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Dermal and Eye Irritation Studies on Bactonematicide, *Photorhabdus temperata* Isolated from *Heterorhabditis megidis* (Nematoda: Heterorhabditidae) in Rabbit*

Park, Sun-Han*** · Chung, Nam-Jun**** · Choo, Young-Moo***** · Kim, Young-Joon****** · Kim, Jin-Ho**

Heterorhabditis megidis (Nematoda: Heterorhabditidae)에서 분리한 Bactonematicide, *Photorhabdus temperata*의 토끼 피부 및 눈자극 시험

박순한 · 정남준 · 추영무 · 김영준 · 김진호

The acute dermal and eye irritation tests of *Photorhabdus temperata*, a symbiotic bacterium of *Heterorhabditis megidis* Gwangju strain, were carried out in New Zealand white rabbit (*Oryctolagus cuniculus*), following the guidelines of OECD and Rural Development Administration (RDA) of Korea. In both tests, neither dermal nor eye responses were found from all the *P. temperata* treated rabbits and the results were classified as non-irritating. That is, erythema, eschar, edema, and

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** Corresponding author, Emeritus Professor, School of Ecology and Environmental System, College of Ecology and Environmental Sciences, Kyungpook National University, Gyeongbuk 37224, Republic of Korea (kimjinho@knu.ac.kr)

*** Ph.D. Candidate, School of Ecology and Environmental System, College of Ecology and Environmental Sciences, Kyungpook National University, Gyeongbuk 37224, Republic of Korea

**** R&D director, Nambo Co., Ltd., Gyeongnam 52840, Republic of Korea

***** R&D manager, Jinju Bioindustry Foundation, Gyeongnam 52839, Republic of Korea

****** Ph.D. Student, Department of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

any other dermal critical signs were not observed from all the experimental rabbits in the dermal irritation test. In the acute eye irritation test, no clinical signs of cornea, iritis, conjunctiva (redness, edema, lacrima, and chemosis) were observed from all the experimental rabbits. Individual ocular irritation, mean ocular irritation, and acute ocular irritation were calculated as 0.0. The results of dermal and eye irritation studies on *P. temperata* indicated that this bacterium could be a safe and effective alternative bionematicide against the most serious plant-parasitic root-knot nematodes in the genus *Meloidogyne*.

Key words : *dermal irritation, eye irritation, Photorhabdus temperata, entomopathogenic nematodes, Heterorhabditis megidis*

Ⅰ. Introduction

Entomopathogenic nematodes (EPNs) *Heterorhabditis* and *Steinernema* in the families Heterorhabditidae and Steinernematidae, respectively, are globally distributed and ubiquitous soil-dwelling obligate biological control agents of many economically important insect pests, and thus being utilized in classical, conservation, and augmentative biological control programs of above and below-ground insect pests (7, 10, 21, 31, 35, 41). They possess several attributes such as high virulence to broad insect hosts, fast insecticidal activity of their hosts, safety to vertebrates, plants, and other non-target organisms without negative effects on the environment, high reproductive potential, and long-span persistence in the field and raised bed soil (21, 22, 29, 35, 38). EPN's lethality is closely linked to the metabolic and toxin-producing abilities of their symbiotic bacterial partners, *Photorhabdus* and *Xenorhabdus* for *Heterorhabditis* and *Steinernema*, respectively, causing septicemia and insect death (44). The bacterial cells, namely, are carried by the infective third-stage juveniles of EPNs in their intestinal tract propagate and kill the insect host by septicemia within 48 hours (21, 35). Moreover, symbiotic bacteria have nematicidal activity as well (32, 61, 65). Thus, *Xenorhabudus* and *Photorhadus* can be used independently for the control of plant-parasitic nematodes by their nematicidal activity, especially against root-knot nematodes *Meloidogyne* spp. (1, 32, 33, 61, 65, Park, unpublished data).

For example, *X. nematophila*, *X. bovieni, X. budapestensis*, *X. szentirmaii*, and *P. luminescens* isolated from *S. carpocapsae*, *S. feltiae*, *S. bicornutum*, *S. rarum*, and *H. bacteriophora*, respectively, reduced egg hatching of *M. javanica* and *M. incognita* (46, 59, 60). Twenty-six species of *Xenorhabdus* and 19 species of *Photorhabdus* have been identified up to the present (57). *P. temperata* is a symbiotic bacterium of *H. megidis*, *H. bacteriophora*, *H. zealandica*, and *H. downesi* (8, 13, 34, 57). *P. temperata* which was also isolated from Korean indigenous *H.*

megidis Gwangju strain and *P. luminescens* isolated from *H. bacteriophora* Hamyang strain showed high nematicidal activity against M. incognita and root-lesion nematode *Pratylenchus penetrans* (Park, unpublished data).

Root-knot nematodes in the genus *Meloidogyne* are the most serious plant-parasitic nematodes and an economically important polyphagous group of highly adapted obligate plant parasites parasitizing nearly every species of higher plants (45). More than 90 *Meloidogyne* species have been described from over 3,000 plant species designated already as hosts to root-knot nematodes in 2003 (26, 53, 62). *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* have been frequently separated from many a kind of economic crops in Korea (15, 16, 17). These ubiquitous nematodes severely damage economic crops, either alone or in combination with plant pathogens causing complex diseases (19, 20). A variety of means have been adopted for the control of root-knot nematodes. Among them, chemical nematicides are the main tools for the control of these serious nematodes. However, the indiscriminate and repeated application of pesticides leads to loss of biodiversity, pest resistance, and other ecological imbalance (40). Adverse effects on the environment including human health from excessive and inappropriate use of agrochemicals have necessitated alternatives (27, 39). Biological control agents are promising alternatives including symbiotic bacteria of EPNs (2). Nematicidal activity of EPN-associated bacteria has received interest in their use for the control of root-knot nematodes. *Xenorhabdus* and *Photorhabdus* produce a variety of antibacterial and antifungal compounds, some of which are also active against plant nematodes (23, 28, 32, 33, 65). Moreover, these bacteria are no hazard to vertebrates and environment (9). For example, *X. nematophila*, *X. bovienii*, and *P. luminescens* associated with *S. carpocapsae*, *S. bibionis*, and *H. bacteriophora*, respectively, produced no disease symptoms, signs of infectivity, pathogenicity, toxicity, and/or harmful effects on experimental animals, such as leghorn chicks, adult albino mice (52), guinea pigs, and rats (47). Many benefits of EPNs and their symbiotic bacteria have brought tremendous research and commercial interest in many parts of the world (24, 29, 37, Kim unpublished data). The nematicidal activity and safety of *P. temperata* and *P. luminescens* have aroused interest in their use as bionematicide development for the control of root-knot nematodes in Korea (Park, unpublished data).

Despite these bacteria are safe to environment and vertebrates (8, 47, 52), all the organic agricultural materials have to be registered by submitting safety data obtained from acute toxicity tests to the Rural Development Administration (RDA). EPNs have been exempted from registration in many countries like the United States and most European countries (4, 25, 29, 63). However, the registration of symbiotic bacteria of EPNs as organic agricultural materials is rarely reported. Besides toxicity evaluation, the evaluation of eye and skin irritation potential is

essential to ensure the safety of individuals in contact with a wide variety of substances designed for industrial use of diverse types of chemicals, including pharmaceuticals, cosmetics, household products, industrial chemicals, and agrochemicals (64). Exposure to these chemicals can be incidental, accidental, or intentional. One of the possible effects of the exposition and accidental contact with new chemicals is skin and eye irritation because the skin and eye are significant portals of entry of hazardous agents and vulnerable target organ system (12, 64).

Accordingly, acute dermal and irritation tests have to be performed on symbiotic bacteria of EPNs under the guidelines of the RDA to register them as bionematocides against plant-parasitic nematodes, especially against root-knot nematodes.

Therefore, acute dermal and eye irritation tests of *P. temperata*, a symbiotic bacteria of *H. megidis* Gwangju strain in rabbits were carried out, following the guidelines of RDA (55, 56) and Organization for Economic Cooperation and Development OECD (48, 49).

Ⅱ. Materials and Methods

1. *P. temperata* and Symbiotic Nematode

P. temperata was isolated from *H. megidis* Gwanju strain which was separated from the sawtooth oak (*Quercus acutissima*) forest of Mt. Mudeungsan in Gwangju, Gwangju Metropolitan City using the *Galleria mellonella* (L.) trapping method (6, 18, 34). The infective juveniles that emerged from *G. mellonella* cadavers were harvested in White traps and stored at 10℃ for no more than 3 weeks before they were used (36). *G. mellonella* larvae were exposed to 20 infective juveniles of H. megidis per larva to isolate and culture *P. temperata*. Two days later, the hemolymph was collected from surface-sterilized *G. mellonella* cadavers with a sterilized 26G × 1/2” syringe (Jung Rim Medical Industrial Co. Ltd., Korea). A drop of collected hemolymph was streaked on NBTA and cultured at 28℃ for 48 h (3, 66). The *Photorhabdus* colonies were selected 48 h later and cultured on the same medium for 48 h. This culture was transferred into a 250 ml Erlenmeyer flask containing 20 ml tryptic soy broth (TSB) and cultured at 25℃ for 48 h (33, 44).

2. *P. temperata* Formulation Used in the Tests

P. temperata cultured in TSB at 25℃ for 48 h was transferred into 500 ml Erlenmeyer flask

containing 300 ml modified nutrient broth (MNB) (g/liter: egg yolk 0.625, cholesterol 0.025, lecithin 0.125, sweet whey powder 2.5, NaCl 2.5, KH_2PO_4 0.625, yeast 1, peptone 3.75) and cultured at 25℃ initially. Mass production was made in fermenter by transferring culture gradually into a larger fermenter as follows; 300 ml of culture was transferred into 30 L optimized MNB in a 50-liter fermenter and cultured for 48 h. Again, 30 L of culture was transferred into a 3,000 L culture medium in a 5,000-liter fermenter and cultured under antibiotic and optimal culture conditions for 48 h. Then, 3,000 L of culture received 1.5% Na-alginate and 1.0% starch as microcapsule substance and stabilizer, respectively. Finally, 3,000 L of microcapsule formulation culture was diluted with the same amount of 3,000 L of water containing efficacy enhancer and penetrant at the ratio of 50:50 to quantify. This formulation was used in the dermal and eye irritation studies in the rabbit.

3. Acute Dermal and Eye Irritation Tests of *P. temperata*

For the acute dermal and eye irritation tests of *P. temperata*, New Zealand white rabbit (*Oryctolagus cuniculus*) which is widely used in the bioassay of agrochemical toxicity test was used. The rabbits were obtained from the Hanrim Laboratory Animal Research Institute (Hwaseong, Gyeonggi province). The tests were performed at the Korea Bio-safety Institute (KBSI) (Eumseong, Chungbuk province).

Acute dermal irritation test was carried out using 6-day acclimated healthy rabbits in compliance with the guidelines of RDA (54, 55). The weight of rabbits was 1.9 - 2.0 kg at the time of receipt and 2.4 - 2.5 kg at the time of treatment. The rabbits were acclimated in a stainless steel cage (50 × 38 × 40 cm) for 6 days. The acclimation and test were administrated under the environmentally controlled conditions of 23 ± 2℃, 50 ± 10% relative humidity, air conditioning, 12-hour light (from 7:00 am to 7:00 pm), and dark cycle, and illuminance of 200 - 300 lux (54). One rabbit was kept in the cage during the test period and a certified rabbit diet (Cargill Agri Purina Korea Inc.) and filtered groundwater were provided. Three healthy rabbits were included in each test unit. One day before treatment, each of a group of three rabbits was clipped 15 × 15 cm in size with an electric clipper (Joas Co., Namyangju, Gyeonggi, Korea). Then, a 6 cm^2 (2 × 3 cm) cotton gauze patch containing a quantity of 0.5 ml of *P. temperata* product was directly administered to the skin onto the clipped part (54). The patch was secured in position by wrapping with non-polar tape (Tegader™, 3M) and Coban™ self-adherent wrap (3M Health Care Ltd., USA). The control group received distilled water, instead of the culture medium with efficacy enhancer because it's known to be non-toxic [the registration standard for pesticide and

raw material: [asterisk 12] standard and method for human and animal toxicity test (Rural Development Administration notification No.2021-33)]. Four hours after administration, the patch was removed from the rabbit, and the treated part was cleaned with distilled water and dried up with medical cotton wool in turn. Then, general toxic symptoms and death cases were investigated for 72 hours. Bodyweight of rabbits was measured before administration and at 48 and 72 h after administration. Erythema, eschar, and edema were examined from the administered spot at 1, 24, 48, and 72 h after exposure, and dermal responses were scored according to the standardized visual assessment scale (48, 54, 56) The result was decided by primary dermal irritation index (PDII) (43, 48, 49, 54, 56).

4. Acute Eye Irritation Test of *P. temperata*

The acute eye irritation test of *P. temperata* was also performed in New Zealand White rabbits under the guidelines of RDA (54, 55). The rabbits were obtained, maintained, and fed as described above. The weight of rabbits was 1.9 - 2.0 kg at the time of receipt and 2.1 - 2.5 kg at the time of treatment. The rabbits were acclimated for 5 days. The acclimation and test were administrated under the environmentally controlled conditions of 23 ± 2℃, 50 ± 10% relative humidity, air conditioning, 12-hour light (from 7:00 am to 7:00 pm), and dark cycle, and illuminance of 200 - 300 lux (54). Food and water were provided as described above.

Each test unit included three healthy rabbits. Both (left and right) eyes of each rabbit were checked 24 h before administration and rabbits having healthy eyes were used. A 0.1 ml volume of *P. temperata* product was instilled to the bottom of the left conjunctival sac, while the right eye was remained untreated for control (54). The eyelids were kept together over the next few seconds. Both eyes of each rabbit were examined at the time of treatment and 48 and 72 h after treatment.

General toxic symptoms and death cases were examined for 72 hours. The weight of survived rabbits was measured at the time of treatment and 48 and 72 h after administration. Cornea opacity (degree of opacity and diffuse-areas of opacity), iris abnormity (iritis), conjunctival redness, edema, and lacrima were recorded at 1, 24, 48, and 72 h after treatment (54). The eye responses were evaluated under the ‘Grading of ocular lesions (48, 50, 54). Individual ocular irritation index (IOI) and mean ocular irritation index (MOI) were calculated by grading of ocular lesions. The maximum value of the mean ocular irritation index was used as the acute ocular irritation index. The degree of irritation was classified using the classification criteria of ocular lesions (43, 54).

Ⅲ. Results

1. Acute Dermal Irritation of *P. temperata* in Rabbit

Neither mortality nor dermal responses (erythema, oedema, maculated crusts, and desquamation) in rabbits were observed during the acute dermal irritation test of *P. temperata*. Erythema, eschar, edema, and any other critical signs were not found from all the tested rabbits (Table 1). The PDII (primary dermal irritation index) was calculated to be 0.0 in both the control and the treated groups (Table 2). Weight increment of rabbits was normal. The weight of rabbits was

Table 1. Evaluation of skin irritation in acute dermal irritation test of *Photorhabdus temperata* (1/2)

Skin reaction score	Animal number	Observation time (days)[a]							
		0		1		2		3	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
Erythema and eschar	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Edema	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0

[a] Time after topical treatment

Table 2. Evaluation of skin irritation in acute dermal irritation test of *Photorhabdus temperata* (2/2)

Sites		Control site								Treated site							
Change		Erythema and eschar				Edema				Erythema and eschar				Edema			
Phases (hours)[a]		1	24	48	72	1	24	48	72	1	24	48	72	1	24	48	72
Animal number	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sum[b]		0				0				0				0			
P.I.I.[c]		0				0				0				0			

Dermal responses were scored according to the standardized visual assessment scale.
Classification of primary dermal irritation index (PDII) followed the criteria of RDA and OECD.
[a] Time after topical treatment
[b] Sum of means at 24 and 72 hours
[c] P.I.I., Primary Irritation Index = mean score at 24 hours + mean score at 72 hours/2

Table 3. Body weight (g) changes of rabbits in acute dermal irritation test of *Photorhabdus temperata*

Animal number	Days after treatment			
	0	2	3	Gain
1	2434.5	2479.0	2508.0	73.5
2	2479.0	2499.0	2520.5	41.5
3	2409.0	2530.5	2560.5	151.5
Mean ± SD[a]	440.8 ± 35.4	2502.8 ± 26.0	2529.7 ± 27.4	88.8 ± 46.2

[a] Standard Deviation

2440.8 ± 35.4 g on the 1st day, 2502.8 ± 26.0 g on the 2nd day, and 2529.7 ± 27.4 g on the 3rd day (Table 3). *P. temperata* was concluded and classified as a non-irritant substance.

2. Acute Eye Irritation of *P. temperata* in Rabbit

Neither mortality nor clinical signs were observed from all the experimental rabbits during the acute eye irritation test. No clinical signs of cornea (degree of opacity and diffuse areas of opacity), iritis, and conjunctiva (redness, edema, lacrima, and chemosis) were observed from all the experimental rabbits in both the control group, right eye, and the treated group, left eye (Table 4 and 5). Individual ocular irritation (IOI), mean ocular irritation (MOI), and acute ocular

Table 4. Non-eyes washed evaluation of eye irritation in acute eye irritation test (Non-treatment)

Times (hours)	Animal number	Cornea		Iris (C)	Conjunctiva			I.O.I.[a]	M.O.I.[b]	A.O.I.[c]
		Degree of opacity (A)	Diffuse areas of opacity (B)		Redness (D)	Edema (E)	Lacrima (F)			
1	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
24	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
48	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
72	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		

[a] I.O.I. (Individual Ocular Irritation) = (A × B × 5) + (C × 5) + (D + E + F) × 2

[b] M.O.I. (Mean Ocular Irritation)

[c] A.O.I. (Acute Ocular Irritation) = the maximum value of M.O.I.

Table 5. Non-eyes washed evaluation of eye irritation in acute eye irritation test (treatment)

Times (hours)	Animal number	Cornea		Iris (C)	Conjunctiva			I.O.I.[a]	M.O.I.[b]	A.O.I.[c]
		Degree of opacity (A)	Diffuse areas of opacity (B)		Redness (D)	Edema (E)	Lacrima (F)			
1	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
24	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
48	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
72	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		

[a] I.O.I. (Individual Ocular Irritation) = (A × B × 5) + (C × 5) + (D + E + F) × 2

[b] M.O.I. (Mean Ocular Irritation)

[c] A.O.I. (Acute Ocular Irritation) = the maximum value of M.O.I.

Table 6. Ocular irritation scores of rabbit

Times (hours)	Animal number	I.O.I.[a]		M.O.I.[b]		A.O.I.[c]	
		Non-treatment	Treatment	Non-treatment	Treatment	Non-treatment	Treatment
1	1	0	0	0	0	0	0
	2	0	0				
	3	0	0				
24	1	0	0	0	0		
	2	0	0				
	3	0	0				
48	1	0	0	0	0		
	2	0	0				
	3	0	0				
72	1	0	0	0	0		
	2	0	0				
	3	0	0				

a I.O.I. (Individual Ocular Irritation)

b M.O.I. (Mean Ocular Irritation)

c A.O.I. (Acute Ocular Irritation = the maximum value of M.O.I.)

Table 7. Body weight changes of rabbits in acute eye irritation test

Group	Animal number	Days after application (g)			
		0	2	3	Gain
No eye washed	1	2455.0	2484.0	2518.5	63.5
	2	2439.0	2467.5	2498.5	59.5
	3	2104.0	2206.0	2241.0	137.0
	Mean ± SD[a]	2332.7 ± 198.2	2385.8 ± 156.0	2419.3 ± 154.8	86.7 ± 35.6

[a] Standard Deviation

irritation (AOI = the maximum value of MOI) were calculated as 0, 0.0, and 0.0, respectively, in both the control group and the treated group (Table 6). *P. temperata* produced 0.0 of a maximum group mean score (AOI). The weight of rabbits was increased normally. The weight of rabbits was 2332.7 ± 198.2 g on the 1st day, 2385.8 ± 156.0 g on the 2nd day, and 2419.3 ± 154.8 g on the 3rd day (Table 7). *P. temperata* was classified as non-irritation according to the classification criteria of ocular lesions (43, 48, 50).

Ⅳ. Discussion

In the acute dermal and eye irritation tests of *P. temperata* using rabbits following the guidelines of RDA and OECD, this bacterium was concluded as a safe organic agricultural material. Neither mortality nor adverse responses, that is, were observed in all the experimental rabbits. Both dermal clinical signs including erythema, eschar, edema, and eye irritating signs including cornea (degree of opacity and diffuse areas of opacity), iritis, and conjunctiva (redness, edema, lacrima, and chemosis) were not observed from all the rabbits in the acute dermal and eye irritation test, respectively. The IOI, MOI, and AOI (the maximum value of MOI) were calculated as 0.0 in both non-treatment and treatment groups.

The skin and eye are significant portals of entry of hazardous agents and vulnerable target organ systems (12) because one of the possible effects of the exposure and accidental contact with new chemicals is eye and skin irritation (64).

The toxicity criteria of skin and eye irritation were classified into four groups based on the skin primary irritation index (SPII) and eye irritation index (EII); non (≤1.0 in both SPII and EII), mild (1.1 - 2.0 in SPII, and 10.0 - 30.0 in EII), moderate (2.0 - 5.0 in SPII and 30.1 - 60.0 in EII), and strong (≥5.1 - 8.0 in SPII and ≥60.1 - 110.0 in EII) (43, 56).

Accordingly, *P. temperata* was classified as a non-irritating substance and can be developed as a new potential bionematicide of root-knot nematodes. *Photorhabdus* and *Xenorhabdus* possess antimycotic and antimicrobial properties such as nematicidal, insecticidal, fungicidal, antibacterial, and antiviral activities (8, 11, 30, 61, 65).

Indole and stilbene derivatives of *Photorhabdus* are well-known compounds contributing to the nematicidal activity (32, 33, 61, 65) However, the secondary metabolites and derivatives of *Photorhabdus* and *Xenorhabdus* related to the nematicidal effects are not fully clarified. Thus, further investigations of metabolites and derivatives of *Photorhabdus* and *Xenorhabdus* have to be continued to detect more detailed nematicidal properties for the development of innovative bionematicides. *P. temperata* isolated from *H. megidis* Gwangju strain was effective against *M. incognita* and *P. penetrans*. The effectiveness of *P. temperata* has prompted the development of a commercial product from this potential bacterium (Park, unpublished data). However, any organic materials or substances can ont be produced as commercial agro-pesticides without their toxicity assessment (56). Evaluation of skin and eye irritation potential is essential to ensuring the safety of individuals in contact with a wide variety of substances designed for industrial use of diverse types of chemicals, including pharmaceuticals, cosmetics, household products, industrial chemicals, and agrochemicals (64). As no dermal and eye irritation data on *P. temperata* have been made to-date, dermal and eye irritation tests of some organic agricultural materials have been performed. The number of approved items of organic agricultural materials in use was 1,590 in the year 2018, among which 15.6%, 11.6% and 4.5% are for pest control, disease control, and disease and pest control, respectively (43).

In the acute dermal and eye irritation tests of ca. 200 materials related to the management of insect pests and plant diseases, 164 and 152 items, respectively were classified as a category ‘none’, i.e., non-toxic materials ($\leq$1.0 for skin primary irritation index and $\leq$10.0 for eye irritation index) with mild 5, moderate 6, and strong 10 in the dermal irritation and mild 19, moderate 2, and strong 11 in the eye irritation (43, 51). No toxic symptoms were also observed from skin and eyes of experimental rabbits at the treatments of 0.5 g and 0.1 g in the acute dermal and eye irritation tests, respectively. Namely, mortality, erythema, incrustation, and edema on skin and cornea, iris, and conjunctiva in eyes were not observed from all the experimental rabbits except one rabbit showing erythema after 1 hour of administration (51). *Burkholderia pyrrocinia* CAB08106-4 is a microbial control agent of garlic black rot mycosis caused by *Sclereotium cepivorum* and *Sclereotium* sp. Local irritation and skin sensitization were evaluated using New Zealand white rabbits for 72 hours and 7 days, respectively. General clinical signs, skin irritation, and eye irritation, and normal increase in body weight were not observed during

the entire test period of the skin and eye irritation tests (42). *Bacillus thuringiensis* (Bt) is a well-known biological pesticide. Bt has been widely used as a natural insecticide and sometimes as a bionematicide. In the dermal and eye irritation experiments of Bt var. *israelensis* (Bti) SH-14 using rabbits according to the United States Environmental Protection Agency guidelines 870.2500, Bti SH-14 showed low eye irritation but not showed dermal irritation (5). Like above, dermal and eye irritation reactions are variable depending on organic materials currently in use.

Evaluation of dermal and eye irritation is an essential process for the development of agro-pesticides. Consequently, the results obtained from the present acute dermal and eye irritation tests of *P. temperata* in rabbits strongly demonstrated that *P. temperata* could be a safe and potential biological alternative control agent of root-knot nematodes.

Therefore, further researches are needed to develop the production and utilization technologies of EPN-associated bacterial nematicides for the control of plant-parasitic nematodes including root-knot nematodes, *Meloidogyne* spp.

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