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MOSQUITO LARVICIDAL EFFECTIVENESS OF ECOBIO-BLOCK® S: A NOVEL INTEGRATED WATER-PURIFYING CONCRETE BLOCK FORMULATION CONTAINING INSECT GROWTH REGULATOR PYRIPROXYFEN

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ABSTRACT. EcoBio-Block® S, a novel controlled release system (CRS) for the insect growth regulator pyriproxyfen, uses a water-purifying concrete block system (EcoBio-Block) composed of a porous volcanic rock and cement, and it incorporates the aerobic bacterial groups of *Bacillus subtilis natto*. EcoBio-Block S showed high inhibitory activity against mosquito emergence as well as a water-purifying effect. Chemical analysis and bioassay showed that EcoBio-Block S provides a high-performance CRS that controls the release of pyriproxyfen at low levels according to "zero order kinetics."

KEY WORDS Pyriproxyfen, EcoBio-Block®, *Aedes albopictus*, water purification, insect growth regulator

INTRODUCTION

Juvenile hormone mimics (JHMs), which have been developed from natural sources, are among the most studied and effective chemicals and are categorized as insect growth regulators (IGRs). These chemicals have a unique mode of action that is insect-specific, stage-specific, slow acting, and not neurotoxic (Miyamoto et al. 1993). Methoprene (Henrick et al. 1973) and pyriproxyfen (Hirano et al. 1998) are the most successful JHMs. Almost 40 years have passed since Williams (1967) suggested that JHMs could be the 3rd generation insecticides that will not adversely affect the ecosystem because of their target-specificity, and against which pests theoretically have no potential of developing resistance. However, the above-mentioned ideas have been proved incorrect or have changed during the 3 decades since the first successful JHMs, methoprene and hydroprene, were commercialized. Insect resistance to JHMs has become common among agricultural and nonagricultural pests (Zhang et al. 1998, Cornel et al. 2002, Ishaaya et al. 2005), and several reports have demonstrated that JHMs may adversely affect the ecosystem if they are overused (Miyamoto et al. 1993, Trayler and Davis 1996). Therefore, utmost care and high-level expertise are a requisite for the biorational use of JHMs, and application of the minimum dose in the most effective manner will result in maximum benefits both to humans and the ecosystem.

EcoBio-Block® is a concrete block composed of porous volcanic rock and cement, and it incorporates the aerobic bacterial groups of *Bacillus subtilis natto*. When EcoBio-Block is deployed in the riverbeds of polluted rivers, sewage treatment plants, and drainage ditches, aerobic bacteria decompose the organic matter; this results in purification of the polluted water. EcoBio-Block has already been commercialized in Japan and is being used in a nationwide, large-scale project for purification of Melaka River in Malaysia since 2001. Recently, Matsunaga et al. (2006) carried out laboratory experiments to determine the ability of EcoBio-Block for mineralizing organic matter and nitrifying ammonium nitrogen (NH_4-N). They reported that EcoBio-Block had a very high ability for mineralization of organic matter and nitrification of NH_4-N, although both reactions depend strongly on the water temperature. For the dual purpose of adding mosquito larvicidal capacity to the block and controlling the release rate of the larvicide into the ecosystem at the minimum effective dose, we have developed a new integrated block, EcoBio-Block S, impregnated with pyriproxyfen. In this study, we report on the mosquito larvicidal activity and water-purifying effect of EcoBio-Block S under laboratory and simulated field conditions.

MATERIALS AND METHODS

Test samples

EcoBio-Block S was produced by mixing neutralized cement (40.6% w/w), aggregate (pumice stone, 52.1% w/w), a mixture of aerobic bacteria and nutrient medium (4.1% w/w), 0.5% granular formulation of pyriproxyfen (Sumilarv® 0.5 G, 3.2% w/w; Shinto Fine Co., Ltd., Osaka,

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Japan). The content of pyriproxyfen in the block was 0.016% (w/w).

Laboratory evaluation of emergence inhibition and release rate of pyriproxyfen after treatment

One liter of deionized water was poured into a glass jar (12 cm in diam, 15 cm in height). One hundred and twenty-five grams of EcoBio-Block S or 2.0 g of Sumilarv 0.5 G were placed at the bottom of the jar to achieve 10 ppm pyriproxyfen concentration. To avoid disturbing the sample, the water in the jar was carefully exchanged daily. Using 4th instars of *Aedes albopictus* (Skuse), inhibition of emergence was examined with the daily exchanged water. Twenty larvae were released into 400 ml of water and were reared in the solution until adult emergence. After emergence of all adults, emergence inhibition was calculated as the percentage of dead pupae per total number of pupated mosquitoes (2 replicates). The pyriproxyfen concentration in the water that was exchanged daily was determined by chemical analysis until 14 days after treatment and thereafter by bioassay. For the chemical analysis, pyriproxyfen in the water was absorbed to Sep-Pak® (Nihon Waters K.K., Tokyo, Japan) and extracted with methanol. The extracted sample was analyzed by a high-performance liquid chromatography (HPLC) system equipped with a YMC-PACK ODS-A column (YMC, Kyoto, Japan) under the following conditions: detector wavelength, 230 nm; and liquid phase methanol/water (85:15 v/v) at a flow rate of 0.8 ml/min. The bioassay for the detection of pyriproxyfen concentration was performed using the percentage of inhibition of emergence in the 4th instars of *Ae. albopictus* with the diluted solution of the original water (principally 100×, occasionally 1,000× to 10,000× dilution). The concentration was calculated by substituting the percentage of inhibition of emergence (2 replicates) into the dose–inhibition regression line for *Ae. albopictus* obtained by probit analysis (Bliss 1934) by using another set of dose–inhibition bioassay data. The above-mentioned procedures were repeated at intervals of several days until 143 days after treatment of the samples.

Evaluation of emergence inhibition by using EcoBio-Block S under simulated field conditions

Plastic buckets (70 liter) were covered with stainless steel mesh nets and used for the test. The buckets were placed under the tree shade on the campus of Nagasaki University, Nagasaki, Japan. Sixty liters of water was poured into each bucket, and 3 small holes were drilled in the bucket walls along the water level to allow precipitation overflow and maintenance of the volume of the water at 60 liters. Approximately 200 g of fallen leaves was put into the bucket as food for the mosquito larvae 22 days before treatment with EcoBio-Block S. Seventeen days before the treatment, approximately 100 eggs of *Ae. albopictus* were inoculated in the water; thereafter, approximately 20 eggs were inoculated at intervals for several days. EcoBio-Block S samples weighing 60 g each were placed into each of the 3 buckets containing water to achieve 0.16 ppm pyriproxyfen concentration, and 3 other buckets were used as controls. Before and after the treatment with the block, pupae were collected from each bucket and were reared in the laboratory until adult emergence. Emergence inhibition was calculated as the percentage of dead pupae per total number of pupae collected. The collection of pupae was done at intervals of 3 to 4 days. The chemical oxygen demand (COD), pH, nitrate nitrogen (NO_2-N), and ammonia nitrogen (NH_4-N) were estimated at the time of pupae collection by using a simple test kit used for water quality check (Packtest®, Kyoritsu Chemical-Check Lab., Corp., Tokyo, Japan). The biological oxygen demand (BOD) was estimated by using a BOD set for river water (WA-BOD, Kyoritsu Chemical-Check Lab.). The precipitation in the test area was recorded daily. The test was carried out from June 3 to October 22, 2003.

RESULTS AND DISCUSSION

Inhibition of emergence in mosquito larvae and release rate of pyriproxyfen with daily exchanged stagnant water after treatment

Figure 1 shows the changes in pyriproxyfen concentration in the daily exchanged stagnant water treated with EcoBio-Block S and Sumilarv 0.5 G, when initially treated with 10 ppm total active ingredient. In the first 14 days, a large amount of pyriproxyfen was chemically detected by HPLC in water samples treated with Sumilarv 0.5 G. The amount of pyriproxyfen in EcoBio-Block S, however, was lower than the detection limit (20 ppb) during the first 14 days. Subsequently, the pyriproxyfen concentration was calculated using the percentage of inhibition of emergence by bioassay, by converting the percentage of inhibition of emergence into the dose–inhibition regression line for *Ae. albopictus*: $Y = 1.7271X + 7.3368$, where Y is the percentage of mortality expressed with Probit, and X is the concentration of pyriproxyfen (ppb). The cumulative release of pyriproxyfen with Sumilarv 0.5 G in the first 14 days was calculated by the exponential approximation formula $Y = 101.16e^{-0.0201X}$, where Y is the pyriproxyfen concentration (ppb), and X is the number of days after treatment. The result obtained was 1242 ppb, i.e., 12.4% of the total active ingredient

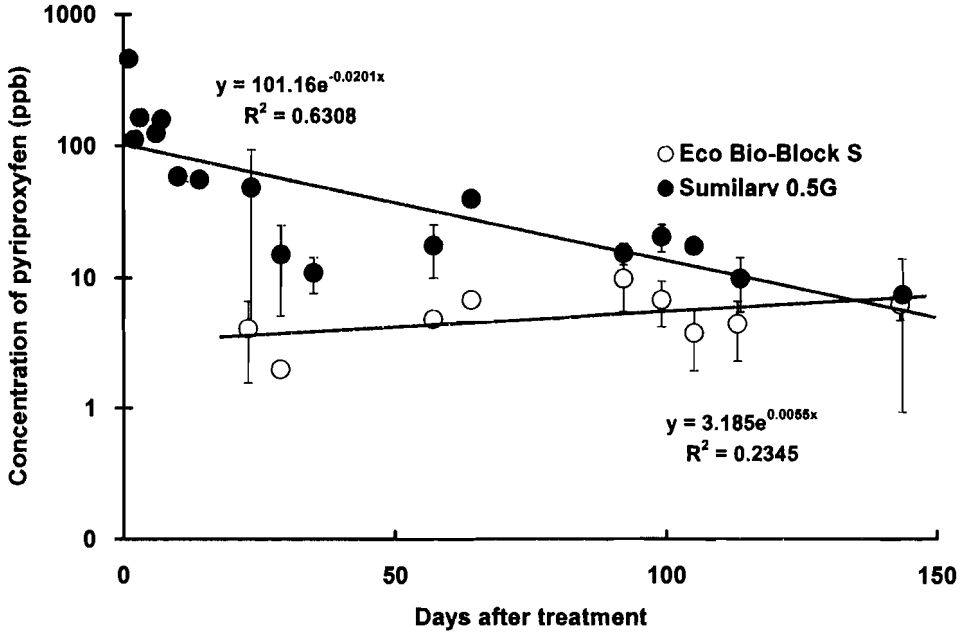

Fig. 1. Changes in concentration of pyriproxyfen in daily exchanged stagnant water treated with EcoBio-Block S and Sumilarv 0.5 G at the rate of 10 ppm as active ingredient. Solid line is exponential approximation curve.

treated. Thereafter, the theoretical cumulative release of pyriproxyfen with Sumilarv 0.5 G calculated by the same formula indicates that 79.6% (7,959 ppb) of the total active ingredient initially treated was released by 150 days after treatment. In constrast, the cumulative release of pyriproxyfen with EcoBio-Block S was calculated by the exponential approximation formula $Y = 3.185e^{0.0055X}$. Cumulative release was less than 8% (784 ppb) of the total active ingredient by 150 days after treatment (Fig. 1), indicating that EcoBio-Block® S provides a high-performance controlled release system (CRS). The exponential approximation formula for EcoBio-Block S seems to indicate that the release of pyriproxyfen is controlled according to "zero order kinetics."

Figure 2 shows the inhibition of emergence in *Ae. albopictus* larvae with 2 formulations of the daily exchanged water. The high inhibition of emergence by both formulations supports the assumption that the chemically synthesized pyriproxyfen in the water is biologically active against mosquito larvae. Compared with natural conditions, the daily exchange of water apparently created difficulties in maintaining the high pyriproxyfen concentration. Furthermore, the

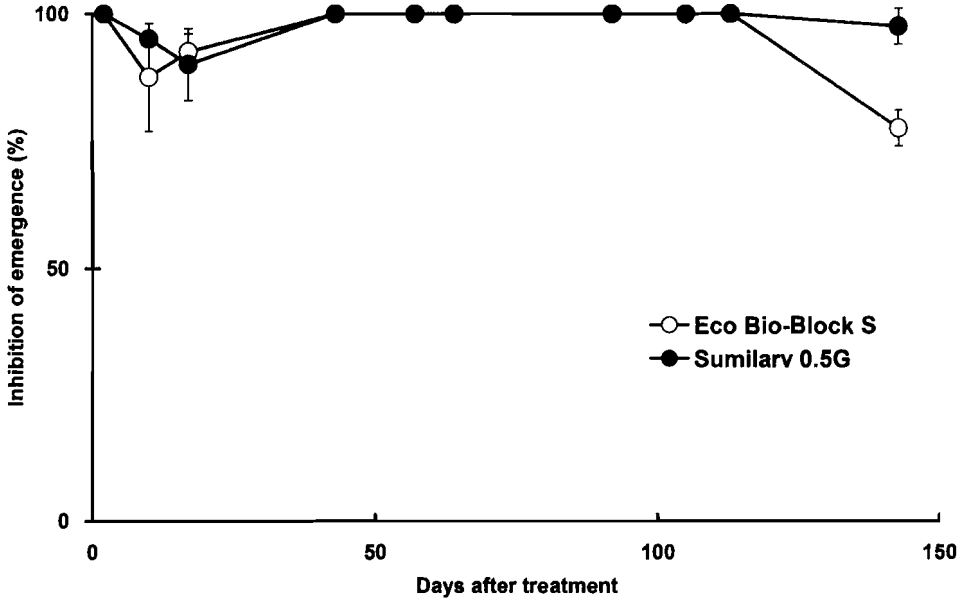

Fig. 2. Changes in inhibition of emergence against 4th instars of Ae. albopictus with daily exchanged stagnant water treated with EcoBio-Block S and Sumilarv 0.5 G at the rate of 10 ppm as active ingredient.

Table 1. Changes in inhibition of emergence of *Ae. albopictus* and water quality after treatment with EcoBio-Block® S at the rate of 1 g/l.

		Days after treatment					
	Sample	−9–0	2–7	8–17	84–94	95–101[3]	102–117
Eco Bio-Block S	No. of pupae collected	133.0 ± 5.3	24.0 ± 4.4	26.7 ± 3.2	23.3 ± 6.7	52.7 ± 17.1	19.7 ± 7.6
	No. of dead pupae	2.7 ± 1.5	17.7 ± 5.7	26.7 ± 3.2	22.0 ± 6.0	52.7 ± 17.1	19.3 ± 7.1
	% inhibition of emergence[1,2]	2.0 ± 1.1	72.9 ± 14.2**	100.0 ± 0.0**	94.9 ± 6.2**	100.0 ± 0.0**	98.8 ± 2.1**
	pH	8.1 ± 0.1	7.9 ± 0.1	7.8 ± 0.2	7.3 ± 0.4	7.3 ± —	7.7 ± 0.2
	NO_2-N	0.040 ± 0.010*	0.025 ± 0.007	0.068 ± 0.044	0.020 ± 0.000	0.020 ± —	0.020 ± 0.000
	NH_4-N	0.20 ± 0.00	0.25 ± 0.07	0.31 ± 0.02**	0.20 ± 0.00	0.20 ± —	0.20 ± 0.00
	COD	16.9 ± 2.3	14.8 ± 4.0	11.0 ± 0.0	8.3 ± 2.4	8.3 ± —	9.7 ± 0.5
	BOD	0.83 ± 0.76	1.42 ± 2.00	0.83 ± 1.44	1.17 ± 0.71	1.67 ± —	0.67 ± 0.94
Control	No. of pupae collected	140.0 ± 21.5	46.3 ± 8.6	47.3 ± 1.5	22.3 ± 10.2	76.3 ± 43.8	77.7 ± 7.5
	No. of dead pupae	4.7 ± 4.0	1.3 ± 2.3	2.0 ± 1.7	1.0 ± 1.7	2.0 ± 2.0	1.7 ± 2.1
	% inhibition of emergence	3.7 ± 3.5	2.5 ± 4.3	4.2 ± 3.6	2.9 ± 5.1	2.8 ± 3.5	2.0 ± 2.4
	pH	8.2 ± 0.2	7.8 ± 0.1	7.6 ± 0.1	6.8 ± 0.2	7.0 ± —	7.3 ± 0.1
	NO_2-N	0.020 ± 0.000	0.020 ± 0.000	0.023 ± 0.006	0.020 ± 0.000	0.020 ± —	0.020 ± 0.000
	NH_4-N	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± —	0.20 ± 0.00
	COD	14.2 ± 3.0	12.5 ± 0.7	14.0 ± 2.8	8.0 ± 1.9	10.0 ± —	10.0 ± 1.4
	BOD	1.06 ± 0.92	1.92 ± 2.71	0.67 ± 1.15	0.83 ± 1.18	1.00 ± —	0.50 ± 0.71

[1] Asterisks indicate significant difference from control (**$P < 0.01$; *$P < 0.05$.)
[2] ± indicates SD.
[3] — indicates that only one datum was available.

initial treatment dose was higher than that recommended for practical treatment, which is less than 0.1 ppm (Hirano et al. 1998). The pyriproxyfen concentration in the daily exchanged water containing EcoBio-Block S (2–7 ppb) seemed to be critical for 100% inhibition of emergence because the LC_{90}s or LC_{95}s for mosquito larvae were reported to be in the range of 0.05–0.65 ppb (Mulla et al. 1986, Schaefer et al. 1988). Under normal conditions, however, pyriproxyfen concentration will be higher than that under the present test conditions because of accumulation of daily released active ingredient.

Emergence inhibition by EcoBio-Block S in simulated field conditions

Changes in percentage of inhibition of emergence in *Ae. albopictus* larvae with and without EcoBio-Block S treatment at 1 g/liter (0.16 ppm active ingredient) are shown in Table 1. The high inhibition of emergence lasted for at least 117 days after treatment with EcoBio-Block S. The inhibition rate was relatively lower (72.9%) in the first 7 days after treatment, indicating that more than 7 days after treatment was required for achieving the required concentration for 100% control of mosquitoes under the present study conditions. The cumulative precipitation during the test period (June 3 to October 22, 2003) was 924 mm, which was equivalent to 177 liters for a bucket; hence, the water in a bucket was replaced 3 times during the test period. Slight increases were observed in NO_2-N and NH_4-N in the EcoBio-Block S-treated water during 17 days after treatment (Fig. 1), which seemed to indicate the mineralization activity of EcoBio-Block as Matsunaga et al. (2006) reported. However, there was no significant differences in BOD and COD between EcoBio-Block S-treated and untreated water. The BOD and COD values indicate that the water quality in both buckets was apparently good during the test period, and we could not distinctively evaluate the water-purifying activity of EcoBio-Block S under the present test conditions.

Controlled release systems that release minute but adequate amounts of active ingredients for an adequately long duration are required for JHMs from the viewpoint that they have to be active at the appropriate time when the larvae mature to the most susceptible stage, which is the late 4th instar in mosquitoes (Kawada et al. 1988, Mulla 1995). Furthermore, release of JHMs has to be minimized to reduce the impact on the ecosystem. Among several formulations tested, a granular formulation of pyriproxyfen (0.5 G) showed the most stable activity in the stagnant water pools under field conditions (Mulla et al. 1986, Kawada et al. 1988). The granular formulation and other types of CRSs are applicable to running water (Kerdpibule 1989), temporary water pools (Okazawa et al. 1991), and water jars (Itoh 1993). Okazawa et al. (1991) reported that treatment with 0.1 ppm of a granular formulation of pyriproxyfen continued to have activity against *Anopheles punctulatus* Dönitz larvae after dry conditions for 50 days; these larvae are found mainly in unshaded, temporary ground water accumulations in the mountainous regions in Solomon Islands. Pyriproxyfen, incorporated in a synthetic polymer slow release formulation, maintained activity for a long duration against *Aedes aegypti* (L.) larvae, although the water in the jar was partially used and replenished (Itoh 1993). Recently, Schwartz et al. (2003) reported a simple CRS technique by which pyriproxyfen was encapsulated in a spongy core material with a coating of a polyurethane or polyurea hydrogel. In this report, EcoBio-Block S showed low-level release pattern of pyriproxyfen in stagnant water followed by release according to zero order kinetics. This CRS technique might reduce the adverse impact of pyriproxyfen on the ecosystem and organisms such as crustaceans and other nontarget organisms in the aquatic environment (Miyamoto et al. 1993); simultaneously, it might reinforce the residual effectiveness of pyriproxyfen. Further studies on larvicidal effectiveness and environmental impact as well as water purification effect of EcoBio-Block S in various field conditions is required before the formulation is used in the field.

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