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Relative Toxicity of Abamectin to the Predatory Mite *Amblyseius womersleyi* Schicha[1] (Acari: Phytoseiidae) and Twospotted Spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

아바멕틴의 긴털이리응애(*Amblyseius womersleyi* Schicha)와 점박이응애(*Tetranychus urticae* Koch)에 대한 選擇毒性

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ABSTRACT The relative toxicity of abamectin was assessed to the predatory mite *Amblyseius womersleyi* Schicha and to dicofol-resistant and -susceptible twospotted spider mite (TSM) *Tetranychus urticae* Koch in the laboratory. Abamectin was much less toxic to the predator than to the spider mite. At 0.12 and 0.6 ppm, all TSM adult females of the two strains were killed within 48 h after dipping in the solutions. The lower concentrations (0.06 and 0.012 ppm) killed more than 77% of TSM female adults of the two strains at 120 h after treatment. However, abamectin did not significantly affect the survival and mobility of *A. womersleyi* female adults at a concentration of 0.12 ppm but the mortality was slightly increased up to 20~23% at 0.6 and 6 ppm. Abamectin did not significantly affect hatchability of one-day old TSM eggs at 0.06~0.6 ppm. The Four-day old eggs were much more susceptible to abamectin than one-day old eggs were. Within 0.006-6 ppm, abamectin did not affect the hatchability of *A. womersleyi* eggs and the development of resulting immature predators. When the predator female adults were dipped in 0.6 and 0.12 ppm solution, their reproduction was not affected, but at 6 ppm it was decreased by 35% However, the reproduction of TSM reduced significantly at concentrations between 0.006 and 0.6 ppm. The differential toxicity of abamectin between TSM and the predator could be of practical importance in managing spider mite populations in the field Abamectin at selective sublethal concentrations (i.e., 0.012~0.06 ppm) could be of value in adjusting predator/prey ratios in integrated management of spider mites

KEY WORDS Twospotted spider mite, *A. womersleyi*, abamectin selectivity. integrated pest management

초 록 긴털이리응애와 dicofol 抵抗性 및 感受性 점박이응애에 대한 아바멕틴의 選擇毒性을 실험실 내에서 葉浸漬法으로 조사하였다. 아바멕틴은 긴털이리응애에 대해서는 毒性이 낮은 반면, 점박이응애에 대해서는 殺蜱效果가 높았다 0 12 ppm과 0.6 ppm의 농도에서 점박이응애는 두 系統 모두 침지 후 48시간 이내에 사망하였고, 0.06 ppm과 0.012 ppm의 낮은 濃度에서도 120시간 이후에는 77% 이상이 사망하였다. 그러나 긴털이리응애의 암컷성충은 0 12 ppm에서는 生存率과 活動力이 영향을 받지 않았고, 0.6 ppm과 6 ppm의 높은 농도에서도 死亡率이 약 20~23%이었다 아바멕틴은 產卵後 1일 이내의 점박이응애의 난에 대해서는 孵化率에 影響을 미치지 않았으나 產卵後 4일된 卵에서는 孵化率이 減少하였다 반면에 0.006~6 ppm 溶液에 긴털이라응애 난을 침지한 경우 난의 孵化率과 그 난에서 부화한 若蟲의 發育에는 영향이 없었다. 긴털이리응애의 암컷 성충을 0.6 ppm과 0 12 ppm에 침지했을 때 산란수가 줄어들지 않았으나 점박이응애의 產卵數는 0 006~0.6 ppm의 농도에서 현저히 減少하였다. 이상과 같이 아바멕틴은 점박이응애와 긴털이리응애에 대한 選擇毒性이 높은 약제로 점박이응애의 綜合防除에 有用하게 利用될 수 있을 것으로 생각되며, 점박이응애에 대한 亞致死濃度(0 012~0 06 ppm)는 긴털이리응애와 점박이응애의 密度를 調整하는 데 利用될 수 있을 것으로 생각된다.

검색어 점박이응애, 긴털이리응애, 아바멕틴 選擇毒性, 綜合防除

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[1]This species was recently reassigned from *A. longispinosus* in Korea by Ryu (1995).

The most serious pests of apple trees in Korea include the twospotted spider mite (TSM), (*Tetranychus urticae* Koch), peach fruit moth, (*Carposina niponensis* Walsingham), apple leaf miner, (*Phyllonorycter ringoniella* (Matsumura)), and two species of aphids (Lee *et al.* 1985, 1994). Most farmers rely almost exclusively on pesticides to control these pests (Ryu *et al.* 1993). TSM has become resistant to various acaricides in Korea, including benzoximate, dicofol, cyhexatin, and propargite (Lee *et al.* 1986, Park *et al.* 1986), and has especially been a serious problem in apple orchards where miticides are sprayed in a calendar-based schedule (Lee *et al.* 1994)

Because integrated pest management programs that do not use chemicals have not yet been developed for apple pests, natural enemies of the spider mites cannot escape from pesticide applications. In the presence of chemical applications, biological control of the spider mites may be achieved by the selective use of pesticides which are less toxic to their natural enemies (Helyer 1985; Hoy 1985).

Amblyseius womersleyi Schicha is a predominant predator of the spider mites in apple orchards in Korea (Lee 1990). Based on field survey of insects, mites, and their natural enemies in apple orchards under different pesticide spray programs, Lee *et al* (1994) estimated that *A. womeraleyi* was less susceptible to pesticide applications than other phytoseiid predators, and had some effect on the reduction of TSM density, because the seasonal occurrence of this predator coincided with that of TSM.

Abamectin (avermectin B_1), a natural product from the soil microorganism *Streptomyces avermitilis* (Burg), is toxic to 84 insect species in 10 orders, most of which are of economic importance (Strong & Brown 1987). Abamectin has promising characteristics for use in integrated mite management One is its intrinsic selectivity Laboratory studies of the selectivity suggested that it was less toxic to *Typhlodromus occidentalis* (Nesbitt) than to the prey *T. urticae* and *Panonychus ulmi* (Koch) (Grafton-Cardwell & Hoy 1983; Hoy & Cave 1985; Zhang & Sanderson 1990). The other is difficulties in developing resistance in spider mites. Hoy & Conley (1987) were unable to detect resistance after 15 selections of three colonies of *T. pacificus* McGregor and two colonies of *T urticae* with abamectin. However, low resistance to abamectin (a 3.8-fold increase in LC_{50}) was obtained after 20 selections of *T. occidentalis* (Hoy & Ouyang 1989). Compared with *T. occidentalis* and *P. persimilis* Athias-Henriot, little is known about the selectivity of abamectin to *A womersleyi*.

Here we report the relative effects of different concentrations of abamectin on the survival and hatchability of both TSM and *A. womersleyi*. The effects of abamectin on reproduction and development of the predator were also evaluated.

MATERIALS AND METHODS

Colony Sources and Conditions

Two strains of TSM were originated from a colony of Korea Research Institute of Chemical Technology (KRICT) in October 1991. The susceptible strain has been reared on kidney bean (*Phaseolus vulgaris* var. *humilis* Alefeld) seedlings (3 wk after germination) in the laboratory without exposure to any insecticide since 1986. The female adults of the resistant strain, when it was obtained from KRICT in 1991, was 471 times stronger in LC_{50} value to dicofol than that of the susceptible one based on leaf disk methods. Since then, the resistant strain has been selected by spraying 100 ppm dicofol solution to kidney bean plants infested with TSM at 10-days intervals, until they were used in this experiment in 1992 The predacious mite, *A womersleyi*, was originally collected from weeds around apple ochards in 1985, and has been maintained on pesticide-free kidney bean leaves infested with TSM in the laboratory. All experiments were done at 22-27°C with 40-70% relative humidity and a 16h : 8h (light : dark) regime.

Serial dilutions of abamectin (not less than 80% avermectin B_{1a} and not more than 20% avermectin B_{1b}) were prepared by diluting 0.6% EC with distilled water Kidney bean leaf disks infested with TSM or *A. womersleyi* were dipped in an aqueous solution of each concentration for 5 s, while the solutions were being agitated. Mites of both prey and predator that did not respond to touches by a fine camel's-hair brush were considered to be dead. Mites that

could walk to more than their body length when stimulated were considered alive. mites that could not walk but move their legs were the immobilized. The number of mites drowned in the water-saturated cellucotton was also counted.

Effect of Abamectin on Survival and Reproduction of TSM

The relative effects of abamectin on the survival and mobility of adult females of TSM were assessed in tests with 50 adult females (5 replicates with 10 mites per replication). TSM females were transferred from the source colony to bean leaf disks (3 by 3 cm) with an aid of a fine camel's-hair brush. The bean leaf disks infested with mites were dipped in distilled water or aqueous solutions of abamectin for 5 s, allowed to dry for 90-120 m., and placed bottom-side up on moist cellucottons in petri dishes (2.2 by 8.8 cm). The number of adults on leaf disks were then counted because a few mites disappeared after dipping. The drowned adults in saturated cellucotton throughout experiment were deducted from the number of tested adults. TSM adults were tested at concentrations of 0, 0.0012, 0.006, 0.012, 0.06, 0.12, and 0.6 ppm. The survival, mobility and reproduction were evaluated at 24, 48, 72, and 120 h after treatment.

Effect of Abamectin on Hatchability of TSM eggs

To assess the hatchability of TSM eggs of susceptible and resistant strains, ten adult females were placed on a leaf disk (3 by 3 cm) for 24 h to allow oviposition, and then all of the adults were eliminated from the disks. The leaf disks with eggs were divided into two groups. Disks of one group were dipped in abamectin solutions at one day after oviposition at concentrations of 0, 0.006, 0.012, 0.06, 0.12, 0.6, and 6 ppm, and those of the other group were dipped in abamectin at 4 days after oviposition at 0, 0.006, 0.06, 0.12, and 0.6 ppm. Leaf disks were treated with abamectin in the same manner as it was for the adult females. Larvae and egg shells were counted daily

Effect of Abamectin on Survival and Reproduction of *A. womersleyi*

The effect of abamectin on adult female *A. womersleyi* was assessed in the same way as it was for TSM adults, with concentrations of 0, 0.12, 0.6, and 6 ppm. The bean leaf disks were infested with predators (4 replicates with 10 adult females per replication), dipped in each concentration of abamectin solution for 5 s, and then allowed to dry until the solution on disks disappeared completely. Those leaf disks were placed bottom-side up on water-saturated cellucottons in petri dishes. A number of fresh TSM adult females (more than 50) were supplied daily to ensure an abundance of food. Survival and reproduction were evaluated at 24, 48, and 72 h after treatment.

Effect of Leaf Residual of Abamectin on Development of *A. womersleyi*

Ten female adults of *A. womersleyi* per leaf disk were transferred from the source colony, allowed to deposit eggs for 24 h, and removed from the disk. Ten eggs were left on each leaf disk and others were removed. Leaf disks containing eggs (five disks per concentration) were dipped in abamectin solution of 0, 0.006, 0.06, and 6 ppm for 5 s, and allowed to dry. Larvae emerging from the eggs were counted daily. To assess the residual effect of abamectin on survival and development of the subsequent life stages, larvae were allowed to develop on the leaf disks on which they emerged until adulthood.

Statistical Analysis

All data on hatchability, survival and egg production at each time period from different concentrations of abamectin in each experiment were subjected to analysis of variance (SAS Institute 1989). Means were separated by Duncan's (1955) multiple range test or the least significant different (LSD) test when the numbers of tested insects were different among replicates. Data in the form of percentages were transformed to arcsine of square root of percentage before they were used in analysis of variance.

A *t*-test was used when only two treatments were compared. The significance level for all statistical analysis was P=0.05.

RESULTS AND DISCUSSION

Effects of Abamectin on Survival and Reproduction of TSM

Leaf disks dipped in abamectin at concentrations more than 0.012 ppm (note that abamectin [1.8% EC] is registered for the control of TSM, European red mite, and aphid species on apple trees at 6 ppm in Korea) significantly affected the survival of dicofol-resistant and -susceptible TSM (Table 1). *T. urticae* females responded rapidly to abamectin. At 24 h after treatment, most adults (>85.7%) died at the highest concentrations of 0.06, 0.12, 0.6 ppm in both strains, and almost all survivals were immobilized. This type of fast response to abamectin was also reported by Grafton-Cardwell & Hoy (1983) for *T. urticae* and *P. ulmi*. At 120 h after treatment, mortalities were more than 84% in dicofol-susceptible strain at concentrations between 0.006 and 0.6 ppm, and were more than 77% in dicofol-resistant strain between 0.012 and 0.6 ppm (Table 1). Generally speaking, TSM mortality increased with increasing abamectin concentrations, especially at lowest concentrations. The dicofol-resistant strain had significantly lower mortality than the susceptible strain ($F=26.39$, $df=1, 56$. $P<0.001$). Preliminary bioassay showed that the dicofol-resistant strain was 471 times as strong as the susceptible in dicofol resistance. However, LC_{50} value calculated with the mortality data of 48 h to abamectin (POLO program; Russell *et al.* 1977) was 0.009 ppm in the dicofol-susceptible strain, and was less than 0.06 ppm in the dicofol resistant strain (resistance ratio<10 times). According to this, it may be said that abamectin has no cross-resistance to dicofol. Kim *et al.* (1994) also reported the same result using laboratory-selected dicofol-resistant TSM strain.

The number of eggs laid showed a similar pattern with the number of surviving adults in abamectin treatment. The number of eggs per TSM female per day was reduced with increasing abamectin concentration, especially at above 0.006 ppm, in both strains (Table 2). There was little difference in the number of eggs deposited between strains. Twospotted spider mites at both strains produced less than 28.8% as many eggs as did control females at above 0.006 ppm, whereas they produced above 94.5% as many at 0.0012 ppm (Table 2). Abamectin did not affect the hatch and development of 1-day old TSM eggs at 0.006 to 0.6 ppm, but these parameters slightly decreased to 79.2% at 6 ppm, although analysis of variance did not show significant differences between treatments (Table 3). El-Banhawy & Anderson (1985) reported similar results that abamectin showed little toxic effect on the egg stage (24 h old) at 16 and 24°C at concentrations as high as 50 ppm. The hatch of 4-day old eggs were, however, affected by dipping in abamectin solutions

Table 1. Survival of adult females of dicofol-susceptible and -resistant *T. urticae* on bean leaf disks dipped in abamectin solution

Conc	Dicofol-susceptible strain				Dicofol-resistant strain			
(ppm)[1]	24 h	48 h	72 h	120 h	24 h	48 h	72 h	120 h
0.6	100a[2]	100a	100a	100a	100a	100a	100a	100a
0.12	100a	100a	100a	100a	87.8b	100a	100a	100a
0.06	90.0a	96.0a	100a	100a	85.7b	93.9a	93.9a	100a
0.012	41.7b	68.8b	75.0b	89.6a	2.1c	12.5b	37.5b	77.1b
0.006	0.0c	36.0c	44.0c	84.0b	6.5c	10.9b	23.9bc	56.5c
0.0012	2.0c	2.0d	2.0d	–	4.2c	6.3b	12.5bc	–
0.0	2.0c	2.0d	6.0d	6.0c	0.0c	2.2b	2.2c	10.9c

1: At each concentration, 37-50 female adults were tested with five replications.

2. In each column, means followed by the same letter are not significantly different (Least Significant Difference Test at P=0.05).

Table 2. Reproduction (No. eggs/female/day) of *T. urticae* adult female on bean leaf disks dipped in abamectin solution

Conc.	Dicofol-susceptible strain					Dicofol-resistant strain				
(ppm)	1st	2nd	3rd	4th	Ave.	1st	2nd	3rd	4th	Ave.
0.6	0.8	—[a]	—	—	0.8	0.2	—	—	—	0.2
0.12	1.0	—	—	—	1.0	0.4	—	—	—	0.4
0.06	1.5	1.0	—	—	1.3	1.3	—	—	—	1.3
0.012	2.0	0.4	—	—	1.2	1.7	1.4	0.5	—	1.2
0.006	2.8	0.7	0.1	—	1.2	1.9	2.0	1.1	0.4	1.4
0.0012	5.4	4.7	4.5	!	4.9	5.4	4.9	5.0	!	5.1
0.0	4.2	5.3	5.6	5.5	5.1	2.9	3.6	5.3	8.3	5.0

a. No eggs were obtained because all TSM females died.
!: No observation.

Table 3. Hatchability of one-day old eggs of *T. urticae* on bean leaf disks dipped in abamectin solution

Conc.	No. of eggs	Cumulative hatchability (%) after				Egg period[a]
(ppm)	tested	10	11	12	13 (days)	(days)
6.0	64	57.1	77.8	77.8	79.2	10.3
0.6	79	52.1	90.0	92.5	92.5	10.5
0.12	81	74.6	92.1	92.1	92.1	10.2
0.06	80	85.0	91.3	91.3	91.3	10.1
0.012	79	73.4	96.2	97.4	97.4	10.3
0.006	73	84.8	97.4	97.4	97.4	10.1
0.0	80	55.0	86.3	86.3	97.5	10.6
F value		3.49	2.46	1.69	1.89	
P(df=6, 20)		0.02	0.24	0.18	0.13	

a: Egg periods of all individuals were averaged

Table 4. Hatchability of 4-day old eggs of *T. urticae* on bean leaf disks dipped in abamectin solution

Conc	No. of eggs	Cumulative hatchability (%) after					Egg period[a]
(ppm)	tested	8	10	11	12	13 (days)	(days)
0.6	75	41.7	50.0	50.0	53.8	53.8	8.3
0.12	80	61.3	65.0	65.0	65.0	65.0	8.1
0.06	77	70.4	78.1	84.8	84.8	87.3	8.4
0.006	58	52.6	81.7	83.5	83.5	83.5	8.8
0.0	53	55.6	87.6	87.6	87.6	87.6	8.7
F value		1.31	1.93	2.28	1.76	1.76	
P(df=6, 20)		0.32	0.17	0.12	0.20	0.20	

a Egg periods of all individuals were averaged.

(Table 4) Their hatchabilities were lower than those of 1-day old eggs were at any concentrations tested ($F=5.92$, $df=4, 15$, $P<0.002$). El-Banhawy & Anderson (1985) said that susceptibility of TSM eggs to abamectin increased gradually up to 96 h and then dropped to 39% at 120 h when eggs of varying stages were dipped in 50 ppm The permeability of the egg chorion to abamectin may change over

Table 5. Susceptibility of adult females of *A. womersleyi* to abamectin on dipped bean leaf disks

Conc[1] (ppm)	Cumulative mortality (%) (% absent from leaf disk) after 24	48	72 (hrs)
6.0	0 0(22 5)a[2]	5.0(22.5)a	20.0(27 5)ab
0.6	7.5(10.0)a	10 0(15 0)a	22.5(20.0)a
0.12	0.0(17.5)a	2.5(17.5)a	7.5(20.0)bc
0 0	0.0(0.0)a	0.0(0.0)a	0 0(2 5)c

1: Number of adult females was 40 for each treatment.
2: In each column, means followed by the same letter are not significantly different (Duncan's multiple range test at P=0.05)

Table 6. Reproduction of adult female *A. womersleyi* on bean leaf disks dipped in abamectin solution

Conc (ppm)	No eggs/female/day 1st	2nd	3rd day
6.0	0 77b[1]	0.81b	1 89b
0.6	0.95b	1 35ab	2.45ab
0.12	1.49a	1 48ab	2.88a
0.0	1 88a	2.08a	2.91a

1: In each column, means followed by the same letter are not significantly different (Duncan's multiple range test at P=0.05)

time, allowing the compound to penetrate and kill more easily at ca 96 h (El-Banhawy & Anderson 1985). The mite embryo is not affected before the nervous system is developed, because abamectin interrupts nervous transmission of the neuromuscular junction (Mellin *et al.* 1983). However the cause of differential susceptibility of TSM eggs at different stages to abamectin has not yet been understood clearly. Further research is required on the ovicidal activity of this chemical.

Effect of Abamectin on Survival and Reproduction of *A. womersleyi*

At 24 h and 48 h, abamectin had no effect on the survival of *A. womersleyi* (Table 5). At 72 h, however, survival rates at 0.6 and 6 ppm were significantly lower than those at other doses. However, mortality was very low as below 22 5% (Table 5), compared with those of TSM adults at the same concentrations (Table 1). At the concentrations tested, 20-27.5% of *A. womersleyi* females escaped from the disks. It is possible that those females will survive and produce the next generation if they could find fresh leaves, as in the case of *T. occidentalis* which has ability to deposit eggs when removed from abamectin residues (Grafton-Cardwell & Hoy 1983). Daily oviposition of female predators was not affected at 0.6 and 0.12 ppm, but it was slightly reduced at 6 ppm (Table 6) Other comparative studies of abamectin toxicity to acarine predator and prey showed similar results (Grafton-Cardwell & Hoy 1983, Hoy & Cave 1985, El-Banhawy & El-Bagoury 1985, Zhang & Sanderson 1990). Grafton-Cardwell & Hoy (1983) said that abamectin was less toxic to *T. occidentalis* than to the spider mites, but few adult females survived and produced fewer eggs at the proposed field rates (4, 8, and 16 ppm). *P. persimilis* was not affected in survival or mobility of adult females, even though their reproduction was reduced at these concentrations (Zhang & Sanderson 1990) Although reproduction declined slightly at 6 ppm, there were no difference between 0.6, 0 12 ppm and control treatment. These results suggest that a common response of the above three predator species to abamectin is reduced reproduction at high concentrations

Effect of Leaf Residue of Abamectin on Development of *A. womersleyi*

Abamectin solutions did not affect the hatch of *A. womersleyi* eggs at any concentration tested (Table 7) In addition, abamectin residues on leaf disks did not affect the immature development of this predator (Table 7). Almost all young larvae survived to adulthood at all concentrations tested.

Our laboratory studies indicate that abamectin is much less toxic to the predator, *A. womersleyi* than to its prey, *T urticae*. Within 0-6 ppm, abamectin did not affect the hatch of *A womersleyi* (Table 7). The larvae of *A. womersleyi* that emerged from eggs dipped in abamectin solution developed normally on leaf disks treated with 0.006 to 6 ppm. More than 92% of the larvae developed to adul-

Table 7. Egg-to-adult survival and development of *A. womersleyi* on bean leaf disks dipped in abamectin solution

Conc. (ppm)	No of eggs tested	Hatchability (%)	Egg period (days)	Active adult (%)	Developmental period (egg to adult (days))
6.0	50	100	2.14	98.0	5.00
0.6	50	100	2.16	92.0	4.89
0.06	50	100	2.28	98.0	5.00
0.006	50	100	2.30	100	5.00
0.0	50	100	2.12	100	5.08

thood and developmental time of immature stages was not influenced at any concentrations. Similar results were reported for other predacious mites, *T. occidentalis* (Grafton-Cardwell & Hoy 1983) and *P. persimilis* (Zhang & Sanderson 1990) at 0-16 ppm. *P. persimilis* larvae also developed normally at 1 and 4 ppm of abamectin solution and there was no difference in developmental period of immature stages at any concentration (Zhang & Sanderson 1990). All of the newly hatched larvae of *T. occidentalis* matured into adults and deposited eggs at 0.001-0.1 ppm. At higher concentrations (4, 8, and 16 ppm), however, all of the larvae quickly became immobilized and died (Grafton-Cardwell & Hoy 1983). This fact indicates that larvae of *A. womersleyi* can also be affected by residues at concentrations of abamectin higher than 6 ppm.

Our results demonstrate that because abamectin appears much more toxic to twospotted spider mites than *A. womersleyi* it could be used as a selective acancide for conservation of this predacious mite. At 0.012 and 0.06 ppm (1/100 and 1/500 of field rate, respectively), most of the decofol-resistant and -susceptible TSM female adults died. However *A. womersleyi* female adults showed very low mortality (7.5%) and the reproduction was not reduced even at 0.12 ppm. In the light of selectivity of abamectin, this sublethal concentration (0.012 or 0.06 ppm) can be used as predator/prey regulating concentration in twospotted spider mite and *A. womersleyi* system. Abamectin at such concentrations might be used to adjust the predator/prey ratio by reducing spider mite numbers while allowing predators to survive by feeding on surviving spider mites, most of which are immobile and unable to cause plant injury.

For long-term biological control and integrated management of spider mite, we would like to conclude that 0.06 or 0.012 ppm concentration of abamectin which showed little effect to predators, should be used in future field trials. However, care should be taken in translating results of laboratory tests into predictions of field performance (Hoy & Cave 1985, Hoy & Ouyang 1989), because laboratory-generated bioassay data alone may not be sufficient to predict response of mites to acaricides in the field (Park *et al.* 1986, Mable & Pree 1993). It is also possible that eggs of *A. womersleyi* are more susceptible to abamectin at higher temperatures as was the case of TSM eggs (El-Banhawy & Anderson 1985).

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