Laboratory study on efficacy of etoxazole formulation having insect growth regulatory activity against the poultry red mite, *Dermanyssus gallinae*

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Abstract: The efficacy of etoxazole emulsifiable concentrate (etoxazole EC) which has insect growth regulatory activity against *Dermanyssus gallinae*, was studied in laboratory. Etoxazole EC did not show acaricidal effects against adult mites. At 2,500-fold or less dilution rates, etoxazole EC showed inhibitory effects on hatchability of eggs and molting to the first instar nymphs. Hatchability and molting rate were less than 30% and 0%, respectively. All of the hatched larvae died within one or two days after hatching. The efficacy of etoxazole EC against *D. gallinae* lasted for at least five months after treatment in the laboratory conditions. The same experiment of etoxazole EC was carried out against *D. gallinae* collected at two different locations to examine the location-related difference in efficacy, and almost similar results were obtained. The above results suggest that etoxazole EC will be an effective agent in controlling *D. gallinae*.

Key words: *Dermanyssus gallinae*, etoxazole EC, insect growth regulator

INTRODUCTION

Northern fowl mite *Ornithonyssus sylviarum* Canestrini et Fanzago, 1877 has been the most serious ectoparasites of poultry at the poultry farming in Japan. In recent years, outbreak of red poultry mite *Dermanyssus gallinae* De Geer, 1778 has become the problem in all parts of Japan. *Dermanyssus gallinae* ranges in all the continents other than Antarctica. *Dermanyssus gallinae* has been the problem in Europe for long period, and many studies have been reported. *Dermanyssus gallinae* hides in a cage or an egg receiver in daytime. In night, the mites climb up on a chicken body and then suck blood. Sucking blood causes anemia in chicken, leading damages to chicken such as de-

crease of egg-laying rate and death in sever case (Wood, 1917; Suzuki, 1988; Cosoroaba, 2001; Ezaki et al., 2002; Pilarczyk et al., 2004; Adachi, 2006). It is also a possible vector of bacterial and viral disease agents (Valiente et al., 2005). Furthermore, the mites may attack humans. Signs of intense pruritus found particularly on exposed areas of the bodies of the people taking care of the flock suggested a mite invasion (Cencek et al., 2000).

The use of commercial acaricides is a common and effective way of controlling *D. gallinae*. However, development of resistance of *D. gallinae* to those commercial acaricides has become the global problem (Zeman and Zelezny, 1985; Zeman et al., 1986; Cooper and Cobb, 1987; Zeman, 1987; Hoglund et al., 1995; Beugnet et al., 1997; Fiddes et al., 2005). As the similar

problem is concerned also in Japan, the authors searched the susceptibility of *D. gallinae* to commercial acaricides on a nationwide scale and found *D. gallinae* resistant to many acaricides (Murano et al., 2006). Five methods other than use of commercial acaricides were proposed for controlling *D. gallinae* by Chauve (1998) who considered that one of the methods using insect growth regulators (IGR) would become a highly contributing way in the future. In this study, Etoxazole, which has IGR activity against mites and ticks (Suzuki et al., 2001; Tamura et al., 2004; Nauen and Smagghe, 2006), was examined for its efficacy against *D. gallinae* in laboratory.

MATERIALS AND METHODS

Acaricides used in the study

Ettoxazole emulsifiable concentrate (containing 2.5% of ettoxazole, YSI-0596W) (ettoxazole EC), was obtained from Yashima-Sangyo Co., Ltd. Etoxazole EC was diluted with water at several dilution rates depending on the test purposes. For commercial acaricides, Sanmako wettable powder (containing 75% of carbaryl, Sankyo Chemical Industries, Ltd.) (Sanmako), ETB emulsifiable concentrate (containing 4% of permethrin, Dainihon Jochugiku Co., Ltd.) (ETB) and Sumithion emulsifiable concentrate (containing 10% of fenitrothion, Yashima-Sangyo Co., Ltd.) (Sumithion) were selected.

Mites used in the study

Dermanyssus gallinae used in Tests I to III were collected in Kyoto Prefecture (2003), and reared on hen at Chiba Prefectural Livestock Research Center. In Test IV, two wild colonies of *D. gallinae*, one collected in Fukushima Prefecture (2004) and the other in Niigata Prefecture (2004) were used.

Susceptibility test

Susceptibility of *D. gallinae* was evaluated according to the methods of Foulk

and Matthysse (1963) and Arthur and Axtell (1983). Disposable Pasteur pipettes (0.5-cm inside diameter $\times$ 11-cm length) were immersed into the acaricide solution for 1 min, and allowed to dry standing vertically on blotting paper. The moisture in the pipette was volatilized using dryer so that the acaricide adsorbs on the internal surface of pipette. The wide end of Pasteur pipette was plugged with a cotton plug. Control pipettes were immersed in water containing no acaricide. Ten blood fed adult female mites were collected into the plugged pipette under reduced pressure and the open end of pipette was sealed with Hemato-Seal (Minato medical KF 120). The pipettes were kept at 25°C and 75% RH for 24, 48 and 72 hours in Tests I and II and for 24 and 48 hours in Tests III and IV before observing live/dead status of mites in the pipettes under the microscope. A mite that stood still or was moribund, was judged to be inactive and struggle. A mite that was completely lethargic, was judged to be dead. A mite that belonged to any other condition than the above cases was judged to be alive. In addition, the number of laid eggs, the number of hatched larvae, and the number of larvae molting to the first nymphs were observed daily under the microscope for one week from the beginning of the incubation. At each concentration level, tests were performed in pentuplicate in Tests I and II and in triplicate in Tests III and IV.

The following four tests were performed according to the test purposes.

1) *Test I: Study on effective dilution rates of ettoxazole EC*

With 1,000, 2,500, 5,000, 10,000, 25,000 and 50,000-fold diluted ettoxazole EC aqueous solutions, acaricidal effect against adult mites, and inhibitions of hatching and molting were examined.

2) *Test II: Comparison of efficacy of ettoxazole EC and three commercial acaricides*

Test II was performed with 50, 100, 200,

400 and 800-fold diluted etoxazole EC aqueous solutions, 150-fold diluted Sanmako, 400-fold diluted ETB and 100-fold diluted Sumithion. The concentrations for the commercial formulations were the stated maximum concentrations for the control of *D. gallinae* on the product labels.

3) Test III: Residual activity of etoxazole EC

Inhibitions of egg-laying, hatching and molting of mites in the etoxazole EC treated pipette stored for five months at 10, 50, 100 and 200-fold dilutions were examined.

4) Test IV: Efficacy of etoxazole against wild colony of *D. gallinae* collected in other prefectures

With 100, 400 and 800-fold diluted etoxazole EC aqueous solutions, the efficacy of the agent against wild colony of *D. gallinae* collected in Fukushima and Niigata were studied.

5) Statistical processing

The 50% inhibition concentration of hatching (IC₅₀) and 95% inhibition concentration of hatching (IC₉₅) of etoxazole were analyzed using a probit procedure (SAS Institute, 1985). The hatching rates, molting rates of larvae and mortalities were compared by Tukey's HSD test (Yoshida and Abe, 1984).

RESULTS

Effective dilution rates of etoxazole EC

Etoxazole EC showed almost no acaricidal effect against adult mites. Even at the maximum concentration of 1,000-fold dilution (25 ppm as etoxazole), the mortality of adult mites was only 8% at 48 hours after the treatment (Table 1).

The hatchability decreased as the concentration of etoxazole increased. The hatchability of mites was 68.5% and 16.1% when treated with etoxazole EC at dilution rates of 50,000 folds (0.5 ppm) and 1,000 folds (25 ppm), respectively. IC₅₀ was 2.23 ppm (approximately 10,000-fold dilution) and IC₉₅ was 134.13 ppm (approximately 200-fold dilution).

No molting from larvae to the first nymphs was observed when treated with etoxazole EC at 2,500-fold (10 ppm) or less dilution rates. Molting rate increased abruptly when treated with 50,000-fold diluted (0.5 ppm) etoxazole EC solution. Mortality of larvae and first nymphs at 50,000-fold dilution was 87.8%. By the treatment with etoxazole EC at 25,000-fold (1 ppm) or less of dilution rates, all larvae and first nymphs died during the observation period (Table 2).

Comparison of efficacy of etoxazole EC and three commercial acaricides

At 48 hours after the treatment, the

Table 1. Mortalities of adult mites of *Dermanyssus gallinae* treated with etoxazole EC* at each dilution rate by contact test.

Test compound	Dilution rate	Concentration of active ingredient (ppm)	Mortality (%)	
			After 24 hours	After 48 hours
Etoxazole EC	1,000	25.0	6	8
	2,500	10.0	0	0
	5,000	5.0	0	2
	10,000	2.5	0	2
	25,000	1.0	0	2
	50,000	0.5	0	4
Control (water)	—	—	0	0

* Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

Table 2. Number of eggs, hatchabilities of the eggs, molting rates to the first nymphs and mortalities of the larvae and the nymphs of the adult *Dermanyssus gallinae* treated with etoxazole EC*.

Test compound	Dilution rate	Concentration of active ingredient (ppm)	Number of eggs per a female	Hatchability (%)	Molting rate (%)	Mortality of larvae and nymphs (%)
Etoxazole EC	1,000	25.0	24.8	16.1	0	100
	2,500	10.0	25.8	23.3	0	100
	5,000	5.0	20.4	34.3	2.9	100
	10,000	2.5	22.2	58.6	6.2	100
	25,000	1.0	22.0	63.1	5.7	100
	50,000	0.5	21.6	68.5	59.5	87.8
Control (water)	—	—	18.4	100	100	0

*Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

Table 3. Mortalities of adult mites of *Dermanyssus gallinae* treated with etoxazole EC at each dilution rate.

Test compound	Dilution rate	Mortality (%)	
		After 24 hours	After 48 hours
Etoxazole EC ¹⁾	50	0 ^{a, 5)}	10 ^{b, 5)}
	100	2 ^a	6 ^{a, b}
	200	0 ^a	0 ^a
	400	2 ^a	2 ^{a, b}
	800	2 ^a	2 ^{a, b}
Sanmako ²⁾	150	44 ^c	82 ^d
ETB ³⁾	400	58 ^c	84 ^d
Sumithion ⁴⁾	100	18 ^b	44 ^c
Control (water)	—	2 ^a	4 ^{a, b}

¹⁾ Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

²⁾ Sanmako wettable powder (containing 75% of carbaryl).

³⁾ ETB emulsifiable concentrate (containing 4% of permethrin).

⁴⁾ Sumithion emulsifiable concentrate (containing 10% of fenitrothion).

⁵⁾ Significant difference was observed between the different letters in the same column ($p < 0.05$).

mortalities of adult mites treated with 50-fold diluted (500 ppm) and 100-fold diluted (250 ppm) etoxazole EC solutions were 10% and 6%, respectively. The mortalities of adult mites at other dilution rates were 0–2%. The mortalities of mites treated with Sanmako (500 ppm), ETB (100 ppm) and Sumithion (1,000 ppm) were 82%, 84% and 44%, respectively, at 48 hours after the treatment. Commercial acaricides showed significantly higher mortality compared to those treated with etoxazole EC ($p < 0.05$) (Table 3).

The hatchability increased as the dilution rates of etoxazole EC increased. However, the hatchability was only 26.4%

when treated with 800-fold diluted etoxazole EC solution. The inhibition rate of hatching of etoxazole EC was significantly higher than those of three commercial acaricides and the control ($p < 0.05$). In addition, no molting to the first nymphs was observed by applications of etoxazole EC at any dilution rates. All of the hatched larvae treated with Sanmako and ETB died at the stage of larvae. In contrast 77.2% of larvae molted to the first nymphs, but all of the larvae and 97.8% of the first nymphs died during the observation period with Sumithion treatment (Table 4).

Table 4. Number of eggs, hatchabilities of the eggs, molting rates to the first nymphs and mortalities of the larvae and the nymphs when the adult *Dermanyssus gallinae* were treated with etoxazole EC and three commercial acaricides.

Test compound	Dilution rate	Number of eggs per female	Hatchability (%)	Molting rate (%)	Mortality of larvae and nymphs (%)
Etoxazole EC ¹⁾	50	30.3	12.2 ^{a, 5)}	0 ^{a, 5)}	100 ^{b, 5)}
	100	30.3	18.7 ^{a, b}	0 ^a	100 ^b
	200	28.7	19.8 ^{a, b}	0 ^a	100 ^b
	400	36.7	22.7 ^b	0 ^a	100 ^b
	800	36.7	26.4 ^b	0 ^a	100 ^b
Sanmako ²⁾	150	11.3	100 ^c	0 ^a	100 ^b
ETB ³⁾	400	2.0	100 ^c	0 ^a	100 ^b
Sumithion ⁴⁾	100	19.0	100 ^c	77.2 ^b	100 ^b • 97.8
Control (water)	—	28.3	100 ^c	100 ^c	0 ^a

¹⁾ Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

²⁾ Sanmako wettable powder (containing 75% of carbaryl).

³⁾ ETB emulsifiable concentrate (containing 4% of permethrin).

⁴⁾ Sumithion emulsifiable concentrate (containing 10% of fenitrothion).

⁵⁾ Significant difference was observed between the different letters in the same column ($p < 0.05$).

Table 5. Number of eggs, hatchabilities of the eggs, molting rates to the first nymphs and mortalities of the larvae and the nymphs when the adult *Dermanyssus gallinae* were treated with etoxazole EC¹⁾.

Months after treatment	Dilution rate	Number of laid eggs per a female	Hatchability (%)	Molting rate (%)	Mortality of larvae and nymphs (%)
0	10	6.3	0 ^{a, 2)}	0 ^{a, 2)}	100 ^{b, 2)}
	50	9.7	3.4 ^a	0 ^a	100 ^b
	100	14.7	13.6 ^b	0 ^a	100 ^b
	200	12.0	13.8 ^b	0 ^a	100 ^b
5	10	6.3	0 ^a	0 ^a	100 ^b
	50	17.3	13.5 ^b	0 ^a	100 ^b
	100	18.7	14.3 ^b	0 ^a	100 ^b
	200	12.0	16.7 ^b	0 ^a	100 ^b
Control (water)	—	16.3	100 ^c	93.9 ^b	6.1 ^a

¹⁾ Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

²⁾ Significant difference was observed between the different letters in the same column ($p < 0.05$).

Inhibition of egg-laying, hatching and molting of mites in the pipette stored for five months after the treatment with etoxazole EC

By the test performed on the day of treatment, etoxazole EC showed 100% inhibition of hatching at 10-fold dilution rate. The hatchability increased as the dilution rates increased, and significantly low hatchability was observed at 10 and 50-fold dilution rate. At all dilution rates, etoxazole showed 100% inhibition of

molting to the first nymphs. Compared to the above results hatchability at 50-fold dilution rate slightly increased at five months after treatment. The molting rate to the first nymphs and mortalities of larva and nymphs were not different between the pipettes on the day of treatment and those after five months (Table 5).

*Efficacy of etoxazole EC against two wild colonies of *D. gallinae**

The acaricidal effect of etoxazole EC

Table 6. Mortalities of adult mites of *Dermanyssus gallinae* collected in two different prefectures when treated with etoxazole EC¹⁾ at each dilution rate by contact test.

Prefecture	Test compound	Dilution rate	Mortality (%)	
			After 24 hours	After 48 hours
Fukushima	Etoxazole EC	100	0	6.7 ^{a, 2)}
		400	0	6.7 ^a
		800	0	0 ^a
	Control (water)	—	0	0 ^a
Niigata	Etoxazole EC	100	0	16.7 ^b
		400	0	16.7 ^b
		800	0	6.7 ^a
	Control (water)	—	0	0 ^a

¹⁾ Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

²⁾ Significant difference was observed between the different letters in the same column ($p < 0.05$).

Table 7. Number of eggs, hatchabilities of the eggs, molting rates to the first nymphs and mortalities of the larvae and the nymphs when the adult *Dermanyssus gallinae* collected in two different prefectures were treated with etoxazole EC.

Prefecture	Test compound	Dilution rate	Number of eggs per a female	Hatchability (%)	Molting rate (%)	Mortality of larvae and nymphs (%)
Fukushima	Etoxazole EC ¹⁾	100	22.7	2.9 ^{a, 2)}	0 ^{a, 2)}	100 ^{b, 2)}
		400	16.3	6.1 ^a	0 ^a	100 ^b
		800	13.7	4.9 ^a	0 ^a	100 ^b
	Control (water)	—	15.3	100 ^b	100 ^b	0 ^a
Niigata	Etoxazole EC	100	23.7	1.4 ^a	0 ^a	100 ^b
		400	18.0	1.9 ^a	0 ^a	100 ^b
		800	31.7	0 ^a	0 ^a	100 ^b
	Control (water)	—	25.3	98.7 ^b	100 ^b	0 ^a

¹⁾ Etoxazole emulsifiable concentrate (containing 2.5% of etoxazole).

²⁾ Significant difference was observed between the different letters in the same column ($p < 0.05$).

was slightly higher against adult mites collected in Niigata than those collected in Fukushima, but the mortality was only 16.7% at maximum (Table 6).

For both wild colonies of *D. gallinae*, the hatchabilities were in the single digits, and the inhibition rates of hatching of etoxazole EC at the dilution rates of 100 to 800 were significantly higher than control ($p < 0.05$). The molting rate to the first nymphs was 0%, and all larvae died during the observation period when treated with etoxazole EC (Table 7).

DISCUSSION

Since IGR exhibits an acaricidal effect

by inhibiting hormone metabolism and chitin biosynthesis, killing effect of IGR cannot be expected in adult insects (Mitsui, 2000). Tebufenozide and methoxyfenozide, which are bisacylhydrazine compounds with molting hormone activity, lowered the hatchability of European corn borer *Ostrinia nubilalis* Hulmer and killed many freshly eclosed larvae because of molting disruption (Trisyono and Chippendale, 1997). In cat flea *Ctenocephalides felis* Bouche treated with pyriproxyfen and methoprene, which have juvenile hormone mimic activity, disintegration of laid eggs and death during embryogenesis and after eclosion were observed (Palma et al., 1993). Treat-

ment of the imago of stable fly *Stomoxys calcitrans* Linnaeus and house fly *Musca domestica* Linnaeus with the chitin synthesis inhibitor, diflubenzuron, reduced the number of eggs laid and the hatchability (Wright and Spates, 1976).

It has been reported that etoxazole showed ovicidal and molting inhibitory activities to two spotted spider mites *Tetranychus urticae* Koch at a low concentration, and hatching inhibition to aphids *Myzus persicae* Sulzer which acquired resistance to various acaricides (Suzuki et al., 2001). Etoxazole also has an inhibitory effect on oviposition and a killing effect against imagos of aphids by shrinking (Tanji et al., 1996). Furthermore etoxazole inhibited the molting of larvae and nymphs and the hatching of eggs of *Haemaphysalis longicornis* Neumann (Tamura et al., 2004). The mode of action of etoxazole in the arthropods described above is unknown. Nauen and Smagghe (2006) treated larvae of fall armyworms *Spodoptera frugiperda* J. E. Smith with etoxazole and suggested that the abnormal amount of chitin content in the integument caused by inhibition of chitin biosynthesis killed the larvae. The present test results confirmed that etoxazole causes reduction of the hatchability and subsequent death of all hatched larvae in *D. gallinae*, and that etoxazole may have effects on *D. gallinae* similar to those on spider mites and animal-parasitic ticks.

Etoxazole EC is thought to have persistent efficacy for at least five months in the laboratory conditions.

Application of pharmaceuticals with IGR activity to *D. gallinae* has not been reported previously in the world. In this study, we evaluated etoxazole as a candidate for controlling *D. gallinae* and obtained good results. In circumstances in which the resistance of *D. gallinae* to commercialized acaricides is increasing throughout the world, etoxazole will be a promising agent. Field tests are under planning for confirmation of practical potency.

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