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EFFECT OF RAINS ON TREATED SOIL WITH TERMITICIDES AGAINST SUBTERRANEAN TERMITES IN EGYPT.

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ABSTRACT

The results of washing on treated soil with insecticides against subterranean termites *Psammotermes hypostoma* (Desnuax),indicated that, the first washed of treated soil were the highly efficacy and quicker in the killed against termites of all tested particle sizes than second and third, respectively, and the small particle size were the quicker and more toxic than others in the first washed of treated soil, while was the lower of toxic than others in third, second and first washed of soil, respectively. Also cypermethrin showed the highly toxic, followed by fipronil and chlorpyrifos, respectively. The treated coarse soil showed more toxic in the second and third than the fine, and to consider the lower safety to the water table, also the washed samples analysis indicated that, the chlorpyrifos showed the higher safety followed by fipronil and cypermethrin. Statistics analysis showed highly significantly between the factors treatments, number of washing and times, with the variable, (mortality %), while low significant recorded for particle sizes.

INTRODUCTION

Subterranean termite is one of dangerous insects due to its hidden life underground. *P. hypostoma* (Desnuax), in Egypt caused highly losses to the national production, it is feed on the all celluloid materials such as wood, wooden constructions, trees, furniture, bricks, mats and paper products (books, carton and government records). The extensive chemicals that used in the termite control can be caused big problems when flow out and contaminate to the water table. The irrigation and rain increases chemicals to go deeply into the soil. The present work was conducted during 2005-2006 to evaluate the effects of three termiticides; chlorpyrifos, fipronil and cypermethrin, against *P. hypostoma* (Desn.).

MATERIAL AND METHODS

Three groups of termiticides were used in the present study; OP group: chlorpyrifos 48% EC, Fiprol or phenylpyrazole group: termidor 5% EC and pyrethroid group: cypermethrin 10% EC.

Subterranean termites, *P. hypostoma* (Desn.) were collected from Ismailia Regional, Agriculture Research Station, by the aid of modified trap (El-Sebay 1991). Termites were separated from the traps by air bump respirator and kept in Petri-dishes provided with pieces of moistened card boards as a source of cellulose and moisture required for termites for seven days in incubator adjusted at 27C◦ ±1C◦. Daily inspection was carried out and eliminated dead or moribund individuals. The healthy workers were used for the tested of control (El-Sebay, 1993a and 1993b; El-Bassiouny, 2001)

To study the effect of excessive water on soil with three particle sizes of 850µm, 425 µm and 250 µm and treated with 500 ppm of each chemical. Each treated soil of each particle size were soaked in 200ml water for two hours and then soaking water was transferred to another 250gm of untreated of same particle size for two hours and then washing water was added to 250gm of untreated soil of the same particle size for two hours. Washed soils were transferred into stainless tray for drying in open air. Each treatment for each number of washing and control were replicated five times. Soils were kept dry in deep freezer for bioassay studies and chemical determination of tested insecticides.

For bioassay experiment, five grams from each treated soil particle size (treated soil replicate), were placed in Petri dish, and provided with moistened cardboard and 20 healthy workers were adopted and liberated. Mortality percentages were recorded for seven days, compared with untreated washed soil (control).

Hundred grams of treated washed soils were used for chemical determination of tested pesticides. Obtained data was analyzed using Proc ANOVA in SAS (SAS Institute 1988).

Residues determination of tested insecticides in treated soil samples:

The original method of **Davis *et al*. (1993),** with some modification was used, and each pesticide was determined separately in the absence of others insecticides. The methods could be concluded as follows:

Three different methods were selected in determination of insecticides, (chlorpyrifos, fipronil and cypermethrin) in soil samples were extracted by the solvents, acetone, dichloromethane and ethyl acetate.

Different solvent systems were used to choose the most suitable and efficient solvent system for extraction of chlorpyrifos, fipronil and cypermethrin from treated soil. These solvent systems were acetone, dichloromethane and ethyl acetate. There ability to extract the tested insecticides successfully was tested by adding the previously mentioned solvents to samples. Blending, cleaning-up and then determining the recovery percentage of the three tested compounds by GLC.

i. Analysis condition of tested insecticides:

- **Chlorpyrifos**

Detector: electron capture
Column temperature: 180 ºC
Detector temperature: 300 ºC
Injection temperature: 250 ºC
Column type: HP – 1 (25 m × 0.23 mm × 0.17µm)

- **Fipronil**

Detector: electron capture
Column temperature: 200 ºC
Detector temperature: 300 ºC
Injection temperature: 250 ºC
Column type: HP – 1 (25 m × 0.23 mm × 0.17µm)

- **Cypermethrin**

Detector: electron capture

Column temperature: 250 °C
Detector temperature: 300 °C
Injection temperature: 270 °C
Column type: HP – 1 (25 m × 0.23 mm × 0.17µm)

RESULTS AND DISCUSSIONS

Rat of recovery from soil:

Results are shown in Table (1), indicate that, extraction of the tested insecticides from treated soil was tested by three different solvents namely: acetone, dichloromethane and ethyl acetate. The most suitable of which was dichloromethane with 94.0, 91.1 and 90.2% percent recovery, and ethyl acetate gave 88.3, 89 and 84.3% while using acetone 85.4, 82 and 85.4% percent recovery, for chlorpyrifos, fipronil and cypermethrin respectively.

As shown in table (2). Data revealed that, the rate of recoveries by 500ppm in soil treated with three insecticides and the rates was follow; the replicates of chlorpyrifos recorded 93.0 and 95.0% with average 94.0%, the fipronil estimated 82.0 and 83.5% with average 82.75%, and the cypermethrin estimated 86.5 and 91.5% with average 89.0%. The obtained data of the insecticide residues were corrected according the previously results.

Table (1): Recovery results of three solvents for the tested insecticides in treated soil samples.

Tested pesticide	Solvent	Recovered 5ppm		% Amount recovered		Average %
		R 1	R2	R 1	R2	
Chlorpyrifos	Acetone	4.21	4.33	84.2	86.6	85.4
	Dichloromethane	4.66	4.74	93.2	94.8	94.0
	Ethyl acetate	4.52	4.31	90.4	86.2	88.3
Fipronil	Acetone	4.15	4.22	83	84	82
	Dichloromethane	4.65	4.46	93	89.2	91.1
	Ethyl acetate	4.33	4.57	86.6	91.4	89
Cypermethrin	Acetone	4.33	4.21	86.6	84.2	85.4
	Dichloromethane	4.57	4.45	91.4	89	90.2
	Ethyl acetate	4.22	4.21	84.4	84.2	84.3

Table (2): The amount recovered for tested insecticides, in soil treated with 500ppm.

Insecticides	Amount recovered in ppm		Recovered %		Average %
	R 1	R2	R 1	R2	
Chlorpyrifos	465	475	93.0	95.0	94
Fipronil	410	417.5	82.0	83.5	82.75
Cypermethrin	432.5	457.5	86.5	91.5	89

Data from the following tables show the effect of treated soil washings on the lower attached soil layers.

As shown in Tables (3), washings of 500 ppm of chlorpyrifos have toxicity of 96% at the first untreated soil followed by 92% at the second wash and decreased to 63% mortality at third wash at soil particle size 850 µm. At particle size 425 µm, toxicity of the first washing gave mortality 98%, followed by 81% in second washing and decreased to 75% to the third washed soil. Toxicity was increased at particle size 250 µm at the first washed soil (98%), followed by second washed (80%) and decreased to the third washed soil (66%).

Data presented in Tables (4), reveled that complete mortality (100%) was given at first washed soil after the third day of treatment, 97% was at the second washed soil after the sixth day and 70% mortality at third washed soil after the sixth day, at particle size 850 µm. Mortality percentages were increased at particle size 425 µm. It was 100% at first washed soil after four days of treatment, 90% at the second one after fifth day and 80% at the third washed soil after the sixth day. At particle size 250 µm, the first washed soil gave complete mortality (100%) after the third day of treatment. Second washed soil, gave 91% mortality after the fifth day and 68% after the sixth day in the third washed soil.

Cypermethrin, in Tables (5), at particle size 850 µm gave washing that contaminated untreated soil with toxicity of 100% after the fifth day of treatment. In the second washed soil, mortality was 99% after the sixth day and 82% at the third washed soil after the seventh day. At particle size 425 µm, mortality was 100% in the fifth day at the first washed soil, 95% at the second washed soil at sixth day and 75% at the third day of treatment in the third washed soil. Mortality was 100% in the first washed soil at 4^{th} day of treatment in particle size 250 µm. The second washed soil gave 95% mortality after the sixth day and 80% at the third washed soil after the sixth day.

In the comparison between the three tested insecticides, data in Table (3), clarify that, at particle size 850µn, the mortality percentages of chlorpyrifos was 96, 92 and 63% for first, second and third washing, respectively. At particle size 425µn, mortality percentages counted 98, 81 and 75%, whereas at particle size 250µn recorded 98, 80 and 66% mortality for the three number of washing, respectively. Time-concentration relationship of the activity of chlorpyrifos proved that LT_{50} values of chlorpyrifos were folded in the first, second and third washings (0.029, 0.57 and 2.016 at 850 µm, 0.218, 0.681 and 1.397 at 425 µm and 0.075, 0.534 and 1.748 at 250 µm), respectively. As shown in Table (6), washing of treated soil with chlorpyrifos (500 ppm), revealed that the first rinsed water which contaminated untreated soil was contained high amount of chemical, 1939.4, 589.4 and 438.5 ppm and caused mortality 96, 98 and 98% at the three particle size (850, 425 and 250µm. (Table 3), The particle size 850µm lost higher residues in the rinsed water more than 425 and 250 µm. On the other hand, data in table (6) showed that, the residues rinsed from second and third washed soils, were less amounts of ppm at all particle size under test (8.01 and 27.6 ppm in 850 µm, 11.1 and 3.1 ppm in 425 µm and 1.3 and 0.41 ppm in 250 µm, respectively).

Table (3): Effect of three washing times of chlorpyrifos treated soil with 500 ppm on *P. hypostoma* termite at different particle sizes.

Washing Soil	Mortality in days							%	LT^{50} values
	1	2	3	4	5	6	7		
Chlorpyrifos 850 µm									
1st	78	89	93	95	96	96	96	96	0.029
2nd	61	79	86	89	90	92	92	92	0.57
3rd	39	50	57	62	63	63	63	63	2.016
Chlorpyrifos 425 µm									
1st	80	90	93	95	98	98	98	98	0.218
2nd	56	66	73	79	81	81	81	81	0.681
3rd	43	56	66	69	72	75	75	75	1.397
Chlorpyrifos 250 µm									
1st	90	97	98	98	98	98	98	98	0.075
2nd	57	69	74	76	78	80	80	80	0.534
3rd	41	53	58	62	65	65	66	66	1.748

The results are agreement with data of Smith and Rust (1993), who mentioned that, the increasing cellulose concentration in sand treated with chlorpyrifos significantly reduced mortality of termite. Apparently, the chlorpyrifos bind to cellulose particles, making them less available to workers, and the addition of cellulose or clay to the sand soil increased mortality caused by cypermethrin. Also, data are agree with Forschler and Townsend (1996), who mentioned that termites were affected by soil type and termiticides tested. Lethal concentrations of chlorpyrifos, cypermethrin and permethrin were estimated at least 7 times lower in sand soil compared with sandy loam or sandy clay loam soils. However, the soil type alone could not be used to predict termite mortality with the termiticides tested. Data are also in agreement with Singh and Kumar (2000), studied the effect of chlorpyrifos on various soil types, and the effect of water co-solvent and organic matter on the insecticide movement. They reported that chlorpyrifos gave the movement of pesticide graded as follow; sandy loam, loam and salty loam soils, when the organic matter washing and removal from the soils caused increased the movement of pesticides, and same effect was noticed when the water co-solvent volume increased the movement of pesticides increased in the soils.

Fipronil, showed different behavior in its washing soil, as indicated from Table (4). Mortality percentages were 100% at the first washing at all particle size under test, which contained 1925.9, 873.7 and 2760 ppm of chemical residues. The LT_{50} values of fipronil were folded in the 1st, 2nd and 3rd washings (0.014, 0.144 and 1.725 at 850 µm, 0.283, 0.524 and 1.331 at 425 µm and 0.348, 0.483 and 2.038 at 250 µm), respectively. As shown in Table (6), the first and second washing contained more residues than the third one at all particle size (1925.9 and 1876.1 ppm at 850 µm, 873.8 and 1940.1 ppm at particle size 425 µm and 2760 and 4129.3 ppm at 250 µm.). While the lowest values of ppm's were at the third washing in all sizes of soil (370.2, 396.2 and 653.8 ppm.), respectively. Waite and Gold (2004), succeeded in using fipronil as an exterior and interior chemical barriers for the control of subterranean termites structures. Data resulted that, in all structures where any points of termite entry were associated with the exterior perimeter wall, the treatment exhibited full control of the infestation within 6 months and when fipronil was applied according to the product label, which required a continuous barrier of fipronil be placed around the perimeter of a structure, as well as to active and potential points of termite entry, treatments were 100% effective. Cox (2005), mentioned that, fipronil a relatively new insecticide in the phenyl pyrazole chemical family and is sold under a variety of brand names including frontline, regent, termidor, combat and maxforce. Resistance and exposure to fipronil, and water contamination are briefly discussed.

Table (4): Effect of three washing times of fipronil treated soil with 500ppm on *P. hypostoma* termite at different particle sizes.

Washing Soil	Mortality in days							%	LT^{50} values
	1	2	3	4	5	6	7		
Fipronil 850 µm									
1^{st}	87	96	100	100	100	100	100	100	0.014
2^{nd}	70	83	89	93	96	97	97	97	0.144
3^{rd}	40	51	61	67	69	70	70	70	1.725
Fipronil 425 µm									
1^{st}	91	97	99	100	100	100	100	100	0.283
2^{nd}	64	74	82	87	90	90	90	90	0.524
3^{rd}	40	61	72	76	79	80	80	80	1.331
Fipronil 250 µm									
1^{st}	95	99	100	100	100	100	100	100	0.348
2^{nd}	66	77	85	89	91	91	91	91	0.483
3^{rd}	41	47	54	60	67	68	68	68	2.038

As shown in Table (5), values of toxicity were very high at the first and second washed soils at all particle size (100,100,100 for first, 99, 95 and 95 at second washed soil). While was low at the third washed soil (82, 75, and 80%). LT_{50} values of cypermethrin were folded and calculated 0.481, 0.661 and 1.525 at 850 µm, 0.302, 0.874 and 1.386 at 425 µm and 0.313, 0.597 and 1.692 at 250 µm), of the first, second and third washings respectively. According to data in Table (6), the percentages values were expressed numerically by 756.7, 2929.7 and 3433.2 ppm at first washed soil through all particle sizes. And were 3135.9, 2482.5 and 4642.1 ppm at second washed and 1824.2, 980.9 and 4438.2 ppm at the third one. Cypermethrin showed highly degradation by water which can wash out to the followed layers of the ground. Smith and Rust (1991), reported that, the insecticides, cypermethrin, dichlorvos, chlorpyrifos and chlordane residues dried deposits produced significant numbers of moribund workers of *Reticulitermes hesperus* (Bank), the insecticides when the termites exposure to vapor from deposits for 12 hr gave significantly greater number of moribund workers than control treatment. He added that, the older deposits were more toxic than fresh deposits for these compounds. Smith and Rust (1993), mentioned that, the addition of cellulose or clay to the sand, increased mortality caused by cypermethrin, while increasing cellulose in sand treated with chlordane or chlorpyrifos significantly reduced mortality of *Reticulitermes hesperus* in direct-exposure experiments. Apparently, chlordane and chlorpyrifos bind to cellulose particles, making them less available to workers.

Table (5): Effect of three washing times of cypermethrin treated soil with 500 ppm on *P. hypostoma* termite at different particle sizes.

Washing Soil	Mortality in days							%	LT^{50} values
	1	2	3	4	5	6	7		
Cypermethrin 850 µm									
1st	81	90	98	99	100	100	100	100	0.481
2nd	68	79	89	94	97	99	99	99	0.661
3rd	41	54	65	75	80	82	82	82	1.525
Cypermethrin 425 µm									
1st	87	93	97	99	100	100	100	100	0.302
2nd	54	69	83	89	92	95	95	95	0.874
3rd	43	56	66	70	75	75	75	75	1.386
Cypermethrin 250 µm									
1st	90	96	99	100	100	100	100	100	0.313
2nd	62	73	81	88	92	95	95	95	0.597
3rd	36	54	65	72	78	80	80	80	1.692

Table (6): Analysis values in ppm, of three insecticides residues in treated soil by 500 ppm, and its washing effect on different particle sizes.

Size /µm	No. of washing	Residues values of pesticides in ppm					
		Chlorpyrifos		Fipronil		Cypermethrin	
		Amount in ppm	Correct value	Amount in ppm	Correct value	Amount in ppm	Correct value
850	First	1823.0	1939.361	1593.75	1925.981	673.5	756.741
	Second	7.569	8.052	1552.5	1876.132	2791.0	3135.955
	Third	25.92	27.574	306.35	370.211	1623.5	1824.157
425	First	554.0	589.361	723.0	873.716	2607.5	2929.775
	Second	10.446	11.112	1605.4	1940.060	2482.5	2789.258
	Third	2.902	3.087	327.85	396.193	873.0	980.898
250	First	412.2	438.510	2283.9	2760	3055.5	3433.146
	Second	1.209	1.286	3417.0	4129.305	4131.5	4642.134
	Third	0.381	0.405	541.0	653.776	3950.0	4438.202

Generally, The first washed of treated soil were the highly efficacy and quicker in the killed against termites of all tested particle sizes than second and third, respectively, and the small particle size were the quicker and more toxic than others in the first washed of treated soil, while was the lower of toxic than others in third, second and first washed of soil, respectively. Also cypermethrin showed to be the highly toxic, followed by Fipronil and Chlorpyrifos, respectively. The treated coarse soil showed to be more toxic in the second and third than the fine, and to consider the lower safety to the water table, also the washed samples analysis indicated that, the chlorpyrifos showed the higher safety followed by fipronil while the cypermethrin showed the lower safety.

The author suggested that, the using of cypermethrin would be unsuitable in the cultivated areas with rainfall and irrigation water, and suggest using in buildings control.

Statistical analysis:

Data in Table (7), indicated that, the mean of treatments, chlorpyrifos, fipronil and cypermethrin, were recorded 77.0635, 82.1746 and 82.8730 respectively, and found highly significantly differences (F. values recorded 22.81). Also the means of particle sizes were 81.5397, 80.7937 and 79.7778 for particle size 850, 425 and 250µm respectively, and the F. values recorded low significant 1.77. The means of times were 63.000, 74.000, 80.852, 84.556, 86.926, 87.778 and 87.815, for 1, 2, 3, 4, 5, 6 and 7 days respectively, and the F value was highly significant 83.01. 1st, 2nd and 3rd washings showed to be highly significant whereas counted 96.2698, 82.5397 and 63.3016 for both respectively, and the F value recorded 621.81

Table (7): Illustration of significantly variables between the treatment, particle sizes, number of washing and time with the variable factor (mortality %)

<table>
<tr><th colspan="7">The means</th><th>F. Value</th><th>Pr > F</th></tr>
<tr><th colspan="7">Treatments</th><td rowspan="3">22.81</td><td rowspan="3">0.0001</td></tr>
<tr><th colspan="2">Chlorpyrifos</th><th colspan="2">Fipronil</th><th colspan="3">Cypermethrin</th></tr>
<tr><td colspan="2">77.0635</td><td colspan="2">82.1746</td><td colspan="3">82.8730</td></tr>
<tr><th colspan="7">Particle sizes</th><td rowspan="3">1.77</td><td rowspan="3">0.1728</td></tr>
<tr><th colspan="2">850µm</th><th colspan="2">425µm</th><th colspan="3">250µm</th></tr>
<tr><td colspan="2">81.5397</td><td colspan="2">80.7937</td><td colspan="3">79.7778</td></tr>
<tr><th colspan="7">Times</th><td rowspan="3">83.01</td><td rowspan="3">0.0001</td></tr>
<tr><th>1 day</th><th>2 days</th><th>3 days</th><th>4 days</th><th>5 days</th><th>6 days</th><th>7 days</th></tr>
<tr><td>63.000</td><td>74.000</td><td>80.852</td><td>84.556</td><td>86.926</td><td>87.778</td><td>87.815</td></tr>
<tr><th colspan="7">number of washing</th><td rowspan="3">621.81</td><td rowspan="3">0.0001</td></tr>
<tr><th colspan="2">First</th><th colspan="2">Second</th><th colspan="3">Third</th></tr>
<tr><td colspan="2">96.2698</td><td colspan="2">82.5397</td><td colspan="3">63.3016</td></tr>
</table>

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دراسة تأثير الغسيل على التربة المعامله ببعض المبيدات ضد النمل الأبيض التحت أرضى فى مصر

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أوضحت النتائج للثلاث مبيدات المختبرة السيبرمثرين والفبرونيل والكلوربيريفوس سمية عالية وسرعة فى القتل فى التربة المغسولة فى المرحلة الأولى ضد النمل الأبيض "ساموترمس هيبوستوما" عن التربة المغسولة فى المرحلة الثانيه والمرحلة الثالثة على التوالى لكل من أحجام التربه الثلاثه المختبرة ٨٥٠ ، ٤٢٥ ، ٢٥٠ ميكرون . وسجل حجم حبيبات التربه ٢٥٠ ميكرون سمية عالية وسرعة فى القتل خاصة فى التربة المغسولة الأولية عن كل من حجم التربه ٤٢٥ ، ٨٥٠ ميكرون حيث وصلت نسبة الموت ١٠٠ % فى اليوم الرابع والخامس ثم الخامس لكل من الأحجام الثلاثه على التوالى . على العكس من ذلك فإن حجم التربه الأقل ٢٥٠ ميكرون كان أقل سمية وبطئ فى القتل عن كل من حجم التربه الأكبر ٤٢٥ ، ٨٥٠ ميكرون فى التربه المغسوله فى المرحلة الثالثة والثانية ثم الأولى على التوالى . وعند مقارنة الثلاث مبيدات المختبره كان مبيد الفبرونيل الأكثر سميه وأسرع فى القتل من السيبرمثرين والكلوربيريفوس على التوالى . وكانت معدلات النسب المئوية للموت فى الكنترول للثلاث أحجام تربه المختبرة ٨٥٠ ، ٤٢٥ ، ٢٥٠ ميكرون كانت على التوالى 1 ، ٠ ، 3% لمبيد الكلوربيريفوس وسجلت ٠ ، ٠ ، ٠% لمبيد الفبرونيل وكانت ٠ ، 3 ، 1% لمبيد السيبرمثرين . واتضح من تحليل عينات التربة المختبرة أن متبقيات مبيد الكلوربيريفوس كانت مرتفعة فى التربة المغسولة الأولية ثم إنخفضت تدريجيا فى التربة المغسولة فى المرحلة الثانية و الثالثة على التوالى لكل من الأحجام الثلاثة للتربة ٨٥٠ ، ٤٢٥ ، ٢٥٠ ميكرون ولذلك إعتبر الأكثر أمانا للمياه الأرضيه ثم كان مبيد الفبرونيل فى المرتبة الثانية أما مبيد السيبرمثرين فقد سجل مستويات تحطيم عالية بفعل الغسيل وازدادت متبقياته فى التربة المغسولة الثانية والثالثة وإعتبر الأكثرسمية والأقل أمانا للمياه الأرضيه. وقد أثبت التحليل الإحصائى وجود فروق معنوية عالية بين المعاملات وبين مراحل غسيل التربة وأيضا مع عامل الوقت لكن كانت الإختلافات ما بين الأحجام أقل فى درجة المعنوية .