0971-4693/94 US $ 5.00
Allelopathy Journal 25 (2): 453-464 (2010) *International Allelopathy Foundation* 2010
Tables: 4, Figs : 3

Effects of *Spirulina platensis* and C-phycocyanin on seed germination and seedling growth of two monocot and dicot plants

P. CHAROENYING[1*], P. CHOTSAENG[1] and C. LAOSINWATTANA[2]

Faculty of Science
King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
E. Mail: kcpatcha@kmitl.ac.th

(Received in revised form: February 20, 2010)

ABSTRACT

In laboratory bioassay the allelopathic effects of *Spirulina platensis* (Nordst) Geitl. were investigated on Chinese amaranth (*Amaranthus tricolor* L.), Chinese mustard (*Brassica campestris* var. *chinensis*), barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.) and rice (*Oryza sativa* L.). The seeds were germinated in Petri dishes with aqueous extract (0.625-5% concentrations) and crude organic extract (250-4000 ppm concentrations) distilled water used as control. All aqueous extracts influenced the seed germination and seedling growth of dicotyledons (*A. tricolor* and *B. campestris*). The aqueous extract of 1.25% and 2.50% concentration completely inhibited the germination in dicots. In monocots (barnyardgrass and rice), the aqueous extract of 2.50% concentration inhibited the root length, while the 5% concentration completely inhibited their germination and seedling shoot length.

The UV-VIS spectra indicated that the aqueous extract contained C-phycocyanin (C-PC), with a purity ratio (A_{620}/A_{280}) of 1.17. The allelopathic activity of biologically active protein pigment, C-PC, was assayed on seed germination and seedling growth at 62.50-1000 ppm concentrations. At 1000 ppm, this compound was most inhibitory to shoot and root length of *A. tricolor* and *B. campestris*, but there was no effect on germination. The crude ethyl acetate extract was most inhibitory to dicots, but all three crude organic extracts were not inhibitory to monocots.

Keywords: Allelopathic effects, aqueous extract, *A. tricolor*, bioassays, *B. campestris*, C-phycocyanin, crops, crude organic extract, *E. crus-galli* and *O. sativa*, *Spirulina platensis*, weeds.

INTRODUCTION

Weeds adversely affects the crop production worldwide. There are many methods to control weeds, the chemical weed control method is most popular but it has resulted in emergence of herbicide-resistant weeds adverse effects on human health and the environment pollution. These concerns are shifting attention to alternative weed control technologies based on natural products (9,10,24,29).

Allelopathy is direct or indirect detrimental or beneficial effects of one plant (including microorganisms) on the germination, growth, or development of other plants

[*]Correspondence author; [1]Faculty of Science, [2]Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

through the production of chemical (allelochemicals) that escape into the environment (25,29). This effect plays a significant role in agroecosystems, growth, quality and quantity of produce (20,33). Use of allelochemicals from plants for this purpose would be environmentally friendly, as natural chemicals are renewable and easily degradable. Although allelopathic phenomena are well recognised in the terrestrial plant kingdom, but very little is known for algae. The prokaryotic cyanobacteria (blue-green algae) produces numerous bioactive substances; their antialgal, antifungal, antibiotic and antiviral activities are associated with cytotoxic, neurotoxic or hepatotoxic actions (8,21,22).

Recently the allelopathic effects of cyanobacteria on other plants, cyanobacteria and microbial species have been reported (11,16,23,31,32,34). *Spirulina platensis* is a photosynthetic cyanobacterium with known biological activity. It is grown to produce biologically active food additives and to treat various diseases (5). Several studies have shown that *Spirulina* or its extracts can prevent or inhibit the cancer in humans and animals (1,4), and that novel *Spirulina*-based medicines are effective (5,7). *In vitro* studies suggest that polysaccharides of *S. platensis* enhances the cell nucleus enzyme activity and DNA repair synthesis (26,27).

This study aimed to investigate the allelopathic effects of *S. platensis* extracts on seed germination and seedling growth of Chinese amaranth (*A. tricolor* L.), Chinese mustard (*B. campestris* var. *chinensis*), barnyardgrass (*E. crus-galli* [L.] Beauv.) and rice (*O. sativa* L.). Since the deep blue fluorescent protein, C-phycocyanin (C-PC), is the major component in the aqueous extract that may exert allelopathic effects on test plants, the study included investigation of the allelopathic effects of C-PC on Chinese amaranth and barnyardgrass.

MATERIALS AND METHODS

The study consisted of 4-factors (i) Test plants: 4 [Chinese amaranth (*A. tricolor* L.), Chinese mustard (*B. campestris* var. *chinensis*), barnyardgrass (*E. crus-galli* [L.] Beauv.) and rice (*O. sativa* L.)], (ii). *S. plantensis* aqueous extracts concentrations: 5 (0, 0.625, 1.25, 2.5, 5% w/v), (iii) crude organic extracts in solvents: 3 (Hexane, ethyl acetate, methanol) and (iv) crude organic extracts concentrations: 6 (0, 250, 500, 1000, 2000, 4000 ppm). The seeds of Chinese amaranth and Chinese mustard were purchased from Thai Seed & Agriculture Co., Ltd., Bangkok and barnyardgrass seeds were collected (August 2006) from paddy fields in the Minburi district, Bangkok, Thailand. Rice seeds were obtained from the Department of Horticulture of our Institute. The germination of test seeds was > 80%.

Dried *S. platensis* was obtained from the Department of Fisheries Science of our Institute. Algal cultures were grown in Zarrouk's medium (37) and maintained in a 0.03% CO_2 atmosphere at 25^{o}C and pH 10.50. The cultivar flasks were illuminated under 400 $\mu molm^{-2}s^{-1}$ light intensity. Cells were harvested at the late exponential phase by centrifugation and dried in an oven at 40^{o}C.

Aqueous extract preparation: Dried *S. platensis* (5 g) was soaked in 95 ml distilled water in 125 ml Erlenmeyer flask. The mixture was stirred for 10 min at room temperature (28-32^{o}C). The flask was sealed with Parafilm and kept in refrigerator at 4^{o}C for 24 h.

Afterwards, the mixture was centrifuged at 5000 rpm for 20 min, and the blue supernatant (5% w/v of stock solution) was used as crude extract in the plant bioassays, the remaining cell debris was discarded. The purity of C-phycocyanin in the supernatant was determined as per the UV-VIS absorption spectra. The stock solution was fifty-folds diluted to prevent the reabsorbtion effects. All spectra were recorded at room temperature.

Effects of aqueous extracts: The stock solution (5% w/v) was diluted with distilled water to get 5%, 2.50%, 1.25% and 0.625% concentrations. Five ml of each concentration was added to each Petri dishes (9 cm in dia) containing germination paper and then 20 seeds of test plant were placed on the germination paper as per treatments. The control received only distilled water. The treatments were replicated 4-times in completely randomized design. All Petri dishes were covered and placed at room temperature (32^{o}C day and 28^{o}C night) and under natural light conditions (0600 h – 1800 h). After 7 days, germination (%), shoot and root length were recorded in all treatment. Inhibition (%) relative to control, was calculated as under:

$$\text{Inhibition (\% of control)} = [1-(\text{sample extracts/control})] \times 100$$

Effects of C-phycocyanin (C-PC): Commercial C-PC was used to represent the pure C-PC from *S. platensis* and purchased from Sigma-Aldrich, USA. Ten mg C-PC was dissolved in 10 ml distilled water in 10 ml volumetric flask to obtain 1000 ppm stock solution, and its UV-VIS absorption spectrum was measured. The stock solution was diluted 62.50, 125, 250 and 500 ppm. Five-hundred µl of each concentration was added in vial (4.50 x 2 cm) lined with germination paper. Ten seeds of each test plant were placed on the germination paper and the vials were sealed with Parafilm and grown for 7 days. The germination test was done in conditions as described above.

Crude organic extracts preparation: The dried *S. platensis* (4 kg) were extracted by hexane-treatment for 7 days at room temperature. The extract was then filtered through a Whatman No. 1 paper. The collected filtrate was evaporated to dryness under reducing pressure at 40°C using a rotary evaporator to yield crude hexane extract. The residue was then similarly extracted with ethyl acetate (EtOAc) or methanol (MeOH) to yield crude ethyl acetate and methanol extracts, respectively. The extraction procedure is shown in Fig. 1.

Effects of crude organic extracts: The 3-dried samples concentrated from hexane, ethyl acetate and methanol were again dissolved in each solvent to compare their inhibitory effects. Five-hundred µl of each crude extract solutions (2500, 5000, 10000 20000 and 40000 ppm concentrations) were added to Petri dishes (9 cm dia) lined with germination paper and the solutions were allowed to evaporate for 3 h at room temperature. After evaporation, 5 ml of distilled water was added on the germination paper to obtain 250, 500, 1000, 2000 and 4000 ppm concentrations. Then, 20 seeds of each test plant were placed on the treated germination paper for 7 days. All germination tests were conducted under similar conditions as described above.

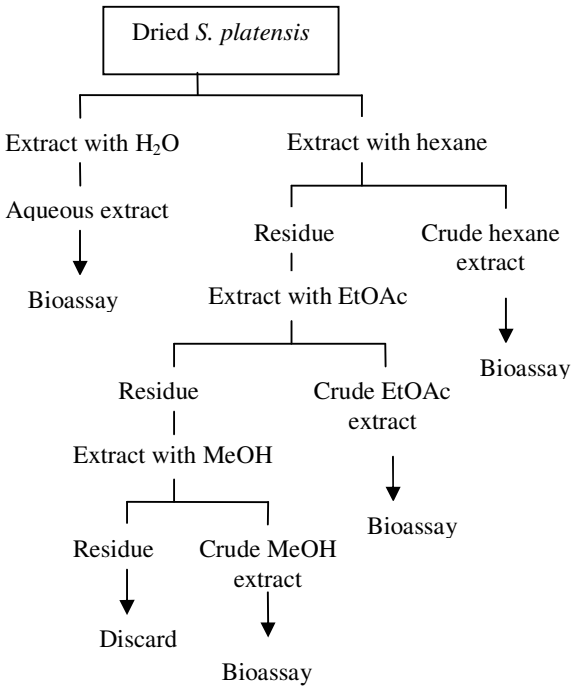

Figure 1**.** Diagram showing the extraction procedure of *S. platensis* to obtain the aqueous extract and various organic extracts.

Spectroscopic measurements: All UV-VIS absorption spectra were recorded on a UV-VIS spectrometer (Thermo Electron, England) with a 1 cm path length. Purity ratio (A_{620}/A_{280}) of aqueous extract and C-phycocyanin (C-PC) were calculated based on the UV-VIS spectra (30).

Statistical analysis: Differences in the percentages of seed germination and root and shoot length were assessed by analysis of variance statistical methods. Comparisons between treatments were made at a 0.05 probability level using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effects of aqueous extracts

All applied concentrations of aqueous extract of *S. platensis* had allelopathic effects on seed germination and seedling growth of dicotyledonous (Table 1). Seed germination of Chinese amaranth and Chinese mustard was completely inhibited at 1.25% and 2.50% concentrations. The 1.25% concentration proved more inhibitory to Chinese

mustard. The 0.625% concentration significantly reduced the seed germination and root length of both test plants, but stimulated the shoot length of Chinese amaranth. Thus aqueous extract influenced the roots growth of test plants.

Table 1. Allelopathic effects of aqueous extract of *S. platensis* on seed germination and seedling growth of Chinese amaranth and Chinese mustard

Concentrations (% w/v)	Chinese amaranth (% Inhibition)			Chinese mustard (% Inhibition)		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
Control	0c	0c	0c	0c	0c	0d
0.625	20b	45.4b	68.4b	5.3b	-12.6d	38.2c
1.25	100a	100a	100a	97.3a	85.4b	95.9b
2.50	100a	100a	100a	100a	100a	100a
5.00	100a	100a	100a	100a	100a	100a

Mean values in each column followed by the same letter are not significantly different at P=0.05 according to the Duncan's multiple range test.

In barnyardgrass, the 0.625% aqueous extract did not influence the germination and shoot length but remarkably inhibited the root length. The 1.25% concentration had moderate effect on the seed germination and seedling growth. While the 2.5% concentration extract inhibited the germination, shoot length and completely inhibited the root length. Beside the highest applied concentration (5.0%) completely inhibited the seedling growth of barnyardgrass. In rice, the 0.625% aqueous extract concentration stimulated the seedling growth but did not influence the germination. The 1.25% concentration moderately inhibited the seedling growth and did not effect the germination. Similar to the effect on barnyardgrass, the 2.50% extract concentration completely inhibited the root length, while, the highest concentration (5.0%) completely inhibited both the seed germination and seedling growth (Table 2). Thus the potent inhibitory activity of *S. platensis* extracts on seed germination and seedling growth of test plants depended on extract concentration.

Table 2. Allelopathic effects of aqueous extract of *S. platensis* on seed germination and seedling growth of barnyardgrass and rice

Concentrations (% w/v)	Barnyardgrass (% Inhibition)			Rice (% Inhibition)		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
Control	0c	0d	0d	0b	0d	0c
0.625	-2.9c	0.1d	16.5c	2.7b	-32.6e	-19.8d
1.25	24.6b	34c	44.9b	5.4b	27.9c	31.0b
2.50	95.6a	93.2b	100a	94.6a	96.2b	100a
5.00	100a	100a	100a	100a	100a	100a

Mean values in each column followed by the same letter are not significantly different at P=0.05 according to the Duncan's multiple range test.

Effects of C-phycocyanin

The aqueous extract of *S. platensis* was very toxic to seed germination and seedling growth of test plants. From the UV absorption spectrum, the purity of the aqueous extract and C-PC aqueous solution was evaluated as per the purity ratio, A_{620}/A_{280}. The fractions that exhibited a purity ratio > 4.0 were considered of high purity C-PC (29). The UV absorption spectrum of aqueous extract showed that *S. platensis* contains C-PC (620 nm peak) and the A_{620}/A_{280} ratio of the blue supernatant was 1.17 (Fig. 2), while the purity ratio of pure C-PC was 4.25. The allelopathic assay of pure C-PC did not inhibit the seed germination of test plants; however, C-PC significantly inhibited the shoot length of Chinese amaranth at 500 and 1000 ppm concentrations by 10.35% and 53.03%, respectively. While 125-1000 ppm concentrations inhibited the root length by 10.37-82.81%. In barnyardgrass, only the highest concentration (1000 ppm) inhibited the shoot and root length (Fig. 3).

The *S. platensis* aqueous extract significantly inhibited the seedling growth in all test plants. The biliprotein is major metabolites in *S. platensis* aqueous extract. (3,12,13,35,36). Biliproteins are brilliantly coloured, water-soluble proteins with linear tetrapyrole prosthetic groups (bilin) chromophores attached. In their functional state, biliproteins are covalently linked to specific cysteine residues. They also form light-harvesting antenna complexes of cyanobacteria, called phycobilisomes (PBS). These are classified in 3-groups based on UV-VIS spectroscopic properties: phycoerythrin (PE) λ_{max} 540-570 nm, phycoerythrobilin; phycocyanin (PC) λ_{max} 610-620 nm, and allophycocyanin (APC) λ_{max} 650-655. Antenna systems of *S. platensis* are composed of C-PC and allophycocyanin (APC) at an approximate 10:1 ratio and the protein fraction may contain up to 20% of PC (2,14,15,36). This supports our hypothesis that one of the major metabolites of *S. platensis* is the fluorescent, deep blue coloured protein, C-PC, and that it can exhibit allelopathic effects on test plant in our study. Jørgensen (17) reported that chlorophyllide pigments, whose structures are closely related to the chromophore of C-PC, were isolated from ether and ethanol extracts of *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, or *Scenedesmus quadricauda*. These compounds inhibited the growth of *Bacillus subtilis* after the chlorophyllides were transformed by light.

Hirata *et al*. (16) investigated the effects of violet pigment nostocine A released from *Nostoc spongiaeforme* blue-green algae. Nostocine A affected the growth of six green algae and seven cyanobacteria. It exhibited growth inhibitory activity comparable to the potent herbicidal compound paraquat (*N*,*N*′-dimethyl-4,4'-bipyridinium dichloride), and the activity was more stronger on green algae than on cyanobacteria. Moreover, nostocine A exhibited a strong inhibitory allelopathic effect on root elongation of barnyardgrass. These reports are in agreement with our results that show C-PC had a greater inhibitory effect on root growth compared to shoot growth.

Jüttner *et al*. (18) reported similar allelopathic effects of cyclic peptide isolated from *Nostoc* 31. The nostocyclamide M peptide contains thiazole and oxazole moieties and shows allelopathic activity to *Anabena* 7120 cyanobacteria. Another cyclic peptide microcystin-LR, was purified from *Microcystis aeruginosa* cyanobacterium, it proved toxic at 50 µg/ml concentration to *Nostoc muscorum* and *Anabaena* BT1 and 6 days after its addition completely inhibited their growth followed by cell lysis (32). The allelopathic

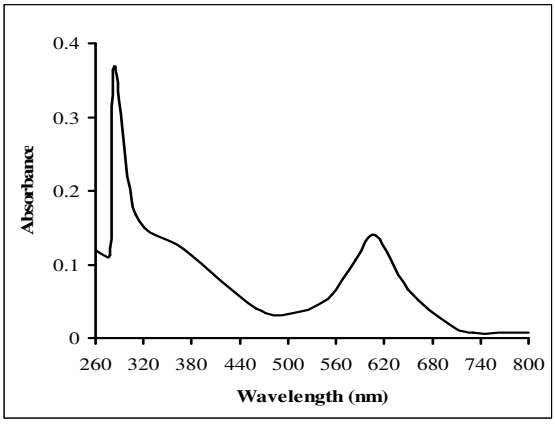

Figure 2. UV absorption spectrum of aqueous extract of *S. platensis*

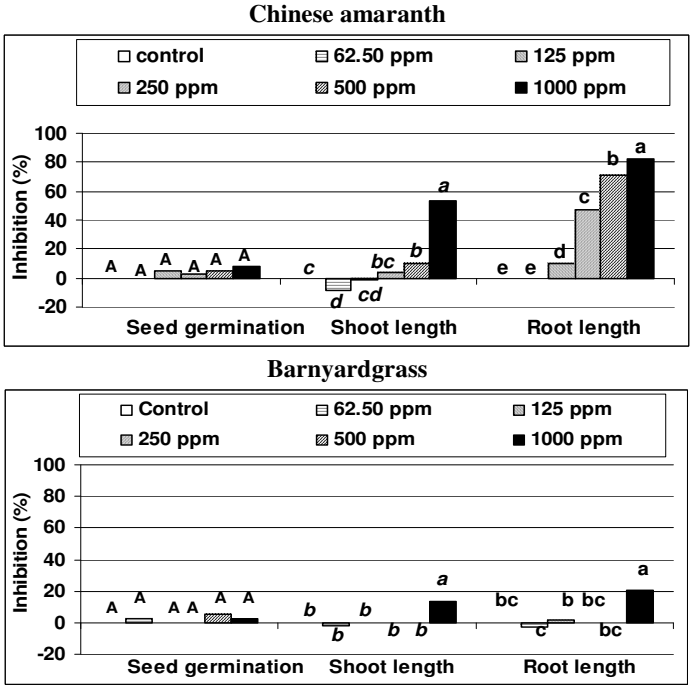

Figure 3. Allelopathic effects of C-PC on (a) Chinese amaranth (b) barnyardgrass; Values with the same letters are not significantly different at P=0.05 according to the Duncan's multiple range test.

Table 3. Allelopathic effects of crude organic extracts of *S. platensis* on seed germination and seedling growth of Chinese amaranth and Chinese mustard

Concentrations (ppm)	Chinese amaranth (% Inhibition)			Chinese mustard (% Inhibition)		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
Control	0ef	0c	0de	0d	0bc	0c
Hexane 250	0ef	-1.8cd	-10.6fg	-2.9d	-15.2e	-0.2c
Hexane 500	-1.4ef	-0.2c	-5.6ef	1.4d	-20.9f	-0.3c
Hexane 1000	0ef	1.4c	-0.4de	1.4d	-28.3g	-0.8c
Hexane 2000	2.9d-f	12.4b	0.9de	14.3c	-19.3f	-2.4c
Hexane 4000	36.2c	21.3b	10.9bc	42.9b	-0.4bc	5.5b
EtOAc 250	1.3d-f	-5.6c-e	-48.9j	1.4d	0bc	-14.1g
EtOAc 500	1.3d-f	-6.3c-e	-45.5j	0d	0.8bc	-10.3f
EtOAc 1000	5.4de	-0.8c	-18.1gh	2.9d	2.9b	-12.7g
EtOAc 2000	50b	13.9b	6.4cd	40b	-0.1bc	-12.4fg
EtOAc 4000	95.9a	76.7a	80.7a	77.1a	8.2a	11.1a
MeOH 250	-2.9f	-12.9d-f	-14.2fg	0d	1.7b	-0.7c
MeOH 500	-1.4ef	-12.8d-f	-23.8h	0d	1.3b	-4.8d
MeOH 1000	-2.9f	-16.2ef	-36.4i	1.4d	-2.9c	-7.5e
MeOH 2000	4.3d-f	-20.3f	-12.5fg	1.4d	-8.1d	0.1c
MeOH 4000	8.7d	-17.1ef	16.2b	0d	-29.8g	-0.1c

Mean values in each column followed by the same letter are not significantly different at P=0.05 according to the Duncan's multiple range test.

Table 4. Allelopathic effects of crude organic extracts of *S. platensis* on seed germination and seedling growth of barnyardgrass and rice

Concentrations (ppm)	Barnyardgrass (% Inhibition)			Rice (% Inhibition)		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
Control	0a	0a-c	0a	0a	0ab	0a
Hexane 250	1.3a	-2.3c-e	2.5a	2.6a	0.4ab	-2.6a
Hexane 500	0a	-1.6b-d	1.2a	2.6a	0.4ab	-10.8b
Hexane 1000	1.3a	0.4a-c	-0.1a	-2.6a	0.7ab	0.1a
Hexane 2000	2.6a	-8.3gh	1.4a	2.6a	-0.7ab	2.8a
Hexane 4000	2.6a	-2.9c-f	0.3a	-2.6a	2.6a	2.4a
EtOAc 250	1.3a	1.5ab	-14.7c	0a	0.05ab	-0.4a
EtOAc 500	1.3a	2.6a	-9.2b	-2.6a	0.6ab	-14.2b
EtOAc 1000	2.6a	-1.4b-d	-12.8c	0a	1.3ab	-1.9a
EtOAc 2000	-1.3a	-1.4b-d	-14.3c	2.6a	1.5ab	2.0a
EtOAc 4000	3.9a	-2.1c-e	-24.9d	2.6a	2.5a	0.7a
MeOH 250	0a	-4.2d-f	2.7a	-2.6a	-5.4c	0.4a
MeOH 500	2.6a	-5.3e-g	-13.5c	0a	-0.3ab	-1.3a
MeOH 1000	0a	-10.4h	-25.7d	-2.6a	-1.7b	0.4a
MeOH 2000	2.6a	-17.1i	-35.4e	0a	2.4a	-0.4a
MeOH 4000	2.6a	-5.9fg	-40.3f	0a	2.1a	0a

Mean values in each column followed by the same letter are not significantly different at P=0.05 according to the Duncan's multiple range test.

effects of isolated proteins, such as elicitins on plants are known. For example, one group of protein elicitors is secreted from plant pathogens of the fungal genus *Phytopthora* and *Pythium*. These compounds can cause a hypersensitive response, including leaf necrosis and cell death, and they can induce systemic acquired resistance in some plant species (19,28). These results indicate that the necrotic proteins had a toxic effect on plant hosts, similar to our results showing that the protein C-PC can inhibit the growth of those plants tested in this study.

It has been reported that in the natural environment some algal toxins are generally present in the algal cells, but are released into the surrounding water when the cell is damaged or dies (6). Likewise, *S. platensis* may cause such allelopathic phenomena when its cells are damaged or died, but may not have an effect when alive and healthy. Our results suggested that the active C-PC pigment may have toxic or allelopathic effects on neighbouring breeding organisms in nature.

Effects of crude organic extracts

The effects of crude organic extracts of *S. platensis* on Chinese amaranth and Chinese mustard, becomes apparent when applied at high concentrations (Table 3). Crude hexane and EtOAc extracts had an inhibitory effect on Chinese amaranth, while crude MeOH reduced the germination and root length but had no inhibitory effect on shoot length. In particular, crude EtOAc at 4000 ppm concentration drastically inhibited the seed germination, shoot and root length of Chinese amaranth (95.95%, 76.70% and 80.69%, respectively). In contrast, crude EtOAc at 250-1000 ppm concentrations and crude MeOH at concentrations of 250-2000 ppm stimulated the root length, while all applied concentrations of crude MeOH stimulated the shoot length of Chinese amaranth. In Chinese mustard, the crude hexane and EtOAc at 2000-4000 ppm concentrations reduced the seed germination. While crude EtOAc at 4000 ppm concentration inhibited the shoot length and crude hexane and EtOAc at the highest concentration (4000 ppm) inhibited the root length. The other applied concentrations had no inhibitory effects on Chinese mustard, but slightly stimulated the seedling growth. The results obtained for barnyardgrass and rice indicate that all the applied concentrations of crude extracts had no inhibitory effects on germination or growth of the test plants, but showed stimulatory effects. Crude MeOH, in particular, had a moderate stimulatory effects on root length of barnyardgrass (Table 4).

CONCLUSIONS

The *S. platensis* aqueous extract had the strongest inhibitory activity, but crude organic extracts had moderate effects. The blue protein pigment, C-PC, possesses allelopathic potential. The biological activity of extracts increased at higher concentrations. All extracts of *S. platensis* and pure C-PC were more inhibitory to the root length than shoot length. Both dicot species were more sensitive to the extracts than monocots. We found that *S. platensis* is a source of bioactive compounds endowed with interesting allelopathic activity. Analysis of possible allelochemicals effects in *S. platensis* should be done in further studies. Isolation and identification of allelochemicals from *S. platensis*, and synthesis of their derivatives, would aid in the development of novel bioactive herbicides.

ACKNOWLEDGEMENTS

This study was supported by Grants from The Research Foundation, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

REFERENCES

1. Babu, M., Rengaswamy, S., Nair, P. P., Cherian, V., Thara, S., Padmavathy, A. B., Sreedevi, A. N. and Madhavan, K.N. (1995). Evaluation of chemoprevention of oral cancer with *spirulina*. *Nutrition and Cancer* **24**: 197-202.
2. Bermejo, R., Talavera, E. M., Alvarez-Pez, J. M. and Orte, J. C. (1997). Chromatographic purification of biliproteins from *Spirulina platensis*: High-performance liquid chromatographic separation or their α and β subunits. *Journal of Chromatography A* **778**: 441-450.
3. Berns, D. S. and MacColl, R. (1989). Phycocyanin in physical chemical studies. *Chemical Reviews* **89**: 807-825.
4. Bin-Meferij, M.M. (2009). Histological and immunohistochemical studies for evaluation of the role of microalgae *Spirulina* sp. against cancer in experimental animals. *International Journal of Cancer Research* **5**: 36 - 43.
5. Blinkova, L. P., Gorobets, O. B. and Baturo, A. P. (2001). Biological activity of *Spirulina*. *Zhurnal Mikrobiology Epidemiology Immunobiology* **2**: 114-118. (In Russian with English abstract).
6. Bourke, A. T. C. and Hawes, R. B. (1983). Fresh water cyanobacteria (blue-green algae) and human health. *The Medicinal Journal of Australia* **1**: 491 - 492.
7. Chamorro, G., Salazar, M., Favila, L. and Bourges, H. (1996). Pharmacology and toxicology of *Spirulina* alga. *Revista de Investigación Clínica; Organo del Hospital de Enfermedades de la Nutrición* **48**: 389-399. (In Spanish with English abstract).
8. Cohen, Z. (1999). *Chemicals From Microalgae*. Taylor & Francis Publishers, London. UK. 419 pp.
9. Dayan, F. E., Romagni, J. G., Tellez, M. R., Rimando, A. M. and Duke, S. O. (1999). Managing weeds with natural products. *Pesticide Outlook* **5**: 185 - 188.
10. Dayan, F. E., Cantrell, C. L. and Duke, S. O. (2009). Natural products in crop protection. *Bioorganic and Medicinal Chemistry* **17:** 4022 - 4034.
11. Doan, N. T., Rickards, R. W., Rothschild, J. M. and Smith, G. D. (2000). Allelopathic actions of the alkaloid 12-*epi*-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix*. *Journal of Applied Phycolology* **12**: 409 - 416.
12. Edwards, M. R., MacColl, R. and Eisele, L. E. (1996). Some physical properties of an unusual C-phycocyanin isolated from a photosynthetic thermophile. *Biochimica et Biophysica Acta* **1276**: 64 - 70.
13. Edwards, M.R., Hauer, C., Stack, R. F., Eisele, L.E. and MacColl, R. (1997). Thermophilic C-phycocyanin: Effects of temperature, monomer stability, and structure. *Biochimica et Biophysica Acta* **1321**: 157 - 164.
14. Gantt, E. (1981). Phycobilisomes. *Annual Review of Plant Physiology* **32**: 327-347.
15. Glazer, A. N. (1981). Photosynthetic accessory proteins with bilin prosthetic groups. In: *The Biochemistry of Plants* (Eds., M. D. Hatch and N. K. Boadman) pp.51-96. Academic Press, New York, USA.
16. Hirata, K., Yoshitomi, S., Dwi, S., Iwabe, O., Mahakhant, A., Polchai, J. and Miyamoto, K. (2003). Bioactivities of nostocine A produced by a freshwater cyanobacterium *Nostoc spongiaeforme* TISTR 8169. *Journal of Bioscience and Bioengineering* **95**: 512-517.
17. Jørgensen, E.G. (1962). Antibiotic substances from cells and culture solutions of unicellular algae with special reference to some chlorophyll derivatives. *Physiologia Plantarum* **15**: 530-545.
18. Jüttner, F., Todorova, A.K., Walch, N. and von Philipsborn, W. (2001). Nostocyclamide M: A cyanobacterial cyclic peptide with allelopathic activity from *Nostoc* 31. *Phytochemistry* **57**: 613-619.
19. Kamoun, S., Young, M., Glascock, C. B. and Tyler, B. M. (1993). Extracellular protein elicitors from *Phytophthora*: Host-specificity and introduction of resistance to bacterial and fungal phytopathogens. *Molecular Plant-Microbe Interactions* **6**: 15-25.
20. Kohli, R.K., Batish, D. and Singh, H.P. (1998). Allelopathy and its implication in agroecosystems. *Journal of Crop Production* **1**: 169-202.

21. Kreitlow, S., Mundt, S. and Lindequist, U. (1999). Cyanobacteria-a potential source of new biological active substances. *Journal of Biotechnology* **70**: 61-63.
22. Legrand, C., Rengefors, K., Fistarol, G. O. and Granéli, E. (2003). Allelopathy in phytoplankton-biochemical, ecological and evolutionary aspects. *Phycologia* **42**: 406-419.
23. Pushparaj, B., Pelosi, E. and Jütter, F. (1999). Toxicological analysis of the marine cyanobacterium *Nodularia harveyana*. *Journal of Applied Phycology* **10**: 527-530.
24. Putnam, A.R. (1983). Allelopathic chemicals: nature's herbicides in action. *Chemical and Engineering News* **61**: 34 - 45.
25. Putnam, A.R. and Tang, C.S. (1986). *The Science of Allelopathy*. Wiley-Interscience Publishers, New York, 317 pp. USA.
26. Qureshi, M.A., Kidd, M.T. and Ali, R.A. (1995). *Spirulina platensis* extract enhances chicken macrophage functions after *in vitro* exposure. *Journal of Nutritional Immunology* **3**: 35-45.
27. Qureshi, M. A. and Ali, R.A. (1996). *Spirulina platensis* exposure enhances macrophage phagocytic function in cats. *Immunopharmacology and Immunotoxicology* **18**: 457-463.
28. Ricci, P., Bonnet, P., Huet, J. C., Sallantin, M., Beauvais-Cante, F., Bruneteau, M., Billard, V., Michel, G. and Pernollet, J.C. (1989). Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco. *European Journal of Biochemistry* **183**: 555-563.
29. Rice, E.L. (1984). *Allelopathy*. 2nd Ed. Academic Publishers, New York. USA. 424 pp.
30. Rito-Palomares, M., Nunez, L. and Amador, D. (2001). Practical application of aqueous two-phase systems for the development of a prototype process for C-phycocyanin recovery from *Spirulina maxima*. *Journal of Chemical Technology and Biotechnology* **76**: 1273-1280.
31. Schlegel, I., Doan, N. T., de Chazal, N. and Smith, G. D. (1999). Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *Journal of Applied Phycology* **10**: 471-479.
32. Singh, D.P., Tyagi, M.B., Kumar, A., Thakur, J.K. and Kumar, A. (2001). Antialgal activity of a hepatotoxin- producing cyanobacterium, *Microcystis aeruginosa*. *World Journal of Microbiology and Biotechnology* **17**: 15-22.
33. Singh, H. P., Batish, D. R. and Kohli, R. K. (2001). Allelopathy in agroecosystems: an overview. *Journal of Crop Production* **4**: 1- 41.
34. Suikkanen, S., Fistarol, G.O. and Granéli, E. (2004). Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermanii* on algal monocultures. *Journal of Experimental Marine Biology and Ecology* **308**: 85-101.
35. Szalontai, B., Gombos, Z., Csizmadia, V., Bagyinka, C. and Lutz, M. (1994). Structure and interactions of phycocyanobilin chromophores in phycocyanin and allophycocyanin from an analysis of their resonance Raman spectra. *Biochemistry* **33**: 11823-11832.
36. Vonshak, A. (1997). *Spirulina platensis* (*Arthrospira*): *Physiology, Cell Biology and Biotechnology*. Taylor & Francis Publishers, London. UK. 254 pp.
37. Zarrouk, C. (1966). *Contribution à l'étude d'une Cyanophycée. Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthèse de Spirulina maxima (Setch. et Gardner) Geitler* (PhD thesis). University of Paris, Paris, France.