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Diversity of Heterocystous Filamentous Cyanobacteria (Blue-Green Algae) from Rice Paddy Fields and Their Differential Susceptibility to Ten Fungicides Used in Korea

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Abstract Cyanobacteria are present abundantly in rice fields and are important in helping to maintain rice fields fertility through nitrogen fixation. Many rice fields soil contain a high density of cyanobactera, and over 50% of cyanobacterial genera that are in existence in rice paddy fields are heterocystous filamentous forms. A total of 142 isolates of heterocystous filamentous cyanobacteria were screened from 100 soil samples taken from rice paddy fields in 10 different locations across Korea, classified according to their morphological characteristics under light microscopy, and their susceptibly to fungicides examined. The collected blue-green alga were classified into a total of 14 genera, including seven genera of filamentous cyanobacteria and seven genera of nonfilamentous cyanobacteria. In particular, 142 heterocystous filamentous cyanobacteria were isolated and classified into six genera, including *Anabaena*, *Nostoc*, *Calothrix*, *Cylindrospermum*, *Nodularia*, *Scytomena*, and *Tolypotrix*. Yet, over 90% of the heterocystous filamentous cyanobacteria isolated from the rice paddy fields belonged to two genera: *Anabaena* and *Nostoc*. The response of 129 N_2-fixing cyanobacterial isolates, 53 *Anabaena* and 76 *Nostoc*, to 10 fungicides was then investigated. The results showed that the *Nostoc* spp. were more tolerant of the ten tested fungicides than the *Anabaena* spp., and among the ten tested fungicides, benomyl showed the highest acute toxicity to *Anabaena* spp. and *Nostoc* spp. In conclusion, although benomyl is a very useful agent to control phytopathogenic fungi, the application of this fungicide to rice fields should be considered because of its toxicity to the heterocystous filamentous cyanobacteria.

Key words: Rice field, fungicides, toxicity, inhibition test, EC_{50}, *Anabaena*, *Nostoc*

Cyanobacteria or blue-green algae belong to a group of ubiquitous photosynthetic prokaryotes possessing the ability to synthesize chlorophyll *a* and carry out an important role in nutrient recycling and the maintenance of organic matter in aquatic systems including lakes, rivers, and wetland. Furthermore, the nitrogen-fixing potentials of these organisms have also been crucial to ecosystems since 1969 [36]. N-fixing cyanobacteria are known to be a prominent component of the microbial population in wetland soils, especially rice fields, contributing significantly to the fertility as a natural biofertilizer [22]. Certain cyanobacterial strains that thrive in rice fields release small quantities of ammonia as the major fertilizing product, and small nitrogenous polypeptides during active growth, whereas most of the fixed products are made available mainly through autolysis and decomposition [14]. Yet, the utilization of cyanobacteria as a biofertilizer in rice field requires that the strains show tolerance to a multiplicity of agrochemicals that are normally used. Recently, the application of fertilizers and pesticide has substantially increased crop production in Korea [15, 19–21, 23, 24, 30].

Pesticides such as fungicides appear to be very effective in controlling a wide range of fungi that infect crop plants including rice, peppers, etc. However, there is little information available on the effect of these pesticides on algal communities [1, 8, 9] and the susceptibility of cyanobacteria to toxicants such as herbicides, fungicides, and heavy metals [3, 4, 10, 12, 22, 24]. Several reports [1, 10, 12, 27, 28] have already been published on the comparative toxicity of herbicides and fungicides toward various test organisms such as blue-green alga [1, 10], green alga [12, 27, 28], rotifer [12], and cladoceran [12]. This study investigated the diversity and strain prevalence of cyanobacteria in rice paddy fields and examined the effects of ten commonly used fungicides in Korea on the cyanobacteria *Anabaena* and *Nostoc*.

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Table 1. The tested fungicides and their chemical structures.

Fungicide	Activity	Solvent	Chemical structure	CAS Registry No.
Benomyl	Benzimidazole fungicide	Distilled water	Methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate	17804-35-2
Bitertanol	Triazole fungicide	Distilled water	1-(Biphenyl-4-yloxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl) 2-butanol (20:80 ratio of (1*RS*,2*RS*)- and (1*RS*,2*SR*)-isomers)	55179-31-2
Carbendazim	Benzimidazole fungicide	Distilled water	Methyl benzimidazol-2-yl carbamate	10605-21-7
Chlorothalonil	Aromatic fungicide	Distilled water	Tetrachloroisophthalonitrile	1897-45-6
Iprodione	Imidazole fungicide	Acetone (<0.05%)	3-(3,5-Dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	36734-19-7
Metalaxyl	Anilide fungicides	Distilled water	Methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate	57837-18-1
Prochloraz	Amide fungicide Conazole fungicide	Distilled water	*N*-Propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide	67747-09-5
Procymidone	Dichlorophenyl dicarboximide fungicide	Distilled water	*N*-(3,5-Dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide	32809-16-8
Thiophanate-methyl	Carbamate fungicide	Distilled water	Dimethyl 4,4-(*o*-phenylene)bis(3-thioallophanate)	23564-05-8
Zineb	Polymetic dithiocarbamate fungicide	Distilled water	Zinc ethylenebis(dithiocarbamate) (polymeric)	12122-67-7

MATERIALS AND METHODS

Chemicals

All of the fungicides tested were purchased from Dongbu-Hannong Co., Ltd. and Kyung Nong Co., Ltd. in South Korea and their chemical structures are given in Table 1. The fungicides tested were dissolved in a small amount of acetone and the concentration of the solvent in the medium kept to a minimum (i.e., less than 0.05%). The U.S. Environmental Protection Agency recommends a maximum allowable limit of 0.05% solvent for acute tests and 0.01% for chronic test [16]; 0.05% acetone has no significant toxic effects on microalgae [28].

Sampling and Morphological Study on Cyanobacteria

As shown in Fig. 1, 200 samples were collected at ten different sampling sites from June to July in 2002. Ten soil cores were taken from the top 0.5 cm at each sampling site, using a soil sampler (Model EP, LaMotte Company, Maryland, U.S.A.) to determine the cyanobacteria. The soil samples were mixed and kept in the dark at 4°C until the experiments were carried out. To isolate the cyanobacteria, 5 g of each soil sample was mixed with 45 ml of a sterilized BG-11 medium including 1.5 g of $NaNO_3$, 0.04 g of $K_2HPO_4{\cdot}3H_2O$, 0.075 g of $MgSO_4{\cdot}7H_2O$, 0.036 g of $CaCl_2{\cdot}2H_2O$, 0.006 g of citric acid, 0.006 g of ferric ammonium citrate, 0.001 g of EDTA (disodium salt), 0.02 g of $NaCO_3$, and 1 ml of trace metal mix A5, 1,000 ml of distilled water litter without nitrogen source [2]. Trace metal mix A5 is composed of H_3BO_3, 2.86 g; $MnCl_2{\cdot}4H_2O$, 1.81 g; $ZnSO_4{\cdot}7H_2O$, 0.222 g; $NaMoO_4{\cdot}2H_2O$, 0.39 g; $CuSO_4{\cdot}5H_2O$, 0.079 g; $Co(NO_3)_2{\cdot}6H_2O$,49.4 mg; and distilled water 1,000 ml. The pH should be 7.0±0.2 after autoclaving and cooling. The soil suspension dilutions were plated onto nitrogen-free BG-11 agar medium. The filamentous heterocystous strains were classified into broad taxa based on the morphological features observed directly on the materials growing in the petridishes, according to Roger [33]. A preliminary morphological study was carried out with a BX-40 Olympus microscope fitted with achromatic lenses ranging from 20 to 40 enlargements, and a 100× plan-apochromatic fluorite lens. The characterization was performed following *Bergey's Manual of Systematic Bacteriology* (2^{nd} Ed.) taxonomy [6] as well as the classification made by Rippka *et al.* [32].

Fig. 1. Sampling sites used for distribution survey of cyanobacteria in rice fields.

Cyanobacterial Cultures

The 142 strains of filamentous heterocystous cyanobacteria, including *Anabaena* spp. and *Nostoc* spp. were isolated and purified by diluting and plating the soil samples from the rice fields. The cyanobacteria were grown in batch cultures at 25–28°C under a constant light intensity of 35–120 $\mu E/m^2 \cdot s$ in a 250-ml Erlenmeyer flask containing a nitrogen-free BG-11 liquid medium [2] at pH 7.0±0.2 with 2.5 mM HEPES, in an orbital shaker at 100 rpm. The growth was determined by measuring the culture density at 680 nm using a spectrophotometer (model HP8453, Hewlett Packard, MI, U.S.A.).

Growth-Inhibiting Test

To obtain enough cells for inhibition tests, 15-ml aliquots of the nitrogen-free BG-11 liquid medium containing the cyanobacteria cells were transferred to sterile 50 ml Erlenmeyer flasks as seed cultures. A wide concentration range of fungicides was investigated to identify the toxicity range for each microalgal species using the method reported previously by Moreno-Garrido *et al.* [29]. For the growth inhibition test, the cyanobacterial cells were cultivated for 7 days to obtain at least a 16 doubling in the cell numbers. The inoculations were made using cyanobacteria precultured for 2 days before the experiments under the same test conditions. The cell concentrations in the precultures were determined using a Coulter Counter (model Ta_{II} multichannel particle counter, Coulter Electronics LTD, Coldharbour Lane, Harpenden, Herts, U.K.) and were in the order of $2-3\times10^6$ cells/ml. The control and test flasks were both inoculated with exponentially growing cyanobacteria to initial concentrations of 2×10^4 and 1.5×10^4 cells/ml for *Anabaena* spp. and *Nostoc* spp., respectively. Serially two-fold diluted fungicides were added to 250-ml Erlenmeyer flasks containing 50 ml of nitrogen-free BG-11, and each flask was then inoculated with an equal volume (50 ml) of the preculture. Thereafter, the flasks were incubated at 25–28°C for 96 h in an orbital shaker at 100 rpm at 28°C under a constant light intensity of 120 $\mu E/m^2 \cdot s$ with a 12:12 (light:dark) diurnal cycle and scored for visible cell growth. The most suitable wavelength to monitor cyanobacterial culture growth was 680 nm, which correlated highly with cell numbers, as confirmed elsewhere [18]. The tested ranges for determining the EC_{50} was from zero to 128 mg/l for all the fungicides. Each measurement with different fungicide concentration was tested in triplicate. Appropriate control system treated with distilled water instead of the tested fungicides was included in each experiment. The control and treated cultures were grown under the same condition of temperature, photoperiod, and mixing conditions. The EC_{50} values for each fungicide were defined as the concentration that caused a 50% reduction in growth, and were calculated using a linear regression analysis based on a natural logarithm of the fungicide concentration versus the percentage inhibition [23]. The percentage inhibition values were estimated using spectrophotometric data at 680 nm. All correlation coefficients were >0.90.

RESULTS AND DISCUSSION

Distribution of Cyanobacteria

The distribution results and morphological characteristics are shown in Table 2. Fourteen genera of cyanobacteria were collected from rice paddy fields at ten different locations in South Korea. *Merismopedia*, *Microcystis*, *Anabaena*, *Nostoc*, and *Oscillatoria* were all distributed nationwide, and high populations of *Anabaena*, *Nostoc*, and *Oscillatoria* were observed in all the rice paddy fields surveyed. Meanwhile, *Aphanocapsa* spp. was recorded in Kimhae, Jinju, and Naju, and a relatively high population of *Spirulina* spp. among the genera at Seosan, which is land that has been reclaimed from the sea (data shown in Table 2). *Synechocystis* spp., *Anabaenopsis* spp., *Calothrix* spp., *Cylindrospermum* spp., *Nodularia* spp., and *Tolypotrix* spp. were isolated in Icheon and Jincheon and the mountain areas (Hongcheon and Ahandong). After comparing cyanobacteria populations at the different sampling sites, no statistically significant differences were found among the sampling sites, although *Microcystis* spp., *Synechocystis* spp., and *Aphanocapsa* spp. were usually detected in Icheon, Jincheon, Kimhae, Jinju, and Naju (refer Table 2). Therefore, it appeared that the cyanobacteria grew rapidly in the rice fields that contained ample organic matters in the soil and water as well as conditions such as pH, temperature, organic sources, etc. that allowed propagation [8, 9, 37].

As regards the nationwide distribution, the appearance and population frequencies were *Nostoc*>*Oscillatoria*> *Anabaena*>*Microcystis*, whereas for the regional distribution, *Nostoc* and *Oscillatoria* were predominant in Icheon and Jincheon, and *Nostoc* and *Anabaena* were the major cyanobacteria species in Jinju, Kimhae, and Naju (Table 2). This means that the heterocystous filamentous cyanobacteria *Nostoc* and *Anabaena*, excreted significant amounts of ammonia into the flood water in the rice fields, resulting in a higher chlorophyll content in the plants and increased the rice grain and straw yields [13, 21, 38]. In addition, as cyanobacterial fertilizers, *Anabaena* and *Nostoc* are a promising alternative that can avoid the soil pollution caused by agrochemicals, while restoring the nutrient content and structure lost after harvest, since they provide the soil with combined N (some of them are N fixers) and exopolysaccharides that improve the soil structure and bioactive substances that enhance seedling growth [17]. Interestingly, in the case of the reclaimed land at Seosan, the cyanobacteria community was relatively simple compared with the other sampling sites. *Microcystis*, *Nostoc*, *Oscillatoria*,

Table 2. Regional distributions (A) and morphological characteristics (B) of cyanobacteria in rice paddy fields.
(A) Regional distributions of the isolated cyanobacteria.

Genus	Population[a]									
	Icheon	Hongcheon	Seosan	Buyeo	Jincheon	Ahandong	Gimjae	Jinju	Kimhae	Naju
Aphanocapsa								–	–	–
Dactylococcopsis	–				–					
Merismopedium	+	–	–	+	+	+	+	+	+	+
Microcystis	+	+	++	+	+	+	+	++	++	++
Synechocystis	–				-					
Anabaena	++	++	+	+++	++	+++	++	++	++	+++
Anabaenopsis		–				+				
Calothrix		–								
Cylindrospermum				-		–				
Nostoc	++	++	++	+++	++	+++	+++	+++	+++	+++
Nodularia		–								
Tolypotrix						–				
Oscillatoria	+++	++	++	++	+++	++	++	++	++	++
Spirulina			++							

[a]Population: (–), very low density (5.0×10^2 cells/cm^{-2} soil); (+), low density (5.0×10^2–1.0×10^3 cells/cm^2 soil); (++), considerable density (1.0×10^3–5.0×10^3 cells/cm^2 soil; (+++), high density (5.0×10^3–1.0×10^4 cells/cm^2 soil); (++++), very high density (1.0×10^4 cells/cm^2 soil).

(B) General morphological characteristics of the isolated cyanobacteria.

Genus	Cell width (μm)	Cell length (μm)[b]	Sheath[b]	Motility[b]	Cellular shape	Family (Order)	Other characteristic
Aphanocapsa	2–6	<12	–		Spherical	(Chroococcales)	Cell division by binary fission
Dactylococcopsis	4–8	35–85	–	–	Elongate cell with pointed ends	(Chroococcales)	Unicellular or colonial
Merismopedia	3–5	6–8	+	+	Spherical	(Chroococcales)	Form flat sheet of cells
Microcystis	3–8	3–8	+	+	Oval, spherical	(Chroococcales)	Unicellular or colonial
Synechocystis	2–5	2–5	–	+	Coccoid	Chroococcaceae	Unicellular or colonial
Anabaena	2–20	NM	–	–	Cylindrical, ovoid	Nostocaceae	Filamentous, heterocystous
Anabaenopsis	4–10	NM	–	–	Helical	Nostocaceae	Filamentous, heterocystous
Calothrix	3–15	<30	+	+	Cylindrical	Rivulariaceae	Unbranched untapered
Cylindrospermum	2–6	NM	–	+	Cylindrical	Nostocaceae	Filamentous, heterocystous
Nostoc	2–8	NM		+	Cylindrical	Nostocaceae	Formation of thallus
Nodularia	4–18	NM	–		Discoid	Nostocaceae	Chemoheterotroph
Tolypotrix	3–6	NM	+	–	Spherical	Scytonematoceae	Rarely geminate false branches single or absent
Oscillatoria	4–15	4–5		+	Ovoid	Ocillatoriaceae	Transverse septa visible
Spirulina	7–9	7–9	–	+	Helical	Ocillatoriaceae	Helical coil

[b]Meanings of symbols: NM, not mearsured; +, present; –, absent.

and *Spirulina* presented the most considerable populations. This result implies that *Microcystis*, *Nostoc*, *Oscillatoria*, and *Spirulina* were already adapted to the saline environments.

Diversity of Heterocystous Filamentous Cyanobacteria

To investigate the diversity and populations of heterocystous filamentous cyanobacteria in rice fields in Korea, 142 isolates of cyanobacteria were obtained from samples taken from rice fields across Korea. Among the 142 isolates, 53 were *Anabaena* (37.3%), 75 were *Nostoc* (52.9%), six were *Calothrix* (4.2%), two were *Cylindrospermum* (1.4%), one was *Nodularia* (0.7%), two were *Anabaenopsis* (1.4%), and three were *Tolypotrix* (2.1%), as shown in Fig. 2. As such, *Nostoc* was the predominant heterocystous filamentous cyanobacteria in most rice fields in Korea. Abundant non-heterocystous cyanobacteria belonging to the genus *Gloethece* [35], *Lingbya*, and *Pseudoanabaena* [7] were also observed. The average heterocystous filamentous cyanobacterial density was 2.98×10^3 cfu/cm^2 soil, as the somewhat acidic pH of the soil can affect the initial cyanobacterial growth [33]. The major filamentous heterocystous cyanobacteria, *Nostoc*, was somewhat expected, as *Nostoc* can withstand desiccation. In fact, *Nostoc* spp. have already been found to be the predominant cyanobacteria in most soil samples

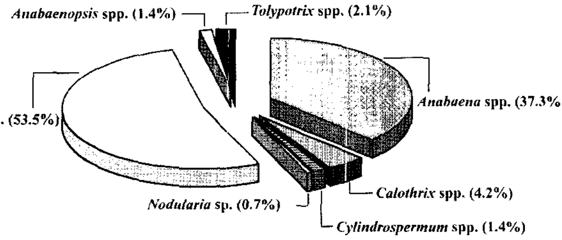

Fig. 2. Prevalence of heterocystous filamentous cyanobacterial isolates. Pie chart of the percentage distribution of cyanobacteria collected from rice fields across Korea.

from rice fields [5]. Moreover, spherical cyanobacterial isolates that belong to the genera of *Synechocystis*, *Dactylococcopsis*, *Microcystis*, *Aphanocapsa*, and *Merismopedia* were also observed. The genus *Microcystis* seemed to be predominant among the spherical cyanobacteria and uniformly distributed nationwide. Furthermore, nitrogen-fixing blue-green algae *Oscillatoria* spp. were also abundant in the soil samples (Table 2). However, high nitrogen fertilizer level with a concentrated population of *Oscillatoria* spp. induces soil-flakes with a blue tint, inhibiting the tilling of rice [23]. These blue soil-flakes increased significantly at temperature between 24–29°C, but 70–90% can be controlled by the application of bensulfuron or bentazon [24].

Growth Inhibition Tests

The susceptibility of the two major heterocystous filamentous cyanobacteria, *Anabaena* spp. and *Nostoc* spp., to the ten fungicides is summarized in Table 3. The EC_{50} values for the tested fungicides with *Anabaena* spp. were in the range of 0.04–36 mg/l, whereas the values for the fungicides with *Nostoc* spp. were much higher, except for those for carbendazim, metalaxyl, procymidone, and zineb. Thus, it was found that the *Anabaena* species were at least four times more susceptible than the *Nostoc* species to six of the tested fungicides, excepting carbendazim, metalaxyl, procymidone, and zineb (Table 3). As for the genus *Anabaena*, eight of the tested fungicides, excepting carbendazim and metalaxyl, were very toxic, with corresponding EC_{50} values of less than 10 mg/l, and the EC_{50} values for benomyl, bitertanol, chlorothalonil, prochloraz, procymidone, and zineb were even lower than 1.0 mg/l, indicating extreme toxicity to the genera. In contrast, the EC_{50} values for the same fungicides with the *Nostoc* spp. exhibited a relatively wide range of 0.02–42 mg/l. Namely, the estimated EC_{50} for benomyl, bitertanol, chlorothalonil, metalaxyl, prochloraz, procymidone, and zineb with the *Nostoc* spp. was 0.2199, 4.0499, 8.0701, 7.5013, 4.0504, 0.6822, and 0.511, respectively (Table 3). Only three fungicides were found to be extremely toxic (EC_{50} values under 1 mg/l) to the *Nostoc* spp.: benomyl, procymidone, and zineb. The *Nostoc* spp. showed a resistance to thiophanate-methyl (NOEC up to 128 mg/l). Therefore, the toxicity of thiophanate-methyl was the lowest among the ten fungicides tested. The toxicities of benomyl, bitertanol, prochloraz, procymidone, and zineb to the *Anabaena* and *Nostoc* species were higher than those of carbendazim, iprodione, metalaxyl, and thiophanate-methyl (data shown in Table 3). Consequently, the use of less toxic fungicides, such as carbendazim, iprodione, metalaxyl, and thiophanate-methyl, is preferable to use in rice fields since cyanobacteria play an important role in nitrogen fixation. Thus, since the EC_{50} values for benomyl with *Anabaena* and *Nostoc* were only 0.04 and 0.22 mg/l, respectively, benomyl should be the last fungicide applied to rice fields. The toxicities of benomyl towards *Anabaena* and *Nostoc* were comparable to that of previously reported photosynthesis-inhibiting herbicides, where atrazine (chlorotriazine herbicide), simazine (chlorotriazine herbicide), and chlorotoluron (phenylurea herbicide) were reported to have an EC_{50} of 0.12–0.15 mg/l, 0.08–0.25 mg/l, and 0.08–1.49 mg/l, respectively, with *Chlorella pyrenoidosa* [26]. However, as in Table 3, the response of the two cyanobacterial strains to various fungicides varied significantly. The strains belonging to the genus *Nostoc* were somewhat tolerant to the tested fungicides when compared with the

Table 3. Differential susceptibilities of two heterocystous filamentous cyanobacteria, *Anabaena* spp. and *Nostoc* spp., on ten fungicides.

Fungicide	*Anabaena* spp. (n=53)		*Nostoc* spp. (n=76)	
	Tested concentration levels (mg/l)	EC_{50} (mg/l)	Tested concentration levels (mg/l)	EC_{50} (mg/l)
Benomyl	0.025–0.1	0.0411	0.05–2	0.2199
Bitertanol	0.125–2	0.8044	0.125–8	4.0499
Carbendazim	8–64	35.0459	8–32	19.0573
Chlorothalonil	0.0125–0.5	0.1002	1–16	8.0701
Iprodione	2–16	6.0407	8–64	42.0011
Metalaxyl	16–64	22.9044	8–32	7.5013
Prochloraz	0.25–4	0.8997	0.25–8	4.0504
Procymidone	0.25–2	0.6799	0.25–2	0.6822
Thiophanate-methyl	1–16	6.7002	4–128	NOEC=128
Zineb	0.125–1	0.5277	0.125–1	0.5011

Anabaena species, as the *Nostoc* spp. were found to be five times more tolerant to six of the tested fungicides, including benomyl, bitertanol, chlorothalonil, iprodione, prochloraz, and thiophanate-methyl, whereas only two or three times more susceptible to carbendazim and metalaxyl, than the *Anabaena* species. Meanwhile, procymidone and zineb exhibited a similar toxicity against the two genera. Therefore, based on the data reported here, the toxicities of the ten tested fungicides was as follows: benomyl> chlorothalonil> zineb>procymidone>bitertanol>prochloraz>iprodione> thiophanate-methyl>metalaxyl>carbendazim for the *Anabaena* spp., and benomyl>zineb>procymidone> bitertanol>prochloraz>metalaxyl>chlorothalonil>carbendazim >iprodione>thiophanate-methyl for the *Nostoc* spp. (shown in Table 3).

However, seven or eight of the tested fungicides that showed a high toxicity against *Nostoc* spp. and *Anabaena* spp. are not normally applied to control phytopathogenic fungi that attack rice plants, whereas thiophanate-methyl, which was less toxic to the *Anabaena* spp. and *Nostoc* spp., is usually applied to rice fields to control the rice disease, *Magnaporthe grisea*. Similarly, metalaxyl, which was also less toxic to the cyanobacteria, is commonly applied to control downy mildew. However, toxicity is affected not only by the types of fungicides, but also by the taxonomic groups and species. Thus, no species can be always identified as the most or least susceptible [34]. Therefore the effect of fungicides on the populations of nitrogen-fixing cyanobacteria in rice fields also depends on other pesticide concentrations, and the water regimes of flooding or non-flooding associated with paddy rice fields [11, 14]. Consequently, for a more thorough understanding of the environmental impact of fungicides on natural nitrogen-fixing cyanobacteria, more detailed field studies are needed. Nonetheless, avoiding the use of extremely toxic fungicides in rice fields, as identified in this study, is still advisable until a better understanding is available. In addition, successful implementation of integrated disease management strategies necessitates the adoption of integrated disease management strategies involving a variety of biological, physical, and chemical methods.

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