, indoxacarb and spinosad recorded the lowest reduction percentage after 30, 60, 90 and 120 days of exposure. Hu (2005) tested the efficiency and non-repellency of indoxacarb when treated the soil against, termites. Spomer *et al.* (2011) showed that, the subterranean termite species *R. flavipes* penetrated in different kinds of indoxacarb treated soils at various depths. The efficiency of spinosad was found normal because it did not kill termite workers on the spot (Saljoqi *et al.*, 2014). They can give time to termites to go back to the colony and allowed feeding their nestmates. Spinosad gave the required percentage mortality; therefore, it was the best insecticide among all tested insecticides. Our findings are in agreement with those of Sattar *et al.* (2002) who found that spinosad was found very effective as a slow-acting toxicant against subterranean termites.

In conclusion, the results of this study indicated that, chlorpyrifos was the most powerful pesticide among all the tested insecticides, and imidacloprid and chlorantraniliprole were highly effective and it is given as unique and hopeful trends in controlling structural infestations of subterranean termite, *P. hypostoma* under field conditions.

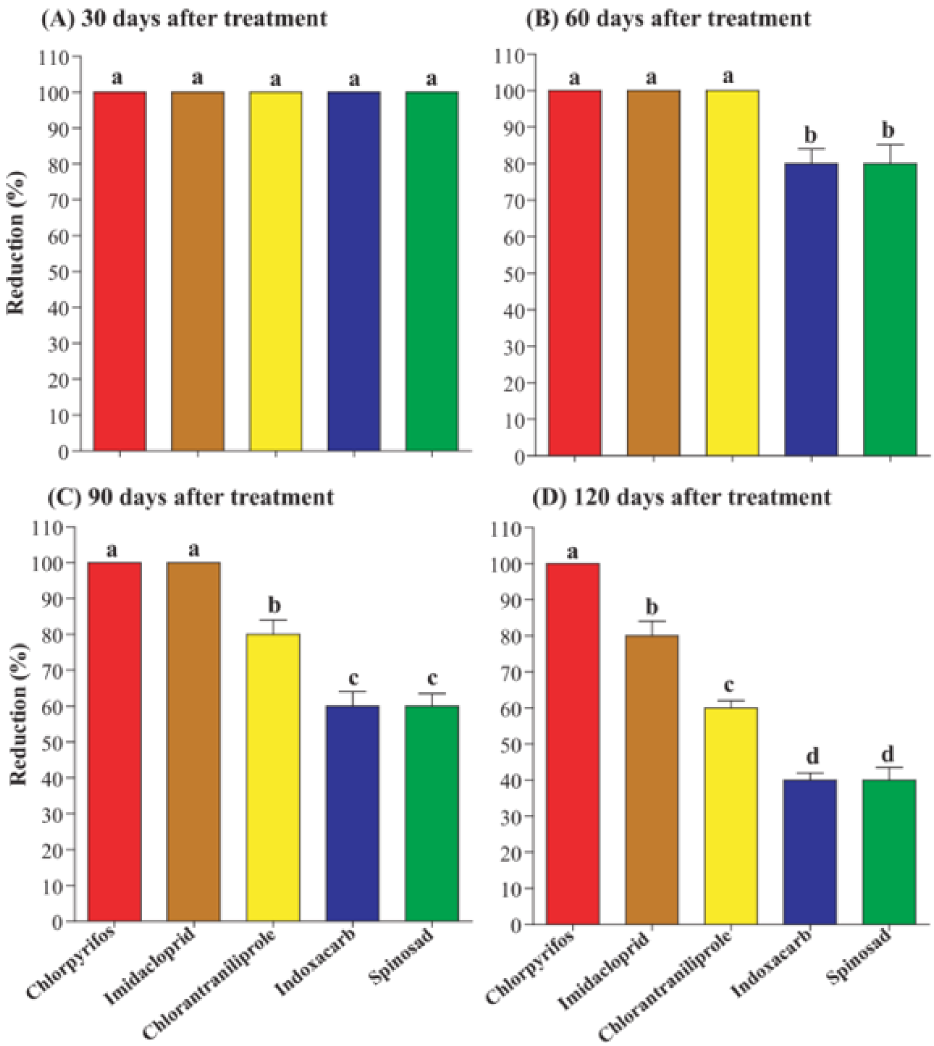


Fig. 2. Field efficiency and persistence of certain insecticides against the subterranean termite, *P. hypostoma* after 30 **(A),** 60 **(B),** 90 **(C)** and 120 **(D)** days after treatment. Reduction (%) ± SE according to Abbott's equation (1925). Columns headed by the same letter (s) within the same figure are insignificantly different (p= 0.05) according to DMRT.

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