The 6th International Conference on Biochemistry and Molecular Biology

Toxicity of paraquat on growth of cyanobacteria (*Nostoc* sp. N1 and *Anabaena* sp. A1) and germination of rice seed (san-pah-twang 1)

Suwakan Tansai[1], Kritchaya Issakul[1], Neti Ngearnpat[2,*]

[1]Department of Environment Science, School of Energy and Environment, University of Phayao, Phayao, Thailand
[2]Department of Microbiology and Parasitology, School of Medical Sciences, University of Phayao, Phayao, Thailand
*E-mail: neti.up1797@gmail.com

Abstract:

This study aimed to determine the toxicity of paraquat on the growth of cyanobacteria (*Nostoc* sp. N1 and *Anabaena* sp. A1) and their potential for reducing paraquat toxicity in rice cultivation (*in vitro*). The results showed that paraquat had a significant negative effect on dry mass, chlorophyll a and phycocyanin contents in both strains. Furthermore, a highly positive correlation was found between chlorophyll a content and dry mass of cyanobacteria. *Nostoc* sp. N1 and *Anabaena* sp. A1 treated with paraquat demonstrated the 50% effective concentration values of 2.735 and 3.456 mg/L, respectively. Rice seeds treated with paraquat and cyanobacterial cell revealed that 5 g/L of *Nostoc* sp. N1 promoted the fresh and dry weight including seedling vigor index compared with the control, while paraquat showed a significant negative effect on the seedling. Nevertheless, the addition of 10 g/L *Anabaena* sp. A1 combined with a 0.05 and 0.1 mg/L of paraquat significantly enhanced the shoot and root lengths, fresh weight, and seedling vigor index. From those results, it can be concluded that *Nostoc* sp. N1 and *Anabaena* sp. A1 can alleviate the toxicity of paraquat with stimulating effect of the shoot and root lengths.

Introduction:

Paddy field cyanobacteria, especially the heterocystous filamentous cyanobacteria such as *Nostoc* and *Anabaena*, are very important for maintaining the rice field fertility through nitrogen fixation.[1] Cyanobacteria has been used as a fertilizer combined with chemical fertilizer for agrochemical cost reduction and rice yield improvement.[2,3] However, they have not been very well accepted for their special activities to be used as biological agents for remediating and improving the soil and water qualities.[4] It was reported that *Nostoc hatei* and *Anabaena lutea* decreased 39.73% of 2,4-D herbicide contaminated water from rice paddy fields after 14 days of receiving.[5] In addition, many strains of cyanobacteria were able to produce extracellular polymeric substances (EPS) comprising mainly polysaccharide,[6] which can be used as a novel soil stabilizer by successfully binding soil particles to improve the soil quality.[7]

Many species of cyanobacteria i.e. *Phormidium*,[8] *Nostoc muscorum* and *Anabaena subcylindrica*,[9] *Anabaena* sp. PCC 7120, *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Anabaena cylindrica* and *Anabaena spiroides*[10,11] have been reported to living and well surviving in heavy polluted areas such as agricultural waste, livestock waste and industrial wastewater.

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) is a bipyridylium-class herbicide, one of the most widely used in the world. In Thailand, the total amount of 13 million kilograms

of paraquat herbicides was imported in 2013.[12] Contamination of paraquat in wetland rice fields posed the negative effects on soil microorganisms, especially the effect on N_2-fixing cyanobacteria such as decreasing of the growth, pigmentation and nitrogen fixation.[13,14,15] According to paraquat residue in soil, a very high amount of 72.15 mg/kg was found in Kalasin province.[16] While a low concentration of paraquat in dry and wet seasons in Chanthaburi province, Thailand, ranged between 3.33-8.28 and 1.30-9.15 mg/kg, respectively.[17]

Therefore, this study aimed to determine the toxicity of paraquat to the growth of *Nostoc* sp. N 1 and *Anabaena* sp. A1 and to evaluate the effect of paraquat and cyanobacteria cells on germination and growth of San-pah-twang 1 rice seedling.

Methodology

Cyanobacteria and culture condition

Resistant paraquat cyanobacteria, *Nostoc* sp. N1 and *Anabaena* sp. A1 were isolated from paddy fields in Phayao province, Thailand. Cyanobacteria were grown in BG-11 liquid medium, shaken at 120 rpm under a room temperature and illuminated with a light intensity of 3000 lux for 3 weeks. Then, each cyanobacterium was transferred to N-free BG-11 mixed with the different concentrations (0, 3, 4, 5 and 6 mg/L) of paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride 27.6% W/V SL) for 96 h of the experimental time.

Determination of the growth of cyanobacteria

Cyanobacterial growth was determined by estimating the cell biomass dry weight after 96 h of the experimental time. Aliquots of 10 mL of cyanobacterial suspension were filtered with filter paper (GF/C). The filters were then washed with distilled water three times, dried at 80 ^{o}C for 24 h, cooled down in a vacuum desiccator, and then weighed.

Determination of cyanobacteria pigments

Determination of Chlorophyll a content

Chlorophyll a content was determined according to the method of Wintermans and de Most.[18] Aliquots of 10 mL of cyanobacterial suspension were filtered with filter paper (GF/C). The chlorophyll a content was extracted by 10 mL of 90% methanol and then inoculated at 70 ^{o}C for 20 min. The extract was centrifuged at 3000 rpm for 10 min. The absorbance was measured at 630, 645, 665 and 750 nm using GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific, USA). The concentration of chlorophyll a was calculated using the following equation:

$$\text{Chl a } (\mu g/ml) = \frac{11.6(A_{665}-A_{750})-1.31(A_{645}-A_{750})-0.014(A_{630}-A_{750})\times \text{The volume of methanol(ml)}}{\text{The volume of filtered water} \times (\frac{1}{\text{cuvette width}})}$$

where A_{630}, A_{645}, A_{665} and A_{750} are the absorbance at 630, 645, 665 and 750 nm, respectively

Determination of Phycocyanin content

Phycocyanin was determined using the techniques of freezing and thawing from Sarada *et al.*[19] A 10 mL of culture was filtered through filter paper (GF/C) to obtain a pellet. The pellet was then added into 5 mL of phosphate buffer (pH 7). Then, phycocyanin was extracted by the freeze-thaw method (5 times). The cell debris was removed by centrifugation at 3,000 rpm and supernatant was then measured by spectrophotometer at 618 nm. Phycocyanin content was calculated by the following equation:

$$\text{Phycocyanin (mg)} = \frac{(OD_{618} \times 1000 \times 5)}{6500}$$

where OD_{618} is the optical density at the absorbance of 618 nm, 1000 is the value for converting the unit to milligrams, 5 is the volume of phosphate buffer and 6500 is the constant.

$$\text{Phycocyanin (mg/g dry weight)} = \text{Phycocyanin(mg)} \times \frac{1000}{\text{sample(mg)}}$$

Effects of cyanobacterial cell and paraquat on rice seedling growth

Growth condition and experimental design

The seed of *Oryza sativa* L. cv. San-pah-twang 1 rice were obtained from Phayao rice seed center, Phayao province. Seeds were surface sterilized using 10% of sodium hypochlorite for 10 min and washed with distilled water. Seeds were germinated in filtered paper on petri dishes, moistened with 10 ml of test solution and placed on growth chamber for 7 days at room temperature with 12:12 h light dark cycle (~15000 lux). This experiment was divided into 24 treatments as described in Table 1. The completely randomized design (CRD) with triplication was performed.

Germination parameters

The germination parameter i.e. germination percentage (GP),[20] germination energy (GE),[21] speed of germination (SG)[22] and the seedling vigor index (SVI)[23] were investigated.

Physical parameters

The shoot and root lengths were measured in centimeter (cm) after 7 days of the application. The fresh weight of each sample was determined, and the samples were then oven-dried at 80 °C for 24 h before the dry weight was obtained.

Table 1. Experimental treatments for the determination of effects of cyanobacterial cells and paraquat on germination and growth of rice seedling.

Treatment	Cyanobacteria (g/L)				Paraquat (mg/L)				Treatment	Cyanobacteria (g/L)				Paraquat (mg/L)			
	Nostoc sp. N1		*Anabaena* sp. A1							*Nostoc* sp. N1		*Anabaena* sp. A1					
	10	5	10	5	0.05	0.1	0.5	1		10	5	10	5	0.05	0.1	0.5	1
Control	-	-	-	-	-	-	-	-	T13	-	/	-	-	/	-	-	-
T1	/	-	-	-	-	-	-	-	T14	-	/	-	-	-	/	-	-
T2	-	/	-	-	-	-	-	-	T15	-	/	-	-	-	-	/	-
T3	-	-	/	-	-	-	-	-	T16	-	/	-	-	-	-	-	/
T4	-	-	-	/	-	-	-	-	T17	-	-	/	-	/	-	-	-
T5	-	-	-	-	/	-	-	-	T18	-	-	/	-	-	/	-	-
T6	-	-	-	-	-	/	-	-	T19	-	-	/	-	-	-	/	-
T7	-	-	-	-	-	-	/	-	T20	-	-	/	-	-	-	-	/
T8	-	-	-	-	-	-	-	/	T21	-	-	-	/	/	-	-	-
T9	/	-	-	-	/	-	-	-	T22	-	-	-	/	-	/	-	-
T10	/	-	-	-	-	/	-	-	T23	-	-	-	/	-	-	/	-
T11	/	-	-	-	-	-	/	-	T24	-	-	-	/	-	-	-	/
T12	/	-	-	-	-	-	-	/									

Statistics

The data were recorded as the mean ± standard deviation of the result in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA). Difference between means was considered using Duncan's New Multiple Rang Test (DMRT) at the significant level of 0.05. The correlations of biomass, chlorophyll a and phycocyanin contents were calculated using the Pearson's correlation coefficient and the toxicity of paraquat (EC_{50}) was calculated using non-linear using GraphPad Prism program.

Results and Discussion

Effect of paraquat on growth and pigments of cyanobacteria

Effects of paraquat on growth of *Nostoc* sp. N1 and *Anabaena* sp. A1 were investigated. It was found that paraquat had a significant negative effect on their dry mass, chlorophyll a and phycocyanin contents. The dry mass of both cyanobacteria significantly decreased with increasing paraquat concentrations (Figure 1). According to its properties, paraquat inhibited the electron transport and CO_2 assimilation while enhanced the synthesis of reactive oxygen species (ROS) of the cell, which disrupts the structure and inhibits the cell growth.[24] According to the previous report, *Nostoc* and *Anabaena* have the highest tolerance to paraquat at 25 mg/L.[25] Generally, *Anabaena* sp. has been reported for its higher yield than *Nostoc* sp. when cultured in nitrogen-free medium.[15,26]

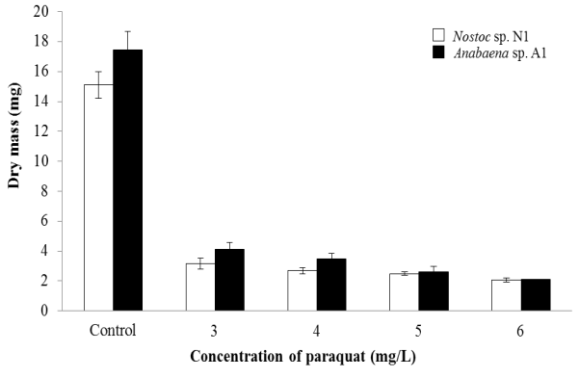

Figure 1. Effect of paraquat on cyanobacterial dry mass.

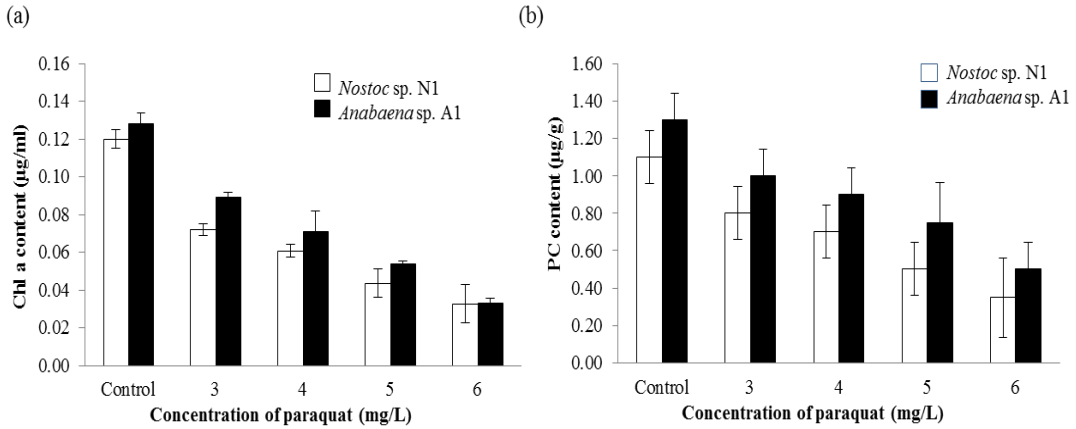

Figure 2. Effect of paraquat on chlorophyll a (a) and phycocyanin (b) contents of cyanobacteria.

Exposure to paraquat under different levels of concentration conditions induced the decrease of pigments (Figure 2). The chlorophyll a and phycocyanin contents tended to decrease continuously with increasing paraquat concentrations. The reduction in chlorophyll a and phycocyanin contents from the paraquat exposure might affect photosynthesis.[24] Other herbicides, such as 2,4-D and glyphosate at concentrations 12 and 0.3 mg/L, respectively, have been reported for their negative effect on chlorophyll a and phycocyanin contents of *Nostoc* and *Anabaena*.[15,27] Similar results of pigment toxic effects were also observed in other herbicides; endosulfan and tebuconazole treatments at various concentrations could cause the reduction in the chlorophyll a and phycocyanin contents of the cells.[28]

Table 2. Correlation coefficient of biomass and pigments of cyanobacteria.

Cyanobacteria		*Nostoc* sp. N1		*Anabaena* sp. A1	
		Correlation coefficient (r)	**Significance level**	**Correlation coefficient (r)**	**Significance level**
Biomass	Chl a[b]	0.992[*a]	0.026	0.880[*]	0.049
	PC	0.816	0.092	0.798	0.104
Chl a	PC	0.973[**]	0.005	0.983[**]	0.003

[a]*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level
[b]Chl a : Chlorophyll a, PC : Phycocyanin

Pearson's correlation coefficient values of dry mass, chlorophyll a and phycocyanin contents of *Nostoc* sp. N1 and *Anabaena* sp. A1 were calculated and presented in Table 2. The results demonstrated a significant positive correlation (P<0.05) between dry mass and chlorophyll a content with the correlation coefficients of 0.992 and 0.880, respectively. Moreover, chlorophyll a and phycocyanin were also significantly correlated (P<0.01) with positive correlation coefficients of 0.973 and 0.983, respectively (Table 2). In contrast, no correlation was found between the biomass and phycocyanin content. This result was supported by Ernst *et al*.[29], who reported that a filamentous cyanobacterium *Planktothrix rubescens* showed a significant correlation between cell counts and chlorophyll a content. Another previous report demonstrated that the chlorophyll a content and dried weight was related to the optical density (OD) of the cell.[30] However, the other study reported that thiobencarb concentration higher than 2 mg/L showed a significant decreasing effect of phycocyanin content but no significant effect on chlorophyll a and biomass yield of *Nostoc sphaeroides*.[31]

Acute toxicity (96 h-EC_{50}) of paraquat to cyanobacteria

The 96-hour EC_{50} values were calculated by the dose-response curve for both cyanobacteria. Paraquat exhibited the EC_{50} values of 2.734 and 3.456 mg/L for *Nostoc* sp. N1 and *Anabaena* sp. A1, respectively (Table 3). The lower EC_{50} value indicates the higher toxicity; consequently, *Anabaena* sp. A1 was more resistant to paraquat toxicity than *Nostoc* sp. N1. As reported previously, *A. variables* also resisted other herbicides i.e. arozin, alachlor, butachlor and 2,4-D.[32] Glyphosate at low concentrations did not affect *Anabaena* sp. However, at a concentration exceeding 1 mM, a significant inhibitory effect was found with the IC_{50} value of 9 mM. While, *Nostoc punctiforme* demonstrated the IC_{50} value of more than 50 mM.[33] Besides, *Anabaena inaequalis* was much more sensitive with their bipyridyl compounds such as diquat, with the EC_{50} values approximately 0.074 mg/L.[34] Therefore, the response of each species of cyanobacteria indicates different sensitivity to each herbicide.

Table 3. Acute toxicity of paraquat to cyanobacteria after 96 h of exposure.

Cyanobacteria	Regression linear	R^2	EC_{50} values (mg/L)
Nostoc sp. N1	Y= -0.01470*X+0.1189	0.974	2.734
Anabaena sp. A1	Y= -0.01556*X+0.1315	0.973	3.456

Effect of paraquat and cyanobacterial cell on germination of rice
Germination parameters

Paraquat and cyanobacterial cells did not pose a significant effect on germination percentage (GP), germination energy (GE), and speed of germination (SG). While, they had a

significant influence on the seedling vigor index (SVI) (Table 4). The addition of both cyanobacteria alone in treatment 1, 2 and 4 gently increased the SVI over the control. Interestingly, addition of 10 g/L of *Anabaena* cells alleviated the effects of paraquat toxicity on rice especially at a low concentration of paraquat (T17 and T18), resulting in the increasing of seedling vigor index. In contrast, the addition of *Nostoc* sp. N1 cells combined with paraquat was not able to increase the SVI compared with the seed treated with paraquat alone. The previous research demonstrated that the SVI of rice and maize increased when cyanobacterial extracellular polymeric substances was applied.[35] Besides, *Anabaena variabilis* and *Nostoc muscorum* posed the positive resultant on the GP, SG and SVI of wheat seeds.[36] According to the residue of paraquat, the previous report revealed that the application of paraquat at 240 g/ha did not affect the GP and SVI of bean seeds.[37] Likewise, the application of paraquat at concentration of 120 to 240 g/ha also did not affect the GP of rice seed.[38]

Table 4. Effects of paraquat and cyanobacterial cells on germination percentage, germination energy, speed of germination and seedling vigor index.

Treatment	Seed germination parameter (Mean±SD[a])			
	GP (%)	GE (%)	SG (%)	SVI
Control	100.00±0.00[a]	90.00±0.00[a]	90.00±0.00[a]	246.60±30.24[a]
T1	100.00±0.00[a]	93.33±5.77[a]	93.33±5.77[a]	288.00±37.27[a]
T2	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	283.93±41.16[a]
T3	100.00±0.00[a]	96.67±5.77[a]	100.00±5.77[a]	238.33±101.79[ab]
T4	96.67±5.77[a]	96.67±5.77[a]	100.00±0.00[a]	292.15±30.64[a]
T5	100.00±0.00[a]	86.67±11.55[a]	86.67±11.55[a]	169.33±33.98[cde]
T6	100.00±0.00[a]	86.67±11.55[a]	86.67±11.55[a]	164.00±14.18[cde]
T7	100.00±0.00[a]	90.00±10.00[a]	90.00±10.00[a]	104.47±38.88[e]
T8	100.00±0.00[a]	86.67±5.77[a]	86.67±5.77[a]	122.00±33.87[de]
T9	100.00±0.00[a]	93.33±5.77[a]	93.33±5.77[a]	157.00±13.11[cde]
T10	100.00±0.00[a]	86.67±5.77[a]	86.67±5.77[a]	167.00±21.93[cde]
T11	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	132.33±32.13[de]
T12	100.00±0.00[a]	93.33±11.55[a]	93.33±11.55[a]	121.00±56.11[de]
T13	100.00±0.00[a]	96.67±5.77[a]	93.33±5.77[a]	157.00±19.43[cde]
T14	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	180.30±41.49[bcd]
T15	96.67±5.77[a]	96.67±5.77[a]	100.00±0.00[a]	128.75±33.73[de]
T16	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	122.00±13.53[de]
T17	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	207.67±31.56[bc]
T18	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	206.00±1.00[bc]
T19	96.67±5.77[a]	96.67±5.77[a]	100.00±0.00[a]	152.48±21.14[cde]
T20	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	142.67±19.14[cde]
T21	100.00±0.00[a]	93.33±11.55[a]	93.33±11.55[a]	184.00±32.23[bcd]
T22	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	151.04±47.73[cde]
T23	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	145.33±20.53[cde]
T24	100.00±0.00[a]	96.67±5.77[a]	96.67±5.77[a]	136.19±19.39[cde]

[a]Means ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan' s Multiple Range Test (DMRT). (GP: germination percentage (%), GE: germination energy (%), SG: speed of germination (%), SVI: seedling vigor index)

Physical parameters of rice seedling

The growth of rice seedling was obviously promoted by *Nostoc* sp. N1 and *Anabaena* sp. A1 cells, resulting in the increasing of fresh and dry weights in comparison to the control. Particularly, the addition of 5 g/L of *Nostoc* cells gave the highest growth in terms of fresh and dry weights. Whereas, the fresh and dry weights of paraquat-treated seedlings were significantly reduced with an increasing of paraquat concentrations. It is interesting that the addition of both *Nostoc* sp. N1 and *Anabaena* sp. A1 cells into a low concentration of paraquat

treatments (T9, T10, T13, T14, T17, T18, T21 and T22) strongly alleviated the paraquat toxicity (Figure 3a) and promoted the seedling growth. A previous paper showed that addition of fresh or dry cyanobacteria as seed pretreatment improved plant nutrients, which supplemented the biochemical partway i.e. exopolysaccharides (EPS), gibberellins, cytokinin, auxin and nitrogenase activity to induce to a more rapid and augmented plant growth.[39]

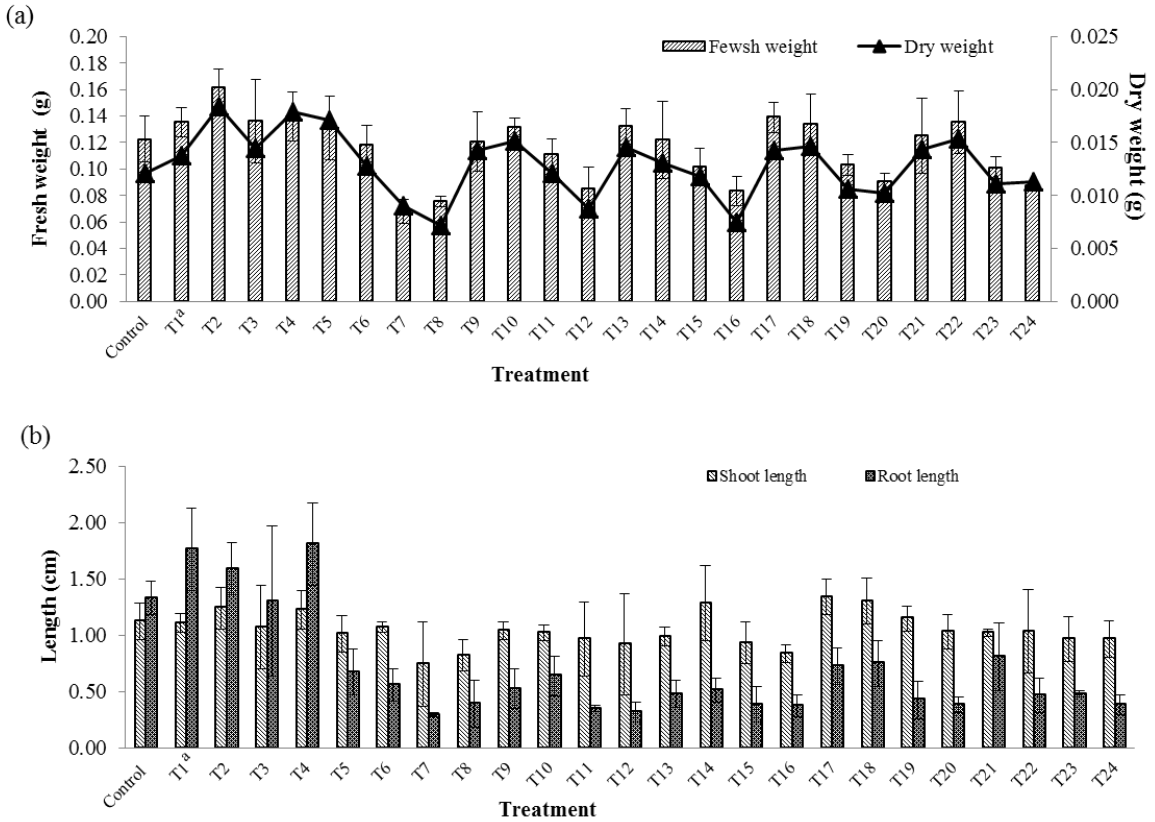

Figure 3. Effects of cyanobacteria cells and paraquat on fresh and dry weights (a), shoot and root lengths (b) of rice seedling.

[a]T1: N 10 g/L T2: N 5 g/L T3: A 10 g/L T4: A 5 g/L T5: P 0.05 mg/L T6: P 0.1 mg/L
T7: P 0.5 mg/L T8: P 1 mg/L T9: T1+T5 T10: T1+T6 T11: T1+T7 T12: T1+T8
T13: T2+T5 T14: T2+T6 T15: T2+T7 T16: T2+T8 T17: T3+T5 T18: T3+T6
T19: T3+T7 T20: T3+T8 T21: T4+T5 T22: T4+T6 T23: T4+T7 T24: T4+T8
Note; N: *Nostoc* sp. N1, A: *Anabaena* sp. A1, P: Paraquat

Nostoc sp. N1 and *Anabaena* sp. A1 cells demonstrated the similar trend in seedling shoot and root lengths as the control. These cyanobacteria and control treatments influenced the longer seedling root than shoot lengths. In contrast, seedling treated with paraquat exhibited a very strong inhibitory effect on the root than the shoot. Addition of both cyanobacterial cells into a low concentration of paraquat treatments significantly increased seedling shoot length compared with the seedling treated with paraquat alone (Figure 3b). However, considering the seedling root length, the addition of these *Nostoc* sp. N1 and *Anabaena* sp. A1 did not pose the positive effect on alleviating of paraquat toxicity. In general, rice seedling treated with cell-free extracts of cyanobacterial strains namely, *Anabaena oryzae* and *Nostoc calcicola* demonstrated the stimulating effect on root and shoot lengths of rice seedlings.[40] Moreover, *Nostoc kihlmani* and *Anabaena cylindrica* significantly increased the root and shoot lengths of wheat.[41]

Conclusion

This study demonstrated that paraquat had a significant negative effect on the dry mass, chlorophyll a and phycocyanin contents of *Nostoc* sp. N1 and *Anabaena* sp. A1. A significantly positive correlation was found between chlorophyll a content and dry mass of cyanobacteria and also between chlorophyll a and phycocyanin contents. *Anabaena* sp. A1 was more resistant to paraquat than *Nostoc* sp. N1 with the EC_{50} values of 3.456 and 2.735 mg/L, respectively.

Paraquat and cyanobacterial cells did not pose a significant effect on most of the germination parameters except for the SVI. The addition of cyanobacteria alone increased the SVI over the control. Addition of both *Nostoc* sp. N1 and *Anabaena* sp. A1 cells into a low concentration of paraquat treatments gradually reduced the paraquat toxicity, resulting in the increasing of fresh weight, dry weight and shoot length of rice seedling.

From those results, it can be concluded that *Nostoc* sp. N1 and *Anabaena* sp. A1 cells can alleviate the toxic of paraquat with the growth stimulating effect on rice seedling. Therefore, these cyanobacterial cells could potentially be one of the natural substances used as a growth promoting substance for rice yield improvement.

References

1. Kim JD, Lee CG. J Microbiol Biotechnol. 2006;16(2):240-246.
2. Chittapun S, Limbipichai S, Amnuaysin N, Boonkerd R, Charoensook M. J Appl Phycol. 2017;30:79-85.
3. Duangkumjan S, Praiwan K, Jantasri R, Pimratch S. Prawarun Agri J. 2013;10:9-17.
4. Prasanna R, Jaiswal P, Kaushik BD. Indian J Microbiol. 2008;48:89-94.
5. Kophimail W, Bunnag S. 15th Khon Kaen Univ Grad Res Conf. 2014;1:804-811.
6. Pereira S, Micheletti E, Zille A, Santos A, Moradas-Ferreira P, Tamagnini P, De Philippis R. Microbiology. 2011;157:451-458.
7. Yuliani E, Imai T, Teeka J, Sekine M. J Water Environ Technol. 2011;9(3):277-287.
8. Canizares-Villanueva RO, Ramos A, Corona AI, Monroy O, de la Torre M, Gomez Lojero C, Travieso L. Water Res. 1994;28(9):1891-1895.
9. El-Sheekh MM, El-Shouny WA, Osman MEH, El-Gammal EWE. Environ Toxicol Pharmacol. 2005;19:357-365.
10. Lee SE, Kim JS, Kennedy IR, Park JW, Kwon GS, Koh S, Kim JE. J Agr Food Chem. 2003;51:1336-1340.
11. El-Rahman Mansy A, El-Bestawy E. World J Microbiol Biotechnol. 2002;18:125-31.
12. Tawatsin A, Thavara U, Siriyasatien P. Medical Research Archives. 2015;3:1-10.
13. Battah MG, Shabana EF, Kobbia IA, Eladel HM. Ecotoxicol Environ Saf. 2001;49:235-239.
14. Singh D, Khattar J, Gupta M, Kaur G. Pestic Biochem Physiol. 2014;110:63-70.
15. Pithukpakorn W, Mala T, Sinma K, Antarikanonda P. J Sci Technol. 2015;4:29-40.
16. Paipard N, Supannatas S, Suttiprapa T. Khon Kaen Agr J. 2014;42(3):301-310.
17. Pataranawat P, Kitkaew D, Suppaudom K. J Sci Technol Humanit. 2012;10(1):17-24.
18. Wintermans J, Demots A. Biochimica et Biophysica Acta. 1965;109:448-453.
19. Sarada R, Pillai MG, Ravishankar GA. Process Biochem. 1999;34(8):795-801.
20. Islam R, Mukherjee A, Hossin M. J Bangladesh Agr Univ. 2012;10(1):15-20.
21. Bam RK, Kumaga, FK, Ofori K, Asieudu EA. Asian J Plant Sci. 2006;5:948-955.
22. Krishnaswamy V, Seshu DV. Seed Sci Technol. 1990;18:147-15.

23. Abdul-Baki AA, Anderson JD. Crop Sci. 1973;13:630-633.
24. Qian H, Chen W, Sun L, Jin Y, Liu W, Fu Z. Ecotoxicology. 2009;18(5):537-543.
25. Pandey FK, Kumar S, Bhatnagar T. Int J Curr Sci. 2011;3:14-16.
26. Rosales Loaiza N, Vera P, Aiello-Mazzarri C, Morales E. Acta Biol Colomb. 2016;2(2):347-354.
27. Shikha S, Singh DP. Curr Sci. 2004;86(4):571-576.
28. Kumar N, Bora A, Kumar R, Amb MK. J Biol Environ Sci. 2012;6(16):67-75.
29. Ernst B, Neser S, O'Brien E, Hoeger SJ, Dietrich DR. Harmful Algae. 2006;5:281-289.
30. Ma J, Tong S, Wang P, Chen J. Int J Environ Res. 2009;4(2):347-352.
31. Xia J. Chemosphere. 2005;59(4):561-566.
32. Singh S, Datta P. Plant Soil. 2007;296:95-102.
33. Forlani G, Pavan M, Gramek M, Kafarski P, Lipok J. Plant Cell Physiol. 2008;49(3):443-456.
34. Peterson HG, Boutin C, Lindsay KE, Martin PA. Aquat Toxicol. 1997;39(2):111-134.
35. Arora M, Kaushik A, Rani N, Kaushik CP. J Environ Biol. 2010;31(5):701-704.
36. Kumar A, Kaur R. Int J Basic Appl Biol. 2014;1(1):11-15.
37. Oliveira CMG, Kryzanowaki FC, Oliveira MCN, Franca-Neto JB, Henning AAJ. J Seed Sci. 2014;36(4):384-391.
38. He Y, Cheng G, Liu L, Li X, Yang B, Zhang H, Wang Z. J Zhejiang Univ-Sci B (Biomed & Biotechnol). 2015;16(10):813-823.
39. Ismail GA, Abo-Hamad SA. Egypt J Bot. 2017;57(3):507-516.
40. Hassan EA, Morcos MM. J Agr Sci. 2006;31(8):5351-5361.
41. Gheda SF, Ahmed DA. Rend. Fis Acc Lincei. 2015;26:121-131.

Acknowledgements

This research work was financially supported by University of Phayao, Thailand. The authors would like to thank the Phayao rice seed center for supporting rice seeds in this experiment.