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Evaluation of Spinetoram against *Odontotermes Brunneus* Hagen (Isoptera: Termitidae) in laboratory and field condition

Vijay Laxmi Rai, AK Karnatak and Shivashankara

Abstract
Field experiments were carried out during 2013-14 in N. E. B. Crop Research Centre of G.B.P.U.A.&T., Pantnagar (Uttarakhand) to evaluate the field efficacy of spinetoram 11.7% SC against termites in sugarcane crop. Sugarcane cultivar CoP 97222 was used for experiment. The plot size was 8 ×5 m^2, row to row distance 90 cm, 3 budded setts were used as planting material and date of planting was 20 March, 2013. The experiment was laid out in a randomized block design with four replications. Six treatments including control were allocated randomly to different plots in each of the three blocks. Foraging workers were collected from termitarium present near Practical crop production, campus of GBPUA&T, Pantnagar. The termite individuals were transferred separately in three glass jars (dia. 20 cm, height 15 cm) having an inner lining of moist filter paper. These jars with termite individuals were kept in laboratory at room temperature (25^{0}C and 80 ± 5% RH) in dark conditions. The top of the jar was covered with slightly moist muslin cloth. All termites were tested within three days of collection and only active and healthy termites were used for the experiments. Spinetoram belongs to spinosyn group, which have novel mode of action and are widely used for the management of various agricultural pest. In this study, Spinetoram 11.7 w/w evaluated for their efficacy in laboratory as well as in sugarcane crop field. In the laboratory test, it was found that at 24HAE, efficacy of Spinetoram (LC_{50} 31.9 ppm) was higher as compare to Imidacloprid (LC_{50} 52.5 ppm) while field evaluation of insecticides application against termites in sugarcane crop showed the order of efficacy of various treatments as Spinetoram (1500 > 750 ml/ha) > Chloropyrifos> Fipronil > Imidacloprid > Control.

Keywords: Termites management, spinosyns, newer molecules

Introduction
Subterranean termites are the major problem which affect the sugarcane crop from its germination through shoot emergence and finally on the quality of canes (Singh and Singh, 2003) [20]. Sugarcane is vulnerable to termites at initial and later stages of growth (Miranda *et al.*, 2004) [9]. At germination stage, the termite losses up to 90-100% have been recorded in sugarcane (Sattar and Salihah, 2001) [17]. Termites fed on inner soft juicy tissue leaving the rind intact. The cavity thus formed is, subsequently, filled with moist soil with galleries in which these insects move freely. The affected canes exhibit yellowing and drying of outer leaves followed by similar withering of inner leaves and ultimately these canes die (Mora *et al.*, 1996) [10].
In spite of various measures of termite management still pesticides happen to be the major effective weapon under modern agricultural technology against various species of termites to obtain maximum yield and additional returns. Those termiticides which are fast in action, usually repelled by termites due to mass mortality (Fei and Henderson, 2005) [4]. To avoid such situation, non-repellent, slow acting termiticides required so that when termites come in contact with the chemicals that result in high and dispersed mortality (Remmen and Su, 2005a,b) [13, 14].
Spinosyns are a family of metabolic compounds derived from the naturally occurring soil actinomycete bacterium, *Saccharopolyspora spinosa* (Kirst 2010) [7]. Spinetoram is considered as second-generation compound of spinosyns. It is the synthetic product of spinosad (also

known as spinosoids) because it is made after combining spinosyn J (major component) and spinosyn L (minor component) with synthetic modification however, Spinosyn J and spinosyn L are also naturally occurring metabolites of *S. spinosa* (Dripps *et al*., 2008) [3]. Spinosyns and spinosoids are primarily broad spectrum, showing activity against insects belonging to order Coleoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Orthoptera, Lepidoptera, Siphonaptera and Thysanoptera, as well as mites (Thompson *et al*. 1995) [23].

They have a primary effect on the binding site of nicotinic acetylcholine receptors and a secondary effect as a GABA neurotransmitter agonist (Salgado 1998, Palumbo and Richardson 2008) [15, 12]. Their mode of action is novel, as they act on receptors of nicotinic and GABA target sites that are different from the target sites of other insecticides (Sparks *et al.* 2001, Palumbo and Richardson 2008, Orr *et al.* 2009) [21, 12, 11]. In addition, a few studies were done to test the effect of spinosad on termites from the families Kalotermitidae and Termitidae. A lab study by Scheffrahn *et al.* (1997) [19] showed that two different species of drywood termites, *Cryptotermes brevis* Walker and *Incisitermes snyderi* Light, actively foraged from an untreated area to a spinosad-treated area, resulting in significantly high mortality. Spinosad applied in wood galleries in lab bioassays at 0.23 and 0.5% resulted in 98–100% mortality for drywood termites, *C. brevis* and *I. synderi* (Scheffrahn and Thoms 1999) [18]. In a comparison of the different termiticides, on *Microtermes mucophagus* Desneux, the LC_{50} of spinosad was low, ranging from 3.24–3.72 ppm (Iqbal and Saeed, 2013) [6].

The insecticides being applied as sett treatments in furrows at the time of sowing of sugarcane gave an increased yield of 46.62 to 55.33 per cent more over untreated, saving considerable amount of loss due to termite attack (Santharam *et al.,* 2002) [16]. But now-a-days many insecticides recommended for termite management in sugarcane are banned

In this context, a new insecticide molecule, spinetoram belonging to spinosyns was taken up in the present study to determine its toxicity against *Odontotermes brunneus* as well as its efficacy in the field.

Materials and methods

Collection of test insect termite, *Odontotermes brunneus* (Hagen)

Foraging workers were collected from termitarium present near Practical crop production, campus of GBPUA&T, Pantnagar. The termite individuals were transferred separately in three glass jars (dia. 20 cm, height 15 cm) having an inner lining of moist filter paper. These jars with termite individuals were kept in laboratory at room temperature (25^0C and 80 ± 5% RH) in dark conditions. The top of the jar was covered with slightly moist muslin cloth. All termites were tested within three days of collection and only active and healthy termites were used for the experiments.

Insecticides

The insecticides tested for the bioassay were Spinetoram (11.7% SC) and Imidacloprid (17.8% SL) while Chlorantraniliprole (18.5% SC) and Chlorpyrifos (20) were standard check against *Odontotermes brunneus.*

Determination of lethal concentrations (LC values) of insecticides:

The efficacy of insecticidal toxicity was studied against termite workers older than third instar. For preliminary screening, seven concentration of each of the insecticides *viz.*, Spinetoram (60, 120, 180, 240, 300, 360, 420 ppm) and Imidacloprid (20, 60, 100, 130, 170, 200 and 240 ppm) were tested against termite workers. Based on the preliminary screening the final dosage regime of the two insecticides selected for the study was: - Spinetoram (360, 300, 240, 180, 120, 60ppm) and Imidacloprid (178, 124, 99.6, 58.7, 19.5 ppm) with Chlorantraniliprole (200 ppm) and Chlorpyriphos (1000 ppm) as standard check. The observations were recorded at 6, 12, 24, 48 and 72 hours after exposure.

Bioassay method

Test concentrations were prepared by serial dilution method in distilled water for each of the four insecticides. The food material, Whatman filter paper No 1 was moistened with distilled water and shade dried for one hour. Then the food materials were thoroughly mixed/ immersed in the different concentrations of test insecticides. Chlorantraniliprole 18.5 SC @ 200 ppm and Chlorpyriphos 20 EC @ 1000 ppm were the standard check. The treated food materials were placed on the bottom of a Plastic trough (18 cm diameter and 6 cm high). Filter paper moistened with distilled water was served as control. Each concentration was replicated three times with 30 numbers of termites introduced by a soft fine brush per replication and thus 90 termite individuals were exposed per concentration. The observation was recorded on mortality at 6, 18, 24 and 48 hours after contact and feeding exposure of insecticides. Workers were considered dead when they showed no movement upon probing with a fine brush.

Statistical analysis

The data thus obtained was subjected to Abbott's formula for the determination of corrected mortality **(Abbott, 1925)1** while Lethal concentration (LC) values were determined by using probit analysis **(Finney, 1971)5.** The relative toxicity (RT) of insecticides was calculated based on LC 30, 50 and 90 values by dividing the LC value of least toxic insecticide to the LC value of candidate insecticide.

Details of field trials

Field experiments were carried out during 2013-14 in N. E. B. Crop Research Centre of G.B.P.U.A.&T., Pantnagar (Uttarakhand) to evaluate the field efficacy of spinetoram 11.7% SC against termites in sugarcane crop. Sugarcane cultivar CoP 97222 was used for experiment. The plot size was 8 ×5 m^2, row to row distance 90 cm, 3 budded setts were used as planting material and date of planting was 20 March, 2013. The experiment was laid out in a randomized block design with four replications.

Six treatments including control were allocated randomly to different plots in each of the three blocks. The treatments were applied on cane setts at the time of planting in furrows. Spinetoram 11.7 SC was used at two dosages *viz.* 750 ml and 1500 ml/ha, Imidacloprid 17.8 SL, Fipronil 0.3 G, and Chloropyrifos 20 EC, as well as untreated check was also there for comparison. The insecticides (except Fipronil) of required concentration were prepared in water and sprayed over setts while Fipronil 0.3G was applied with hand over cane setts in furrows.

Observations on germination percentage were recorded after 45 days of planting for damage assessment due to termites using following formula.

a) Bud damage assessment- Ten spots at random from each plot were dug out to assess the bud damage due to termites after 30 days of planting.

$$\text{Per cent bud damage} = \frac{\text{Damaged buds}}{\text{Total buds observed}} \times 100$$

b) Plant damage assessment- Ten plants at random from each plot were observed to record the plant damage due to termites at 6, 8 and 10 weeks after planting

$$\text{Per cent plant damage} = \frac{\text{Damaged plants}}{\text{Total number of plants observed}} \times 100$$

c) Cane damage assessment- Ten mature canes at random from each plot were observed to record the cane damage due to termites at harvesting time

$$\text{Per cent mature cane damage} = \frac{\text{Damaged canes}}{\text{Total number of canes observed}} \times 100$$

d) Yield estimation- All the canes from net plots were cut close to the ground level. The tops and trash were removed and cane yield per plot was recorded and computed to hectare basis.

Data analysis

The data were subjected to analysis of variance (ANOVA) as prescribed for randomized block design. Data recorded on per cent bud damage and plant damage was subjected to square root transformation.

Results

Determination of toxicity of two insecticides against worker termites, *Odontotermes brunneus* (Hagen)

Data presented in Table 1 and 2 revealed that the LC_{50} value of Spinetoram was 281 ppm with fiducial limit between 205 to 538 ppm and 141 ppm with fiducial limit 78.1 to 200 ppm at 6 and 12h after exposure, respectively. At 18h after exposure, at all three LC levels, Spinetoram was more toxic insecticide (LC_{30} = 22.8, LC_{50} = 62.3 and LC_{90} = 737 ppm) than Imidacloprid (LC_{30} = 36.5, LC_{50} = 119 and LC_{90} = 2209 ppm). Considering the relative toxicity (RT) value of Imidacloprid as unity (being least toxic) a comparative dose mortality response expressed in terms of relative toxicity indicated that Spinetoram was 1.60 (RT_{30}), 1.91 (RT_{50}) and 2.99 (RT_{90}) times more toxic than Imidacloprid at 18h after exposure.

At 24h after exposure, at all three LC levels, Spinetoram was more toxic insecticide (LC_{30} = 13.9, LC_{50} = 31.9 and LC_{90} = 244 ppm) than Imidacloprid (LC_{30} =26.7, LC_{50} = 52.5 and LC_{90} = 276 ppm). The data on relative toxicity indicated that Spinetoram was 1.92 (RT_{30}), 1.64 (RT_{50}) and 1.13 (RT_{90}) times more toxic than Imidacloprid. However, at 48h after exposure, at all three LC levels Imidacloprid was more toxic as values being 10.1 ppm (LC_{30}), 23.9 ppm (LC_{50}), 97 ppm (LC_{90}) and Spinoteram showed less toxicity (LC_{30} = 16.1, LC_{50} = 21.8 and LC_{90} = 170 ppm) and Imidacloprid was 1.59 (RT_{30}), 1.12 (RT_{50}) and 1.08 (RT_{90}) times more toxic than Spinetoram. Thus, under laboratory conditions, Spinetoram was the more toxic insecticide at all three LC levels than imidacloprid against termites.

Field efficacy of Spinetoram against sugarcane termite during 2013-14

Field data presented in Table 3 showed the efficacy of insecticides against termites in sugarcane crop during 2013-14.The damages caused by termites to the planted Setts and sett buds were observed in different treatments and the data obtained were transformed to per cent infestation. The summarized data indicated that all treatments proved to be significantly superior to untreated control.

Germination per cent

Data on germination per cent showed that Spinetoram treatments as spray over setts had not affected the germination adversely instead resulted in increased germination over untreated check. Germination in untreated check in the experiment was 39.32 per cent while it was 51.26 per cent (T_1) and 49.89 per cent (T_2) in the Spinetoram treatments. Similarly, increased germination in other insecticide treatments *viz*., Imidacloprid, Fipronil and Chlorpyrifos, was also observed in the experiment. Germination per cent in Chloropyrifos treatment (T_5) was recorded 49.43 per cent followed by Fipronil treatment (T_4) 48.46 per cent and Imidacloprid treatment (T_3) 46.47 per cent.

Per cent sett bud damage

Results on per cent sett bud damage revealed that nil bud damage due to attack of termite, recorded in Spinetoram treatments (T_1 and T_2). While, maximum sett bud damage (8.4 per cent) was recorded in Imidacloprid treatment (T_3) followed by 6.7 per cent in Chloropyrifos treatment (T_5) and 3.3 per cent in Fipronil treatment. All treatments proved superior as against maximum bud damage 19.9 per cent in untreated check.

Per cent plant damage

Perusal of data presented in Table 3 indicated that no infestations of termites was recorded under Spinetoram, 1500 ml/ ha spray over setts (T_2) while at lower dose of Spinetoram, 750 ml/ ha spray over setts (treatment T_1) the termite infestation was 5.0 per cent at 6 weeks after planting (WAP). The infestation of termites in Imidacloprid treated (T_3) plot was recorded 12.5 per cent, 10 per cent in Cholorpyrifos (T_5) and 7.5 per cent in Fipronil treatment (T_4) while maximum in control, 22.5 per cent at 6 WAP. At 8 WAP, Spinetoram treatment showed termite infestation of 2.5 per cent in T_2 and 7.5 per cent in treatment T_1. In Imidacloprid treatment (T_3) termite infestation of 15.0 per cent was recorded. However no difference in infestation percentage was recorded in other insecticidal treatment compared to previous fortnight. While 25.0 per cent plant damage was observed in control. Slightly higher infestation of termites was observed in different treatments at 10 WAP. Spinetoram treatment showed termite infestation of 5.0 per cent in T_2 while 10 per cent in treatment T_1. In other insecticidal treatments, maximum infestation of 17.5 per cent was recorded in Imidacloprid treatment (T_3) followed by 15.0 per cent in treatment T_5 and minimum infestation of 10.0 per cent in treatment T_4. While 35.0 per cent damage recorded in control plots at 10 WAP.

Per cent incidence of termites at harvest

It is evident from data that at harvest, the nil incidence of termite was recorded in Spinetoram, 1500 ml/ ha spray over setts (T_2) while 2.5 per cent incidence in treatment T_1. The termite incidence in fipronil treated plots showed 10.0 per cent infestation. Slightly higher infestation of 12.5 and 15.0 per cent was recorded in treatment T_5 and T_3, respectively and maximum termite infestation of 27.5 per cent obtained in control plots.

Cane yield

Data showed that cane yield was significantly high in all the insecticidal treatments ranging from 70.83 to 76.10 tonnes per ha while it was 60.89 tonnes per ha in the untreated check. The yield in Spinetoram treatment T_2 was 76.10 t/ha which was at par with Spinetoram treatment T_1 i.e. 75.66 t/ha with 24.97 and 24.25 per cent increase in yield over untreated check. However, in other insecticidal treatments the yield was less than Spinetoram treated plots. The yield in treatment T_5 was recorded 72.60 t/ha with per cent increase over check was 19.23. While in case of treatment T_4, the yield was 71.93 t/ha with 18.13 per cent increase and 70.83 t/ha yield was recorded in treatment T_3 with 16.32 per cent higher yield as against control plots.

Discussion

Efficacy of spinetoram was high against termites under laboratory and field conditions. Iqbaal and Saeed, (2013)[6] found that the order of average toxicity of insecticides against *Microtermes mycophagus*, reported from highest to lowest was: Chlorfenapyr > Spinosad > Thiamethoxam > Fipronil > Indoxacarb > Imidacloprid. In the present study the difference in the toxicity of the tested insecticides might result because of the difference in their modes of action. Su *et al.* (1987)[22] reported that slow-acting toxicants required a longer time to kill termites at low concentrations than at high concentrations. The level of mortality and the speed of death were dependent on concentration. Manzoor *et al.* (2012)[8] observed that *Heterotermes indicola* when treated with Imidacloprid, Chlorfenapyr, Bifenthrin, Fipronil, Bifenthrin, and Cadusafos, it took more than 8 hours to obtain 97% mortality with LC50 values for *H. indicola* were 346.75, 75.86, 14.45, 1.05, 0.46 for Imidacloprid, Chlorfenapyr, fipronil, Bifenthrin, and Cadusafos, respectively after 8 hrs of exposure. He also mentioned that Imidacloprid, Chlorfenapyr and Fipronil are slow acting insecticides and showed less mortality when compared to Bifenthrin. Termites may be intoxicated by a pesticide due to ingestion, contact, or both. Spinosad and spinetoram can act by both routes of entry, but ingestion is more effective to control most pests, especially lepidopterans (Palumbo and Richardson, 2008)[12]**.** Cockroaches can acquire spinosad by contact and ingestion, both routes acting on the nicotinic acetylcholine and GABA receptors (Watson, 2001)[24]. However, the oral route seems to be more effective in administering the toxin to termites because while foraging on the treated area they can directly drink or feed from the treated substrate or move treated particles with their mouthparts.
The results from the field study reveals that spinetoram gave best control against termites compared to other chemicals and control plots. Increase in germination is attributed to the control of termites in the treatments. Such increase in germination of setts due to insecticides treatment has been reported in an earlier study also (Anonymous, 1998) [2].These findings are in concurrence with the findings of Singh and Singh, (2003) [20] who reported that insecticide formulations helps in promoting the shoot growth and millable cane by successfully controlling the termite infestations. Santharam *et al*. (2002) [16] also reported that increased yield of cane in the treatments was attributed to the control of termites damaging setts, resulting in better germination which ultimately led to harvesting of higher number of millable canes.

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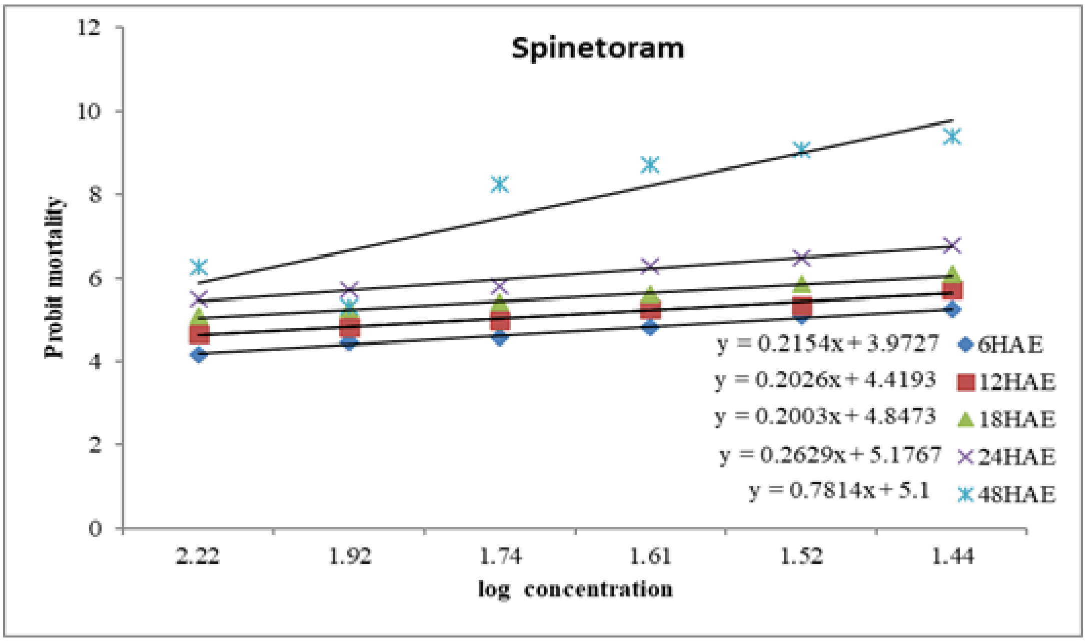

Fig 1: Dosage-mortality response of Spinetoram 12 SC against worker termites *Odontotermes brunneus* (Hagen)

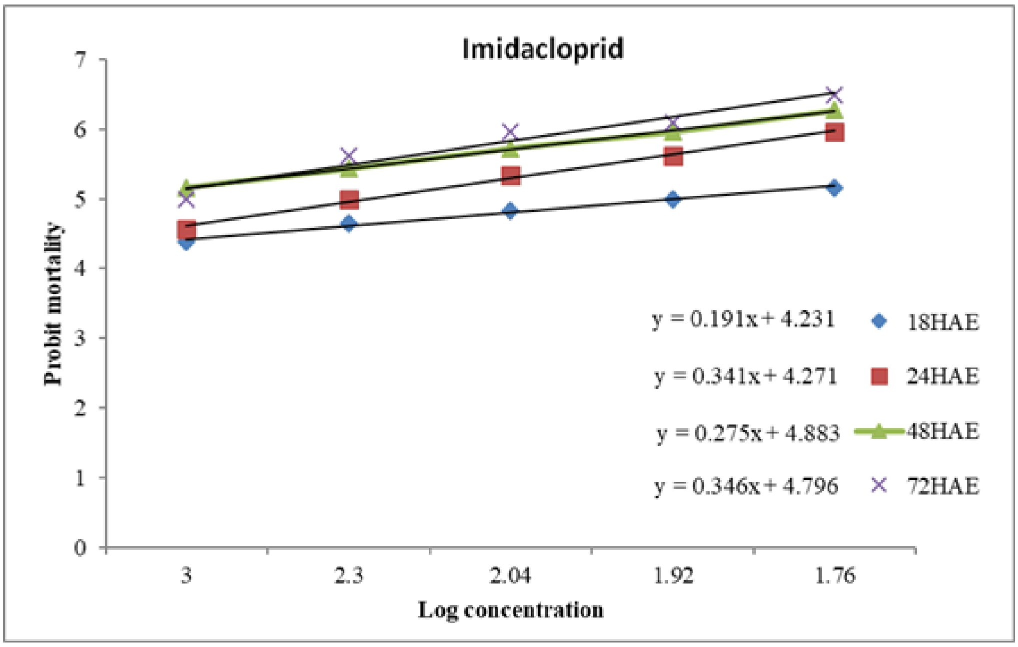

Fig 2: Dosage-mortality response of Imidacloprid 17.8% SL against worker termites *Odontotermes brunneus* (Hagen)

Table 1: Dosage mortality response of worker termites *Odontotermes brunneus* (Hagen) against Spinetoram 12 SC and Imidacloprid 17.8 SL at 6, 12, 18, 24 and 48 hours after exposure

Hrs	Spinetoram 12 SC					Imidacloprid 17.8 SL				
	LC values in ppm			Chi Sq.	Regression equation Y= a+bx	LC values in ppm			Chi Sq.	Regression equation Y= a+bx
	LC_{30}	LC_{50}	LC_{90}			LC_{30}	LC_{50}	LC_{90}		
6	119 (58.5-165)	281 (205-538)	2320 (572-9410)	0.932	Y= 3.972+0.215x	-	-	-	-	-
12	54 (24.9-117)	141 (78.1-200)	1500 (417-5390)	1.581	Y= 4.419+0.202x	-	-	-	-	-
18	22.8 (6.28-83.1)	62.3 (11.1-101)	737 (273-1980)	1.201	Y= 4.847+0.200x	36.5 (2.5-60.8)	119 (74.8-598)	2209 (167-3919)	0.073	Y= 4.231+0.191x
24	13.9 (3.3-58.6)	31.9 (12.0-84.4)	244 (167-608)	2.068	Y= 5.176+0.262x	26.7 (15.7-45.6)	52.5 (33.5-69.3)	276 (143-532)	0.178	Y= 4.271+0.341x
48	16.1 (3.2-34.7)	24.6 (0.2-44.9)	170 (82-432)	8.141	Y= 5.1+0.781x	10.1 (3.06-33.8)	21.8 (4.49-39.4)	157 (12-355)	0.200	Y= 4.883+0.275x
72	-	-	-	-	-	119 (58.5-165)	281 (205-538)	2320 (572-9410)	0.932	Y= 4.796+0.346x

The values in parentheses are fiducial limits; LC= Lethal Concentration, Chi Sq.= Chi Square

Table 2: Relative toxicity of insecticides against worker termites *Odontotermes brunneus* (Hagen) at 18, 24 and 48 hours after exposure

Hours	Relative toxicity(RT) at LC values					
	RT at LC_{30}		RT at LC_{50}		RT at LC_{90}	
	Spinetoram	Imidacloprid	Spinetoram	Imidacloprid	Spinetoram	Imidacloprid
18	1.60	1.00	1.91	1.00	2.99	1.00
24	1.92	1.00	1.64	1.00	1.13	1.00
48	1.00	1.59	1.00	1.12	1.00	1.08

Relative toxicity= LC value of least toxic insecticide/LC value of candidate insecticide
LC= Lethal Concentration

Table 3: Evaluation of bio-efficacy of Spinoteram compared to Imidacloprid, Chloropyrifos and Fipronil against Termites in Sugarcane

S. No.	Treatment	Germination (%)	% Bud damage by termites (30 DAP)	% Plant damage (Weeks after planting)			% Incidence of termites at harvest	Yield (t/ ha)	% Increase over check
				6WAP	8WAP	10 WAP			
1.	Spinetoram 12 SC 750 ml/ ha spray over setts and cover	51.26	0.0(1.0)	5.0(2.2)	7.5(2.4)	10.0(3.0)	2.5 (1.6)	75.66	24.25
2.	Spinetoram 12 SC 1500 ml/ ha spray over setts and cover	49.89	0.0(1.0)	0.0(1.0)	2.5(1.6)	5.0(2.1)	0.0(1.0)	76.10	24.97
3.	Imidacloprid 17.8% SL 350 ml/ ha spray over setts and cover	46.47	8.4(3.0)	12.5(3.3)	15.0(3.9)	17.5(4.2)	15.0(3.9)	70.83	16.32
4.	Fipronil 0.3% G 20 kg/ha soil application	48.46	3.3(1.9)	7.5(2.8)	7.5(2.7)	10.0(3.0)	10.0(3.0)	71.93	18.13
5.	Chloropyrifos 20 EC 5 lit./ ha spray over setts and cover	49.43	6.7(2.7)	10.0(3.0)	10.0(3.0)	15.0(3.9)	12.5(3.6)	72.60	19.23
6.	Untreated check	39.32	19.9(4.5)	22.5(4.8)	25.0(5.0)	35.0(5.9)	27.5(5.2)	60.89	-
CD at 5%		3.35	6.8(0.9)	11.1(2.0)	11.2(1.9)	8.9(1.5)	10.4(1.5)	4.50	-
SEm±		1.11	2.3(0.3)	3.6(0.6)	3.7(0.6)	2.9(0.5)	3.44(0.5)	1.49	-

%= Per cent; WAP= Weeks After Planting; DAP= Days after Planting
Values in parentheses are Square root transformed (x+1.0) value

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