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Insecticidal Activity of Japanese Pine Sawyer (*Monochamus alternatus*) and Pine Sawyer (*Monochamus saltuarius*) Using Abamectin and Emamectin benzoate

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Abstract

Pine wilt disease (PWD) caused by pine wood nematode (PWN), *Bursaphelenchus xylophilus*, which is transmitted by *Monochamus alternatus* and *M. saltuarius*, is a serious threat to coniferous forests in the Northern Hemisphere, including Korea. The efficacy of abamectin and emamectin benzoate for preventing the PWD in the field and its effect on the vectors *Monochamus alternatus* and *M. saltuarius* (Coleoptera: Cerambycidae) were evaluated. An experimental plot was delimited, of which consists of Japanese red pine (*Pinus densiflora*) forest in South Korea, and trunk injection trials were made with abamectin and emamectin benzoate. Branches of each tree were collected, and are subsequently subjected to the analysis of residues for both nematicides. Results obtained in this study showed that abamectin and emamectin benzoate showed over 90% mortality at the recommended concentration after 6 days and 8 days, respectively. Consequently, it was found that both insecticides have a higher effect on the susceptibility and persistence of two vectors of PWD, *M. alternatus* and *M. saltuarius* feeding on branches of the trees, and its application by trunk injection is confirmed as an option for pine wilt disease management programs in Korea.

Key Words: *Bursaphelenchus*, pine wilt disease, pine wood nematode, *Pinus*

Introduction

Pine wilt disease (PWD) is one of the serious forest diseases posing a major threat on forest ecosystems worldwide, especially for pine forests, including China, Japan and Korea (Yi et al. 1989; Ichihara et al. 2000; Heo et al. 2019). Following the first detection from Portugal in 1999 (Soursa et al. 2001), the PWD has also been subsequently spreading across the Europe, including Spain (Mota et al. 1999; Vicente et al. 2011).

Since the first discovery of the PWD from Pusan, South Korea in 1988, it has been recorded that a total of approximately 0.5 million trees from 117 regions of the country were confirmed to be infected by the PWD (retrieved at February, 2020 from the Korea Forest Service; http://www.forest.go.kr). The causal agent of the PWD is *Bursaphelenchus xylophilus* (PWN) vectored by *Monochamus* spp. of which two vectors, *M. alternatus* and *M. saltuarius* are reported currently occurring in South Korea (NIFoS 2007; Han et al. 2016).

Intensive control efforts have been made for the PWD using silvicultural control, physical, and chemical treatment

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(Kwon et al. 2011). In cases where the chemical treatment is adopted for PWD control, logs of infected pine trees are fumigated with metam-sodium covered with vinyl sheets or trunk injection procedures are adopted using nematicides including abamectin (1.8% EC) and emamectin benzoate (2.15% EC) as a preventative control method against the PWN (Kwon et al. 2011), since its nematicidal efficacy (Sousa et al. 2013; Takai et al. 2000).

Given that effective control measures for the PWD vectors, *M. alternatus* and *M. saltuarius*, have still not been devised, we tested the insecticidal efficacy of the vectors using abamectin and emamectin benzoate as a single formulation, respectively.

Materials and Methods

Nematicides

Two nematicides, abamectin and emamectin benzoate were used in this study. Applications of the nematicides was made following procedures made by an Agrochemicals user's guide book use published by Korea Crop Protection Association and manual of Pine Wilt Disease control technique published by National Institute of Forest Science (Go et al. 2016).

Preparations of pine wood nematode and insect vectors

The pathogenic PWN and its fungal food source, Botrytis cinerea were obtained from the NIFoS. The cultured nematodes on 2% potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) were separated from the culture media by the Baermann funnel technique (Baermann 1917) and were collected by centrifugation (1,000 g, 1 min). The final nematode suspension (*c* 10,000 nematodes ml^{-1}) was prepared by appropriated dilution.

Pupal stage of *M. alternatus* and *M. saltuarius* were obtained from Osang Kinsect System, South Korea. Newly emerged adults were kept in iron-screened cages (30 cm long×30 cm wide×30 cm high) on 1-2-year old *Pinus densiflora* twigs at 25°C and under 12:12 h (light/dark) photoperiod.

Trunk injection and residual test

To test the efficacy of two nematicides, abamectin and emamectin benzoate, on the susceptibility and persistence of the two PWD vectors, trunk injections on 20 trees of *P. densiflora* (*c* 15-year-old) were performed between February and March, 2016 in Gumi, Gyeongsangbuk Province of South Korea (36°13'25.5"N 128°23'54.8"E). This was followed by the manual of Pine Wilt Disease control technique published by Go et al. (2016). Branches of the trees were then collected between May and June, 2016 and transported to the laboratory for further study.

Twenty trees in each treatment were selected and two branches from each tree were randomly cut for the quantitative analysis of abamectin and emamectin benzoate in the tissues. Given that the insect vectors feed mainly on young shoots, 1-2-year-old branches were selected for analyzing the residues of each nematicide used in this study.

Adult branch-feeding bioassay in vitro

The adult stage of *M. alternatus* and *M. saltuarius* were kept individually in petri dishes under controlled conditions of temperature (25°C and 70% r.h.). Each beetle was given twigs of the tree (~ 10 cm in length, 2-3 cm in diameter) to feed on, which was replaced weekly with those in similar sizes. Beetles were checked daily and mortalities of the vectors were checked after two weeks. This was replicated 10 times and repeated twice.

Repellent activity of two nematicides against the vectors

To test the repellent activity of two nematicides against the PWD vectors, the amount of weekly feeding by the vectors on the twigs treated with abamectin and emamectin benzoate and untreated as control was measured by recording the surface-feed areas (where the bark had been chewed) into a transparent paper sheet, and afterwards into a square graph paper with a 1 mm grid, allowing the measurement of the grids affected by the insect's feeding activity. Measurements of feeding areas continued until the death of the vectors. The preference defined in this study is as the number of which the vectors are sitting on the twigs. This was replicated three times and repeated twice.

Data analysis

Analysis of variance (ANOVA) with an α-level of 0.05 was used to compare parameters, followed by Duncan's

multiple range test using (SAS Institute 2009).

Results and Discussion

Insecticidal efficacy of two nematicides

High mortality rates were recorded from the twigs treated with abamectin and emamectin benzoate against two vectors of the PWD. Of those used in the study, a higher mortality was found from the twigs when treated with emamectin benzoate (Table 1, Fig. 1). In cases where the nematicides was treated, it took 6, 8 and 13 days for emamectin benzoate, abamectin and control, respectively, reaching 90% mortality at the recommended concentration which is the standard of registration of pesticides in South Korea defined by Rural Development Administration (2020). Two nematicides, abamectin and emamectin benzoate are among the chemical treatments of pine wilt disease to kill the PWN currently used in the field. Although the nematicides tested in this study are currently registered for use only to control the PWN in South Korea, the results obtained in this study showed that these can be another option for the chemical treatment that can be applied for control of the PWD vectors in the country.

Repellent activity of two nematicides against the vectors

Irrespective of treatments, the twigs treated with two nematicides was fed by two vectors of the PWN. There was no significant statistic difference in the feeding preference, number of feeding on twigs given to the vectors and feeding areas, compared to the control (Table 2). Given the fact there was no repellent activity observed between treatments, and two nematicides are also toxic to two insect vectors of the PWN, *M. alternatus* and *M. saltuarius* under labo-

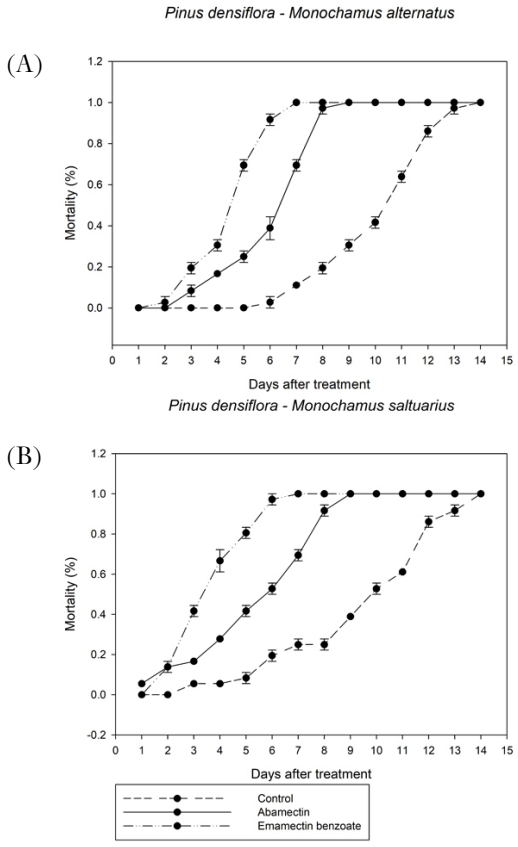

Fig. 1. Residual effect of two different insecticides against two PWD vectors. (A) Residual effect of two different insecticides against *Monochamus alternatus*. (B) Residual effect of two different insecticides against *M. saltuarius*. The values are presented as the mean±SE.

Table 1. Comparative survey on the efficacy of two different nematicides on the susceptibility and persistence of the two PWN vectors

Vector	Nematicide (ppm)	No. of insect vectors (male/female)	Male		Female	
			Weight (mg)	Longevity (days)	Weight (mg)	Longevity (days)
M. alternatus	Control	10/10	185.70±18.36^{a}	10.68±0.69 (7-13)^{b}	181.10±15.39^{d}	11.00±0.51 (8-14)^{e}
	AB 1 ppm	10/10	168.60±19.80^{a}	6.00±0.61 (3-9)^{c}	231.90±19.20^{d}	7.50±0.22 (6-8)^{f}
	EMB 1 ppm	10/10	209.00±22.67^{a}	4.80±0.20 (4-6)^{c}	207.10±35.25^{d}	4.90±0.54 (3-7)^{f}
M. saltuarius	Control	10/10	36.30±7.26^{a}	9.70±1.03 (3-13)^{b}	59.60±15.45^{d}	10.30±0.73 (6-14)^{e}
	AB 1 ppm	10/10	25.00±4.27^{a}	5.30±0.84 (1-8)^{c}	34.20±4.41^{d}	7.10±0.57 (4-9)^{f}
	EMB 1 ppm	10/10	51.80±13.66^{a}	4.40±0.56 (2-7)^{c}	49.50±7.47^{d}	4.30±0.47 (2-6)^{g}

The different letters annotated in the column of the table indicate that it is significantly different ($p < 0.05$).

Table 2. Comparisons on repellent activity of two different nematicides against two PWN vectors

Vector	Nematicide (ppm)	Preference	df	F	p	Number of feeding branches	df	F	p	Feeding area (mm^2)	df	F	p
M. alternatus	Control	2.00±0.58	2	2.333	0.178	8.67±3.17	2	0.846	0.474	141.23±41.08	2	1.202	0.364
	AB 1 ppm	5.00±3.61				2.33±1.86				87.81±68.68			
	EMB 1 ppm	5.33±2.19				7.33±1.86				119.71±38.60			
M. saltuarius	Control	2.00±1.53	2	0.098	0.908	2.00±2.00	2	0.302	0.75	29.32±29.32	2	0.135	0.876
	AB 1 ppm	3.00±2.52				0.33±0.33				3.43±3.43			
	EMB 1 ppm	1.00±0.58				1.00±1.00				11.85±11.85			

ratory conditions, this might result in disturbing insect's feeding appetence and longevity in natural conditions. The fact that the presence of abamectin and emamectin benzoate in the trunk and branches of the pine trees prevented pines from being killed by *B. xylophilus* is important given that the PWD vectors with lower longevity affects the transmission of *B. xylophilus* into the healthy host and the insect's reproductive potential (Sousa et al. 2013).

Taken all together, our results indicate that the application of the abamectin and emamectin benzoate as the preventative control method for the PWD vectors, aligned with Sousa et al. (2013), can greatly improve the conventional control for the PWD carried out in the country by diminishing the population of the PWD vectors in the field.

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