Photodegradation of emamectin benzoate and its influence on efficacy against the rice stem borer, *Chilo suppressalis*

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**Article Info**

**Abstract**

Emamectin benzoate, a novel insecticide with characteristics of translaminar movement into plant leaf tissue, was derived from the avermectin family with improved thermal stability, greater water solubility and a broader spectrum of insecticidal activity than avermectin. To quantify UV photodegradation of emamectin benzoate, HPLC analysis was conducted to quantify residues of MAB1a, the major component in emamectin benzoate, after exposure to UV light for different lengths of time. Results showed both MAB1a concentration and length of UV light exposure (0–120 h) had significant impacts on photodegradation rate. The degradation rate increased as exposure duration increased, but decreased as initial MAB1a concentration increased. Four UV protectants, kojic acid, sodium ligninsulfonate, soybean lecithin and milk, were evaluated for their effect on UV degradation. Results showed that kojic acid could effectively reduce the photodegradation of MAB1a. In addition, the photodegradation of emamectin benzoate was also examined for its influence on the efficacy against the rice stem borer, *Chilo suppressalis*. The results of the bioassays were consistent with those of HPLC analysis. The initial concentration of emamectin benzoate and exposure duration to UV light both had significant influences on the efficacy against *C. suppressalis*. As the UV exposure time increased, the efficacy of emamectin benzoate against *C. suppressalis* decreased. The results indicated that UV photodegradation has a significant effect on the efficacy of emamectin benzoate against *C. suppressalis* and the effect is concentration-dependant. In addition to understanding the critical factor of UV exposure length, this study also showed that maintaining emamectin benzoate concentration above a certain level in the formulation and the application solution, and applying UV protectants may reduce photodegradation and increase efficacy against target pests.

**Keywords:** Emamectin benzoate, Photodegradation, UV light, *Chilo suppressalis*

1. **Introduction**

Avermectin, isolated from fermentation products of the soil microorganism Streptomyces avermitilis (MacConnell et al., 1989), is considered an important component for controlling the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), one of the most serious pests on rice, *Oryza sativa* L., in Asia (He et al., 2008). Many mixtures or formulations of avermectin have been registered for control of the rice borers since 1998 in China (Cao et al., 2003; Qin and Ju, 2004). Emamectin benzoate is a novel macrocyclic lactone insecticide derived from the avermectin family with a substitution of an epi-methylamino (-NHCH3) group for a hydroxyl (-OH) group at the 4a-position on the disaccharide and is produced as a benzoate salt. As with avermectin, emamectin benzoate (MK244) consists of a mixture of two homologous compounds, a major (≥90%) constituent of 4a-deoxy-4a-(epi-methylamino) avermectin B1a (MAB1a) benzoate and a minor (≤10%) constituent of 4a-deoxy-4a-(epi-methylamino) avermectin B1b (MAB1b) benzoate. MAB1a differs from MAB1b only by the presence of an additional methylene unit on the side chain at C-25 (Mushtaq et al., 1998). Emamectin benzoate was further improved with thermal stability and greater water solubility, which then resulted in a broader spectrum of insecticidal activity than avermectin (Jansson and Dybas, 1998). Emamectin benzoate targets...
various lepidopteran pests and is being developed for use on major field crops and vegetables, such as soybean, cotton, cabbage and radish (Lasota and Dybas, 1991; Ishaaya et al., 2002).

Avermectins have relatively shorter residual activities. After the compound has been sprayed on plants, surface residues decompose rapidly under sunlight, resulting in a relatively low toxicity to beneficial insects (Feely et al., 1992). They are rapidly photodegraded in water with a half-life (t½) of approximately 0.5 days or less in summer. When abamectin is applied as thin films on water surface, its half-life was only 4–6 h, suggesting a potential low risk to aquatic organisms (Wright et al., 1985). Despite its rapid decomposition in various systems, abamectin still provides a relatively long residual activity against target pests in field conditions due to its translaminar activity (Wright et al., 1985). Unlike avermectin, the half-life of emamectin benzoate in pond water could reach 7 days, but would be reduced to as short as one day if the water contained a natural photosensitizer such as humic acid.

However, in aqueous solution buffered to pH 7, photodegradation (t½) of emamectin benzoate under sunlight may take 22 days. In darkness, there is almost no degradation and emamectin is stable in soils (Mushitaq et al., 1996, 1998). However, Kong et al. (2009) estimated that the photolytic half-life of emamectin benzoate in water under a Xe lamp with an optical intensity of 2370 lux and ultraviolet radiation intensity of 13.5 μW cm⁻² to be 1.73 h.

This study was conducted to investigate the stability of emamectin benzoate under UV light, to assess the effect of different UV protectants on photodegradation, and to evaluate its toxicity against C. suppressalis, after exposure to UV light for different time lengths. It was expected that results of this study would provide guidelines for improving control efficacy of emamectin benzoate against target pests.

2. Materials and methods

2.1. Chemicals and equipment

Technical grade (94.4%) emamectin benzoate was provided by Syngenta Investment Company (Nantong, Jiangsu, China); Acetoniitrile (HPLC grade), methanol (HPLC grade) and trifluoroacetic acid (HPLC grade) were purchased from Sigma (St. Louis, MO). The UV protectants, kojic acid and sodium ligninsulfonate, were provided by Shanghai Jinchun Chemical Co., Ltd, soybean lecithin was provided by Shanxi Jinfu Chemical Co., Ltd and were reared in the laboratory by using a rice seedling rearing method (Shang et al., 1979). The insects were maintained at 28 ± 1 °C and a photoperiod of 16:8 (L:D) h.

2.2. Insects

The egg masses of the first field generation of C. suppressalis were collected from rice fields in 2009 in Lianyungang (Jiangsu, China), and were reared in the laboratory by using a rice seedling rearing method (Shang et al., 1979). The insects were maintained at 28 ± 1 °C for HPLC column. Injection volume was 20 μl. The mobile phase was acetonitrile and 0.1% trifluoroacetic acid. The start ratio of the mobile phase (acetonitrile: 0.1% trifluoroacetic acid) was 60:40%. Acetonitrile ratio increases to 100% after 30 min and then decrease to 40% after 36 min.

The gradient program of HPLC was listed in Table 1.

2.3. Photodegradation of MAB1a under UV light

2.3.1. HPLC parameters for MAB1a analysis

Flow rate was set at 1.0 ml min⁻¹ and wavelength of the detector was set at 250 nm. Ambient temperature was maintained at 21 °C for HPLC column. Injection volume was 20 μl. The mobile phase was acetonitrile and 0.1% trifluoroacetic acid. The start ratio of the mobile phase (acetonitrile: 0.1% trifluoroacetic acid) was 60:40%.

Acetonitrile ratio increases to 100% after 30 min and then decrease to 40% after 36 min. The gradient program of HPLC was listed in Table 1.

2.3.2. Preparation of MAB1a analytical standard curve

Fifty mg of emamectin benzoate analytical standard (MAB1a: 94.4%) was dissolved in 50 ml acetonitrile. After the solution was treated with ultrasonic bath at room temperature for approx 5 min, 50 ml trifluoroacetic acid (0.1% in acetonitrile) was added to the emamectin benzoate solution to obtain a final concentration of MAB1a at 472 mg a.i. per liter as a stock solution. The stock solution was diluted to a series of the analytical standard solutions at 4.72 mg, 47.2 mg, 141.6 mg, and 236 mg a.i. per liter (MAB1a) with acetonitrile. The diluted and the stock solutions were used to establish the standard curve for analysis of MAB1a content of the technical grade of emamectin benzoate and UV light treated samples. The peak areas were plotted against MAB1a concentrations to generate a regression equation (Fig. 1).

\[ Y = 18.444X - 1.0759 \]  

(1)

or

\[ X = 0.0542Y + 0.0583 \]  

(2)

where:

- Y = Peak area of MAB1a.
- X = Concentration of MAB1a (mg a.i. per liter).

The correlation coefficient of the regression was high (\( R^2 = 1 \), Fig. 1), which indicated a good linear relationship between the peak areas and the MAB1a concentrations from 4.72 mg a.i. per liter to 472 mg a.i. per liter. Therefore, the analytical method and standard curve were suitable for this analysis. The technical grade of emamectin benzoate was analyzed, and the content of MAB1a was calculated by using the Eq. (2) above.

2.3.3. Preparation of photodegradation solution

Exactly 2650 mg of technical grade emamectin benzoate (with the content of 94.3% MAB1a) was placed in a 250 ml volumetric flask. One hundred ml of methanol was used to dissolve emamectin benzoate. After the solution was treated with ultrasonic bath for approx 5 min, additional methanol was added to the solution to obtain a stock solution with 1000 mg a.i. per liter of MAB1a. The stock solution of MAB1a was diluted with methanol to 100 mg a.i. per liter, 10 mg a.i. per liter, and 1 mg a.i. per liter. Ten ml of the MAB1a was transferred into a quartz vial (diameter: 25 mm, height: 100 mm). A set of 9 vials were prepared and placed in UV light cabinet for 3 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h, 96 h, and 120 h, respectively. The vials with MAB1a were removed from UV cabinet after certain time. The remaining content of MAB1a was immediately analyzed with HPLC. An additional vial was prepared immediately for HPLC analysis as a no UV exposure treatment (0 h).
exposure was repeated twice, and each data point represented an average of the two HPLC readings.

2.3.4. Analysis of MAB1a photodegradation samples

The setup of the HPLC system was as described above and gradient program in Table 1. The standard solutions and the sample solutions treated with UV light were injected into HPLC. The first injection was repeated until the difference between the peak areas of the two consecutive injections was not more than 1%. The sequence of injection was as:

...standard, sample 1, sample 1, sample 2, sample 2, standard...

The standard sample was used as a control, and the response factor of two standard injections should not vary by more than 1%.

2.3.5. The calculation of photodegradation rate (%)

After the HPLC peak areas were obtained from UV light treated samples, Eq. (2) (above) was used to calculate the concentration of the MAB1a solutions. The photodegradation rate of MAB1a was calculated by using the following equation:

\[
\text{Photodegradation rate \%} = \left( \frac{C_0 - C_1}{C_0} \right) 
\times 100
\]

where:

- \(C_0\) = MAB1a concentration before UV light exposure.
- \(5C_1\) = MAB1a concentration after UV light exposure.

2.4. Photodegradation of MAB1a solutions mixed with UV protectants

One hundred and six mg of analytical standard emamectin benzoate (MAB1a: 94.4%) was dissolved in 50 ml methanol. After being held in an ultrasonic bath at room temperature for approx 5 min, the solution was brought up to 1000 ml with acetonitrile to obtain a final concentration of MAB1a at 100 mg/l using blank solution. Exactly 0.05 g of kojic acid, sodium ligninsulfonate, soybean lecithin and milk were added separately into 100 ml of MAB1a solution to obtain 0.05% (w/v) of the UV protectants in 100 mg/l MAB1a methanol solutions. The content of MAB1a was determined after the mixtures were exposed to UV for 3, 6, 12, 24, 36, 48, 72, 96, and 120 h. To test the concentration effect of UV protectant on MAB1a photodegradation, 0.05%, 0.10%, 0.20%, 0.50% and 1.00% of kojic acid was added to 100 mg/l MAB1a solutions. The content of MAB1a was determined using the same procedures described above.

2.5. Bioassays

The topical application method (FAO, 1980) was used to conduct bioassays on C. suppressalis. Middle fourth instars with body weights ranging from 6 to 9 mg per larva were used as a standard larval stage in the bioassay (Cao et al., 2001). Larvae were placed into Petri dishes (5 cm) containing a piece of artificial diet (1 × 1 × 0.3 cm). The artificial diet was prepared based on the improved recipe (Tan, 1987), which was originally reported by FAO (1980). Insecticides were diluted into a series of concentrations with acetone. A droplet of 0.04 μl of insecticide solution was topically applied to the dorsal side of larval middle abdomen with a capillary microapplicator (FAO, 1980). Ten larvae were treated for each replication, and three replicates were used for each concentration. Control insects were treated with acetone only. The rearing conditions for treated larvae were controlled at 28 ± 1 °C and a photoperiod of 16:8 (L:D) h. Mortality was recorded 72 h after treatment with emamectin benzoate. Larvae were considered dead if they were unable to show response after being probed with a fine brush. PoloPlus software (LeOra Software, 2007) was used for probit analysis of dose–response data and calculations of LD₅₀ values. Relative toxicity (the ratio of the LD₅₀ of no UV treatment to the LD₅₀ for UV treatment) was used as a relative potency (%) against C. suppressalis (Fig. 2).

3. Results

3.1. Photodegradation of the active ingredient of emamectin benzoate (MAB1a)

By using Eq. (3), the average photodegradation rate of each treatment was calculated. After being exposed to UV light for 0–120 h, all of the emamectin benzoate solutions at four different concentrations were significantly degraded with increasing effect as the exposure time increased (Fig. 3). After the lowest concentration (1 mg a.i. per liter) was exposed to UV light for 3 h, 70% of the MAB1a was photodegraded, and 100% of the MAB1a was degraded after 48 h of UV exposure. The photodegradation rates of the 10 mg a.i. per liter MAB1a sample solution were 66.5% after the UV light treatment for 3 h, and the 100% of the MAB1a was degraded after being exposed to UV light for 120 h. After the 100 mg a.i. per liter MAB1a solution was treated with UV light for 9 different time durations (3–120 h), 40.9%–93.7% of the MAB1a was degraded. When 1000 mg a.i. per liter MAB1a solution was exposed to UV for the 9 time durations, the degradation rates were the slowest, ranging from 9.0% to 58.3%. Results indicated that UV light had significantly degraded emamectin benzoate under laboratory conditions. Among the four sample solutions with different initial concentrations of MAB1a, the photodegradation was quicker in the low concentrations of MAB1a, while it was relatively slower in higher concentrations of MAB1a (Fig. 3).

3.2. Influence of UV protection reagents on photodegradation of MAB1a

Results showed that the addition of UV protectants could reduce photodegradation of MAB1a (Fig. 4). After 24 h exposure to UV light, the degradation rates of MAB1a mixed with kojic acid, sodium ligninsulfonate, soybean lecithin, and milk were 41.0%, 49.3%, 52.8% and 56.2%, which were substantially lower than 62.2% degradation rate in control (no UV protectant). After 120 h, approximately 90% of MAB1a was photodegraded in the mixtures of MAB1a with sodium ligninsulfonate, soybean lecithin, and milk, while kojic acid maintained the degradation rate below 55%. Without the UV protectants, it took about 5 h to degrade 50% of the MAB1a. The
addition of milk, soybean lecithin, sodium ligninsulfonate, and kojic acid prolonged the 50% degradation time to 9, 17, 24 and 48 h, respectively (Fig. 4). Among the four additives tested, kojic acid exhibited the best UV protection of MAB1a from UV degradation. The experiment with different concentrations of kojic acid revealed that increase of the additive concentration could further suppress UV degradation (Fig. 5). After 120 h exposure to UV, kojic acid at 0.50% and 1.00% could reduce the photodegradation to 29% and 18%, respectively. The other three concentrations, 0.05%, 0.10%, 0.20%, had similar (53–55%) photodegradation ratios (Fig. 5).

3.3. Effect of UV photodegradation on the efficacy of emamectin benzoate against C. suppressalis

After the emamectin benzoate sample solutions at 10, 100 and 1000 mg a.i. per liter were exposed to UV light for 0, 12, 24, 36, 48,
72, 96 and 120 h, the topical application method was used to examine the efficacies of emamectin benzoate against the fourth instar larvae of *C. suppressalis* (Table 2). The LD$_{50}$ values of each concentration of emamectin benzoate without UV light exposure were used as control values for calculating the relative toxicity index of each concentration after UV light exposure.

Without UV light exposure, the emamectin benzoate sample solutions of 1 mg a.i. per liter caused the mortality of only 13.3% (72 h) to *C. suppressalis*. Dose-response equations and LD$_{50}$ values could not be calculated because of lack of sufficient data points. After the emamectin benzoate sample solutions (1 mg a.i. per liter) were exposed to UV light for 12, 24, 36, 48, 72, 96 and 120 h, the mortalities of treated *C. suppressalis* for all UV treatment lengths were nearly 0%. These results showed that exposing low concentration of emamectin benzoate (i.e. 1 mg a.i. per liter) to UV light for as short as 12 h, the chemical completely lost its toxicity against *C. suppressalis*.

After the 10 mg a.i. per liter of emamectin benzoate was exposed to UV light treatment for 12 h, the contact toxicity (LD$_{50}$: 0.00050 mg a.i. per larva) was significantly lower than that of the 0 h treatment (LD$_{50}$: 0.00018 mg a.i. per larva), with a relative toxicity of 36%. After the 10 mg a.i. per liter of emamectin benzoate solution was exposed to UV for 24 h, the mortality of *C. suppressalis* was reduced to 13.3%. Because of reduced efficacy, the dose–response (LD$_{50}$) could not be established for the same concentration (10 mg a.i. per liter) of emamectin benzoate solution after longer than 24 h exposure to UV light.

When the emamectin benzoate concentration was increased to 100 mg a.i. per liter, the 12 h, and 24 h long UV light treatments substantially reduced toxicity (LD$_{50}$: 0.00025, 0.00022 mg a.i. per larva) of emamectin benzoate against *C. suppressalis*, as compared with the no-UV treatment (LD$_{50}$: 0.00011 mg per larva). The relative toxicities were 44.0% and 50.0% for the 2 different exposure durations, respectively. After the same concentration of emamectin benzoate had been exposed to UV for longer, i.e. 48, 72, 96 and 120 h, emamectin benzoate became less effective against the insect (LD$_{50}$ values were 0.00055, 0.00058, 0.00168, 0.00181 mg a.i. per larva, respectively). Meanwhile, there also were substantially lower relative toxicities of 20.0%, 19.0%, 6.5% and 6.1% than for the same chemical without UV treatment.

When emamectin benzoate, with initial deposit treatment of 1000 mg a.i. per liter, was exposed to UV for 12 h, and 24 h, its toxicity (LD$_{50}$ values were 0.00017 and 0.00023 mg a.i. per larva) didn’t show significant differences from that without UV treatment (LD$_{50}$ = 0.00021 mg a.i. per larva), because of overlapping of the 95% FL of their LD$_{50}$. After extended exposure to UV light for 48, 72, 96 and 120 h, the relative toxicities of emamectin benzoate at 1000 mg a.i. per liter (LD$_{20}$ values were 0.00040, 0.00038, 0.00060, 0.00074 mg a.i. per larva, respectively) were 52.5%, 55.3%, 35.0% and 28.4%, respectively.

The bioassay data indicated that the efficacy of emamectin benzoate against *C. suppressalis* was related to the UV exposure time length and the initial concentration of emamectin benzoate. As the exposure time increased, the efficacy of each emamectin benzoate sample solution against *C. suppressalis* decreased, and the higher initial concentration solutions of emamectin benzoate exhibited slower reduction rates of their relative toxicity.

### 4. Discussion

Emamectin benzoate is a glycoside consisting of a 16-membered ring macrolide and a disaccharide (oleandrose) via substitution of an
epi-methylamino (-NHCH₃) group for a hydroxyl (-OH) group at the 4′-position on the disaccharide. Previous studies indicated that the bonds in emamectin benzoate, such as ether and ester, are vulnerable to photodegradation, which may produce many photodegradation byproducts under UV light (Mushtaq et al., 1998; Feely et al., 1992; Wrzesinski et al., 1996; Crouch and Feely, 1995).

By exposing emamectin benzoate to UV light in this study, the active ingredient (MAB1a) of emamectin benzoate was substantially degraded. The degree of degradation was correlated with chemical concentration. At the low initial concentration (1 mg a.i. per liter), the half-life of emamectin benzoate was less than 3 h. As the initial concentrations of emamectin benzoate were increased, the half-life of the chemical due to photodegradation became longer. However, even if the concentration of the emamectin benzoate solution was increased to 1000 mg a.i. per liter, the long period of UV exposure (up to 120 h in this study) could still result in more than half (up to 57%) of the chemical being photodegraded.

Bioassay is a practical alternative to evaluate the effect of UV light on chemical stability. In addition to HPLC-measurement of emamectin benzoate content, bioassay was also conducted in this study to examine the effect of photodegradation on toxicity reduction of the chemical. Our data indicates that the photodegradation of emamectin benzoate could reduce the control efficacy against C. suppressalis. The toxicity reduction tendency was also closely correlated with the concentration of emamectin benzoate and the UV light exposure time. As the concentration of emamectin benzoate was increased, the efficacy reduction caused by the UV light treatment was relatively slowed down. These findings also consistently confirm that UV light exposure might be the major reason for the reduced control efficacy of emamectin benzoate in the field.

Our HPLC analysis and bioassay clearly indicate that UV light exposure duration is a key factor in emamectin benzoate photodegradation. The photodegradation rate was closely associated with the exposure time. The photodegradation increased as the UV exposure extended. More interestingly, we also found that the photodegradation rate of emamectin benzoate was concentration-dependent. Within the range of the tested concentrations (1–1000 mg a.i. per liter), the photodegradation rate of emamectin benzoate decreased rapidly as its initial concentration increased. These results showed that a low content of emamectin benzoate formulation and low rate in field application could result in short residual activity and low control efficacy against the target pest. Considering the potential risk of resistance development and economic cost, however, the results from this study do not constitute a recommendation of high concentration of emamectin benzoate for formulation or for field application. Instead, an alternative approach to protect emamectin benzoate from UV damage was evaluated.

Table 2

<table>
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<th>Concentration (mg a.i. per liter)</th>
<th>Exposure time (h)</th>
<th>Slope (SE)</th>
<th>α²</th>
<th>β²</th>
<th>LD₅₀ (95% CL) (mg a.i. per larva)</th>
<th>Relative toxicity (%)</th>
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<td>10</td>
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<td>180</td>
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a Number of insects tested.

b Relative toxicity was calculated by dividing LD₅₀ of non-UV-control by LD₅₀ of UV-treatment.

c The χ² value without “*” indicate good fit of the data to the probit model (P < 0.05).

d Not established (due to reduced efficacy or lack of enough data points).

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