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Biological Activity of the Organic Pesticide Baicao No. 1 Against the Red Spider Mite *Tetranychus urticae* Koch

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ABSTRACT

The efficiency of the organic pesticide "Baicao No. 1" (active ingredient matrine) was studied against the different stages of the red spider mite *Tetranychus urticae* Koch. It showed high efficacy against mite adult female as its LC_{50} values were 1.14 and 0.08 ppm when mortality was observed after one and four days, respectively. Less efficacy was observed against protonymphs, as the LC_{50} value averaged 26.14 ppm. Slight ovicidal efficacy was recorded on egg hatchability, as LC_{50} value was 168.25 ppm; while further high mortality was observed in hatched larvae which was reflected in a reduction in LC_{50} value to 8.03 ppm. Applying LC_{50} concentration (21.07 ppm) on protonymphs resulted in some changes in biological aspects of the survived mites which reduced finite rate of increase from 1.33 to 1.20 female per female per day. Matrine also showed antifeedant effect; as shrinkage in feeding area was recorded when mites fed on treated leaves.

Key Words: *Tetranychus urticae*, Matrine.

INTRODUCTION

Matrine is a botanical tetracyclo-quinolizindine alkaloid obtained from *Sophora flavescens, S. japonica, S. alopecuroides* and *S. subprostrata.* It was isolated and identified in 1958 (http://www.itmonline.org/arts/oxymatrine.htm).
It was used intensively in pharmacological and medical studies (Cho, 1986; and Wang 1994). Matrine also demonstrated biological activity against various pests. The nematicidal activity of matrine was recorded (Matsuda *et al* 1991, BoGuang 1998 and 1999). GuoHua & XiaoMei (2001) recorded the insecticidal effect of matrine against tea geometrid *Ectropis obliqua* and cabbage worm *Pieris rapae* in a field trial with no significant influence on natural enemies. Also the insecticidal effect of matrine against *Bemisia tabaci* was reported by ShuangYan & DunXiao (2001), against rice water weevil *Lissorhoptrus oryzophilus* (XiaoGuang *et al* 2004) and against *Atractomorpha sinensis* by FangWen (2005). WanChun & Qiang (2003) reported that matrine could inhibit the activity of acid phosphoresterase on metabolic esterases of the larvae of the diamondback moth *Plutella xylostella*. YuZhe *et al* (2004) demonstrated that matrine could inhibit tetradotoxin-sensitive sodium current (INa) in central neurons of the cotton bollworm, *Helicoverpa armigera*. The aim of the present work is to evaluate the efficiency of Matrine against the red spider mite *Tetranychus urticae* Koch.

MATERIALS AND METHODS

The organic pesticide "BaicaoNo. 1" was obtained as aqueous solution formulation (0.36% active ingredient matrine fig. 1).

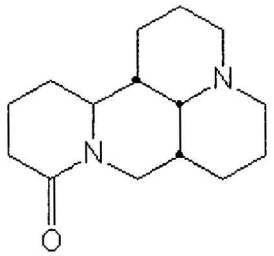

Figure 1. Matrine

Mite culture

The original sensitive colony of the red spider mite *T. urticae* was supplied from Acarology Laboratory in Plant Protection Research Institute. It was maintained on mulberry *Morus alba* L. leaves, placed upside-down on moist cotton pads in plastic plates (20 x 12 cm) and kept at 25°C and 60 ± 5% RH. Mulberry leaves were replaced with fresh leaves when it was needed.

Toxicity tests

Series concentrations of "BaicaoNo. 1" were prepared. To evaluate the acaricidal activity of tested material on protonymphs and adult females, twenty newly emerged protonymphs or adult females were transferred to the lower surface of each mulberry leaf disc (2.5 cm in diameter). Thereafter five discs, as replicates for each concentration, were sprayed with different concentrations at rate of deposit 0.025 ml/disc. Check discs were sprayed with water. All discs were placed on moist cotton wool in Petri dishes. The treated mites were kept under constant temperature of 25 ± 0.5 °C and 60% ± 5 R.H. The mortality of adults was estimated after 1, 2, 3 and 4 days; while, treated protonymphs were observed until they reach deutonymphal stage. Their mortality was estimated before reaching the quiescent protonymph, and for those failed to molt to

deutonymphal stage. To investigate the ovecidal activity of "BaicaoNo. 1", twenty adult females were placed on each mulberry leaf disc (2.5 cm in diameter). Discs were put on wet cotton wool in Petri dishes and left for 24 hours to deposit eggs then adults were removed. Thereafter, discs were divided into two groups, one was treated just after removing the adult females (0 - 1 day old), while the other group was treated after 2 days (2 - 3 days old). Check discs were sprayed with water. Treatment was conducted, with series of concentrations, as described above, then eggs were incubated at 25 ± 0.5 °C and 60% ± 5 R.H. till hatching and the percentage of hatchability was determined. Mortality was corrected by Abbott's formula (1925); LC_{50}, LC_{90} and slope values were calculated according to Finney (1971) using "LdP Line"® software.

Effect on the biological aspects

Biological aspects of *T. urticae* were studied after treatment of protonymphs with LC_{50}. Sufficient number of protonymphs were transferred singly to mulberry leaf discs then sprayed as described above. Their development was observed until death. Finite rate of increase (a life table parameter) was calculated according to Birch (1948).

Residual effect

The effect of fresh and 2 days after treatment residues was investigated on adult females. Leaf discs were sprayed with 4, 10 and 15 ppm of "BaicaoNo. 1" while check discs were sprayed with water. Treated discs were divided into two groups. Newly emerged adult females were transferred instantly to the first group, while the second group was kept in incubator for two days then 20 females were transferred for each disc. Five discs were used as five replicates for each treatment. Discs were incubated at 25 ± 0.5 °C and 60% ± 5 R.H. Mortality, fecundity (no. of eggs per no. of alive females each day) were counted daily for five days. Mortality and reduction of fecundity were corrected according to Abbott's formula (1925).

Feeding deterrent effect:

Acalypha marginata leaves were used for this study, as such leaves obviously reveal injury symptom of mite feeding. Upper surface of leaf discs was sprayed with 4, 10 and 15 ppm, then discs were left to dry. Twenty adult females were transferred to each disc then incubated as described above. Survived mites were counted and disks were photographed every 24 h. for five days using Canon® PowerShot A400 camera; photos were taken with appropriate close-up, in order to recognize symptom. Mites symptom area on each disc were measured using "Compu Eye, Leaf & Symptom Area" software, Bakr, 2005. Mite feeding area was calculated on basis of area/female/day.

RESULTS AND DISCUSSION

High efficacy was obtained when mite adult females were sprayed with BaicaoNo. 1 in the laboratory. As shown in Table (1) LC_{50} value was 1.14 ppm when mortality was counted after one day. Additional mortality was obtained when mortality was counted later and expressed by a reduction in LC values. LC_{50} value decreased to 0.44, 0.17, and 0.08 ppm when mortality was estimated after 2, 3 and 4 days, respectively. Also LC_{90} value decreased from 11.23 to 1.39 ppm when mortality was estimated after one and four days, respectively. On base of confident limits, it could be observed that LC_{50} value reduced significantly every day until the third day then insignificant difference was observed between third and fourth day. Considering LT_{50} values in Table (1), it indicated that, 2.32 days were required to kill 50% of the population when mites were sprayed with 0.25 ppm of Baicao No. 1.; while only 0.44 day was needed to kill 50% of the population when mites were sprayed with 4 ppm.

When *T. urticae* protonymphs were sprayed with BaicaoNo. 1, results in Table (2) indicated that protonymphs were more tolerant than adult females. The LC_{50} value was 26.67 ppm when mortality was estimated during protonymph duration, while when the observation was extended those individuals failed to quit the subsequent quiescent stage, LC_{50} reduced to 21.07 ppm, while LC_{90} values were 64.14 and 52.16 ppm, respectively.

When (0 - 1) and (2 - 3) days old eggs of *T. urticae* were sprayed, non-hatching eggs and mortality in subsequent larval stage were counted as well. As it is shown in Table (3), based on non hatching eggs, LC_{50} values were 168.25 and 156.41 ppm after spraying (0 - 1) and (2 - 3) days old eggs, respectively. In addition to the reduction in hatchability, high additional mortality was observed in the subsequent larval stage. When the additional mortality in larval stage was considered (non-hatching eggs + mortality in larval stage), LC_{50} values were highly reduced to 8.03 and 7.70 ppm after spraying (0 - 1) and (2 - 3) days old eggs, respectively, but the difference was not significant.

Change in biological aspects of survived mites were determined after spraying protonymphs with LC_{50} (21.07 ppm). As shown in Table (4), survived protonymphs showed significant elongation in protonymph and pre-oviposition durations compared with untreated mites. Durations of protonymph and pre-oviposition were elongated from 0.47 and 0.72 to 0.70 and 1.11 days, respectively. But the most considerable change was the significant reduction in the number of eggs laid per female, as the treated survived mites laid 61.56 eggs per female while

Table (1): Mortality % after direct spray of BaicaoNo. 1 on *T. urticae* adult females

Con. (ppm)	Days after treatment				LT_{50}	LT_{90}	Slope
	1	2	3	4			
0.0	3.00	6.00	8.00	11.00			
0.25	25.51	44.90	64.29	72.45	2.32 (2.01-2.69)	9.47	2.10
0.5	29.79	52.13	69.15	82.98	1.89 (1.63-2.14)	6.58	2.36
1	47.52	69.31	80.20	90.10	1.16 (0.87-1.40)	4.81	2.07
2	63.64	84.85	89.90	94.95	0.60 (0.27-0.87)	3.52	1.67
4	78.00	91.00	96.00	98.00	0.44 (0.18-0.66)	1.85	2.04
LC_{50}	1.14(0.93-1.42)	0.43(0.32-0.54)	0.17 (0.09-0.26)	0.08 (0.02-0.15)			
LC_{90}	11.23	3.68	2.37	1.39			
Slope	1.29	1.37	1.12	1.03			

Table (2): Efficiency of Matrine on *T. urticae* protonymphs

Con. (ppm)	Mortality % of mites before reaching given stage	
	Quiescent protonymph	Deutonymph
15	16.13	27.42
30	63.16	77.19
60	88.89	90.12
100	95.38	98.46
LC_{50}	26.67 (23.75-29.63)	21.07 (18.36-23.67)
LC_{90}	64.14	52.16
Slop	3.36	3.26

Table (3): Efficiency of direct spray of Matrine on *T. urticae* eggs at (0-1) and (2-3) days old

con. (ppm)	Mortality % of eggs (non-hatching eggs)		Total mortality % (non-hatching eggs + mortality of larval stage)	
	(0-1) day old eggs	2-3 days old eggs	0-1 day old eggs	2-3 days old eggs
0	8.60	9.60	8.60	9.91
5	19.72	18.57	36.62	34.76
10	21.43	24.62	60.32	70.15
50	32.71	39.05	99.63	97.63
500	64.22	77.45	99.57	100.00
5000	95.85	85.61	100.00	100.00
LC_{50}	168.25 (120.22-240.97)	156.41(108.79-230.61)	8.03 (6.94-9.27)	7.70 (6.55-8.96)
LC_{90}	4376.24	6062.29	22.57	23.73
Slope	0.91	0.81	2.85	2.62

Table (4): Effect of Matrine on the biological aspects of *T. urticae*

	Average durations in days								Eggs Number	Finite rate of increase
	Pre-treatment duration	Proto-nymph	Q. Proto-nymph	Deuto-nymph	Q. Deuto-nymph	Pre ov. Period	Ov. period	Post Ov. period		
Treated	6.98	0.70	0.79	0.57	0.74	1.11	14.22	0.72	61.56	1.20
Check		0.47	0.67	0.65	0.88	0.72	12.91	0.70	116.65	1.33
P		8.05E-5**	0.093	0.157	0.093	0.004**	0.350	0.948	1.46E-6**	

Q.: Quiescent, Ov.: Oviposition,

Table (5): Effect of initial and 2 days Matrine residues on *T. urticae* adult females

Criteria	con. (ppm)	Initial residues						2 days residues					
		days of exposure					Mean	days of exposure					Mean
		1	2	3	4	5		1	2	3	4	5	
Mortality* (%)	4	0.00	0.00	0.00	10.61	14.06	-	0.00	0.00	0.00	4.69	0.64	-
	10	9.20	12.39	27.16	60.49	70.48	-	2.53	20.22	22.14	38.13	42.26	-
	15	17.26	46.71	74.92	90.49	90.26	-	2.82	18.76	64.04	80.58	78.58	-
Reduction* (%) E/F/D	4	12.22	13.73	18.20	21.10	22.31	17.51	13.27	7.44	11.12	13.22	12.86	11.58
	10	25.94	45.87	56.41	58.86	61.93	49.80	33.51	44.15	53.91	50.76	51.77	46.82
	15	85.94	79.68	82.22	83.25	84.39	83.09	37.17	49.38	60.07	64.19	67.12	55.59

* Corresponding data was corrected by Abbott's formula (1925). E/F/D: Eggs/female/day.

Table (6): Effect of Matrine residues on feeding area of *T. urticae* adult females

Con. (ppm)	Feeding area (mm^2) of mite female on given day					mean	Feeding reduction* %
	1st day	2nd day	3rd day	4th day	5th day		
0	0.0582	0.0602	0.0622	0.0621	0.0651	0.0616	0.00
4	0.0212	0.0209	0.0208	0.0245	0.0277	0.0230	62.61
10	0.0078	0.0136	0.0144	0.0180	0.0213	0.0150	75.60
15	0.0110	0.0080	0.0107	0.0105	0.0107	0.0102	83.46

* Feeding area reduction % was calculated according to Abbott's formula (1925) based on mean values.

untreated mites laid 116.65 eggs per female. The overall effect of treatment: mortality and changes in biological aspects of the survived mites could be expressed in a reduction of finite rate of increase. As shown in Table (4) sprayed protonymphs with LC_{50} reduced finite rate of increase from 1.33 to 1.20 female per female per day.

Residual activity of Matrine was bioassayed by transferring recently emerged adult females to mulberry leaf discs previously treated with different concentrations of "BaicaoNo. 1". The effect of fresh and two days after treatment residues was studied in two separate tests. Results in Table (5) showed that mortality percentage increased by increasing days of exposure; recorded 14.06, 70.48 and 90.26 % when mites were exposed for five days to fresh residues of 4, 10 and 15 ppm respectively. Less mortality was obtained when residues left for two days before exposure, it reached only 0.64, 42.26 and 78.58 % after five days of exposure to the same concentrations respectively. In addition, a reduction in fecundity per female was observed when mites were exposed to residues of "BaicaoNo. 1". As shown in Table (5) the percent of eggs reduction per survived female per day, was 17.51, 49.80 and 83.09 % after exposure for 5 days to initial residues of the same concentrations, respectively. Less reduction was observed when females were reared on leaves treated two days before as the average of reduction values were 11.58, 46.82 and 55.59 % for the after mentioned concentrations, respectively.

When mites were fed on leaves treated with "BaicaoNo. 1", a reduction in feeding area was observed. As shown in Table (6), increasing concentration of "BaicaoNo. 1" reduced the feeding area per female. Average of five days feeding area of one female was 0.0616 mm^2 when mites were fed on untreated leaves. Feeding area shrank along with increasing concentration to 0.0230, 0.0150 and 0.0102 mm^2 when mites were fed on leaves pretreated with 4, 10 and 15 ppm, respectively.

Toxicity of Matrine against many insects was previously proved. Sun-ShuangYan and Hu-DunXiao (2001) proved the toxicity of Matrine against B-type *Bemisia tabaci*. Wei *et al* (2003) mentioned that Matrine oxide liquid (0.18%) showed sustainable control effects against diamondback moth - *Plutella xylostella* in field experiment.

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