EFFECT OF *SPIRULINA MAXIMA* AQUEOUS EXTRACT ON SEED GERMINATION AND SEEDLING GROWTH OF MUNG BEAN, *VIGNA RADIATA* AND RICE, *ORYZA SATIVA* VAR. JAPONICA

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ABSTRACT

The effects of blue green algae, (*Spirulina maxima*) extract concentration and extracting solvent on seed germination and seeding growth of *Vigna radiata* and *O. sativa* var. Japonica were determined from the 2013 to 2014 . *S. maxima* was extracted using three different solvents; distilled water, methanol and hexane at 0, 2.5, 3.5, 4.5 and 5 g L^{-1}. The extracts were treated at three different stages of seed development, the dry stage, the radicle emergence stage and the vegetative growth stage. Data gathered were the percentage germination, the average length of the shoots and roots at the three stages and the calculated inhibition percentage of shoot and root length. Both distilled water extract and methanol extract inhibited germination resulting in an inverse relationship between the concentrations of the solvent extracts and the germination percentage. However, the hexane extract showed no effect on seed germination. When the extracts were applied at the dry seed stage, both methanol and distilled water reduced the average length of shoots and roots of *V. radiata* compared to the control. When the extracts were applied at the radicle emergence and the vegetative growth stages, the distilled water extract reduced average shoot and root length more than the methanol extract. In contrast, for *V. radiata*, the hexane extract increased the shoot length when treated at the dry and radicle emergence stages, but inhibited the root length slightly in all stages. For *O. sativa*, both the methanol and distilled water extracts treated at the dry seed and radicle emergence stages reduced the average length of shoots and roots. In contrast, the hexane extract increased the shoot length of *O. sativa* when treated at the dry and growth stages, but these extracts inhibited the root length slightly when treated at all three stages. *S. maxima* extract has the potential as a natural herbicide. Further work needs to be done to prove that this bioefficacy can be maintained under field environmental conditions.

Key words: allelopathy, biochemicals, extract, seed germination, solvent

INTRODUCTION

Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. These biochemicals are known as allelochemicals and can have a beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. Not only plants can produce allelochemicals but also many cyanobacteria have the ability to produce these secondary products that affect biochemical processes within the cells of living organisms.

From the past to present day, human beings have widely utilized algae, which is a rich source of new bioactive compounds. For example, the extract from *Scytonema hofmanni* can inhibit growth of bacteria and the blue-green algae *Aphanocapsa Synechococus*, but it can induce Actinomyces growth (Mason and Gleason, 1981).

Moreover, it has been found that *Scytonema hofmanni* can produce cyanobacterin, a secondary metabolite, with both antibiotic and allelopathic characteristics by inhibiting algal and multicellular plant growth (Gleason and Paulson,1984). Chotsang and co-workers (n.d.) isolated C_{11} and C_{13} norisoprenoids from *Spirulina platensis* for the first time. They found that these substances were dihydroactinidiolide: (1) 4-oxo-β-ionone (2) 3-hydroxy- β -ionone (3) 3-hydroxy- 5 α, 6 α-epoxy-β-ionone (4) 3-hydroxy-5β,6β-epoxy-β-ionone, and (5) loliolide. C_{11} and C_{13} norisoprenoids were demonstrated to restrain Chinese spinach germination and growth at concentrations between 250-1,000 ppm.

The various benefits of algae led the authors to study the extract from *Spirulina maxima* in order to identify bioactive compounds with allelopathic effects on germination and growth of *Vigna radiata* and *Oryza sativa* var. Japonica (hereafter called Japanese rice) as the test plants. Results from this study can be used as basic information for further development of herbicides and pesticides, which are bioactive compounds and naturally degradable. This will reduce the use of synthetic chemicals that degrade slowly and leave some toxic chemical residues in the environment.

MATERIALS AND METHODS

Starter culture preparation

S. maxima was cultured in Zarrouk medium until the OD (optical density) value was equal to 1. A sample weighing 2 kg of *S. maxima* were harvested using a plankton net, washed with distilled water and freeze dried for 24 hours.

Algae harvesting and extraction

Freeze dried *S. maxima* was then extracted by three different solvents, from high to low polarity: distilled water, methanol and hexane, respectively. For each solvent, the concentrations used were 0, 2.5, 3.5, 4.5 and 5 g L^{-1} of the freeze dried *S. maxima*. Cell disruption was performed using a homogenizer at 2000 rpm for 5 minutes. The resulting solutions were shaken for 2 hours and then stored at 4 º C for 12 hours. The solutions were then centrifuged at 3500 rpm for 30 minutes. This process was repeated 3 times until the extract was derived. Five mL of the extract was placed in filter paper within a Petri dish and then in a desiccator for 24 hours to evaporate the solvent.

Bioassay experiment

Five mL of distilled water was added to the Petri dish with filter paper containing the extracts. *V. radiata* and Japanese rice seeds at the dry stage, the radicle emergence stage and the growth stage, 10 seeds for each. The bioassay was conducted for 7 days in a dark room at ambient temperature. The experiment was laid out following the Complete Randomised Design with 4 replications.

Sample preparation

The seeds at the radicle emergence and growth stages were placed on Petri dishes with filter paper containing distilled water under the same conditions until each treatment was applied. Data were collected by measuring the length of shoots and roots.

Statistical analysis

Calculations of inhibition and germination percentage were determined by the formulae given below and statistical analyses were done using Tukey's test.

Inhibition percentage = [1-(Mean of root or shoot length of the samples/Mean of root or shoot length of the control) x 100]

Germination percentage = 100 – inhibition percentage

RESULTS AND DISCUSSION

Dry seed stage

For *V. radiata* with the methanol extract at 5 g L^{-1} showed the lowest germination at 33% (Fig.1). Japanese rice with distilled water extract at 4.5 g L^{-1}, had a 38% reduction in germination as compared with negative control with germination of 98%. However, the hexane extract had no effect on the inhibition of seed germination.

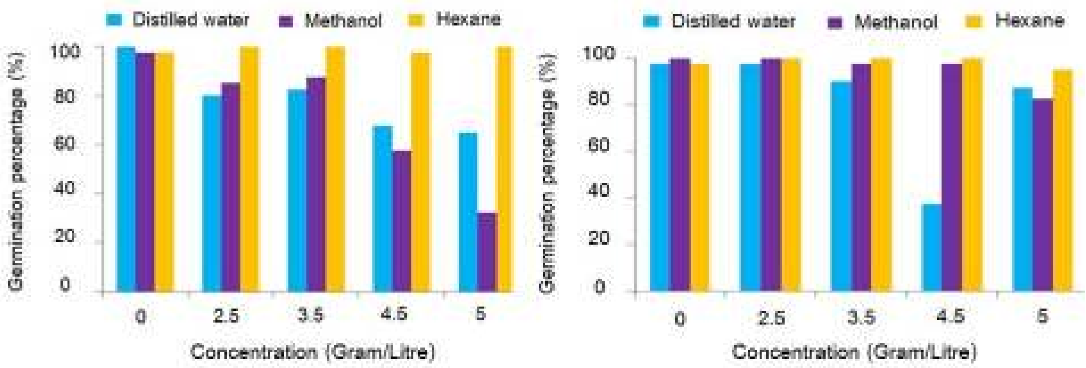
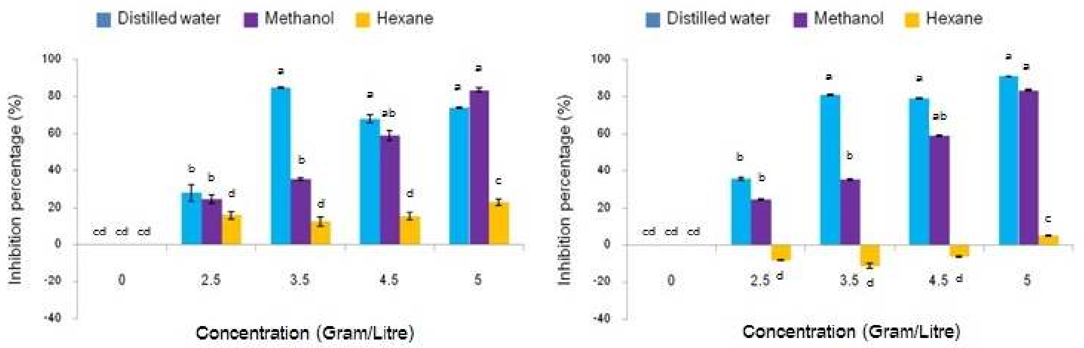


Fig. 1. Effect of the concentration of *S. maxima* and the solvent of extraction on germination percentage of the seeds treated at the dry stage in *V. radiata* (left) and *O. sativa* var. Japonica (right).

The effect of *S. maxima* extraction by distilled water and methanol on seed germination of *V. radiata* and Japanese rice showed that, as the concentration of the extract increased the percentage of seed germination reduced. This finding is in line with the Zungsontiporn (1992) study, where the extract from *Eupatorium adenophorum* Spreng inhibited seed germination of monocots and dicots, with stronger effect on germination of root than that of shoot. These results were similar to those of Charoenying et al. (2010) who reported that the high concentration of distilled water extract from *Spirulina platensis* had an inhibiting effect on seed germination of plants. Chotsang and co-workers (n.d.) reported that the C_{11} and C_{13} norisoprenoids, extracted from *Spirulina* can inhibit the seed germination and the growth of *Amaranthus tricolor* Linn. Therefore, the alkaloid compound extract from *S.maxima* may be involved in the seed germination process. However, the *S. maxima* extract with hexane had no effect on the seed germination of both plants. It can be implied that the substances inhibiting seed germination are the polar compounds, because distilled water and methanol can extract polar substances (Gross et al. 2003). The hexane extract contains non-polar compounds that do not inhibit seed germination of either plant.

For *V. radiate,* the distilled water extract at 5.0 and 3.5 g L^{-1} showed the highest inhibition effect on the length of shoot (92%) and root (85%) respectively, when these were treated at the dry seed stage (Fig. 2). The hexane extract inhibited root growth, but increased shoot length by 11% at 3.5 g L^{-1} when these were treated at the dry seed stage.

Figure 2 shows that Japanese rice applied with Spirulina crude extract by distilled water at concentration of 4.5 g L^{-1} significantly gave the best result, by inhibiting root length by 99% and shoot growth by 78% at a concentration of 5.0 g L^{-1}. For the hexane extract, it was observed that root length was inhibited by 33% at 5 g L^{-1}, but it inhibited shoot length by 19% at 2.5 g L^{-1}.



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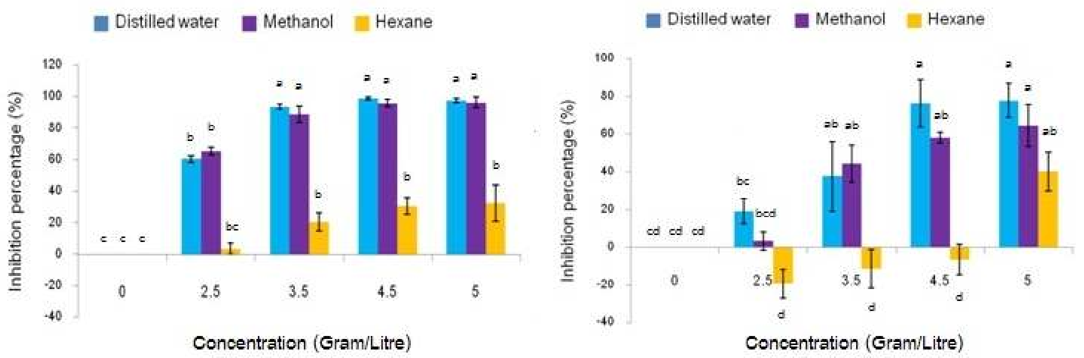

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Fig. 2. Effect of the concentration of *S. maxima* and the solvent of extraction on the inhibition percentage of the root (left) and shoot (right) grown from the seeds treated at the dry stage in *V. radiate* (A) and *O. sativa* var. Japonica (B). Values are given as means ± SD of four experiments in each group. Values not sharing a common marking (a,b,c,…) differ significantly at $P<0.05$ (Tukey's test).

The radicle emergence stage

For *V. radiata,* when the distilled water extract at 5.0 g L^{-1} was treated at the radicle emergence stage, the inhibition percentage of root and shoot growth was 98% and 93% respectively (Fig. 3). When the hexane extract was applied at this stage, root growth was inhibited slightly, whereas it increased the shoot length at 2.5 g L^{-1} (-33% inhibition).

For Japanese rice, the distilled water extract at 5 g L^{-1} inhibited the root length by 98%, and the methanol extract at 5 g L^{-1} inhibited the shoot length by 87%. The hexane extract at 3.5 g L^{-1} inhibited root growth 46% and shoot growth 51%.

The growth stage

For *V. radiata,* the distilled water extract at 5.0 g L^{-1} showed the highest inhibition percentage in root and shoot growth when treated at the growth stage (99% and 83% respectively) (Fig. 4). Hexane Spirulina crude extract has a slight effect on inhibition of the shoot and root growth. For Japanese rice, the methanol extract at 5 g L^{-1} reduced root length by 93%. The distilled water extract at 5.0 g L^{-1} also inhibited shoot length by 69%. However, the extract from Spirulina by methanol and hexane at 2.5 g L^{-1} concentration induced shoot growth by 18% and 20% respectively.

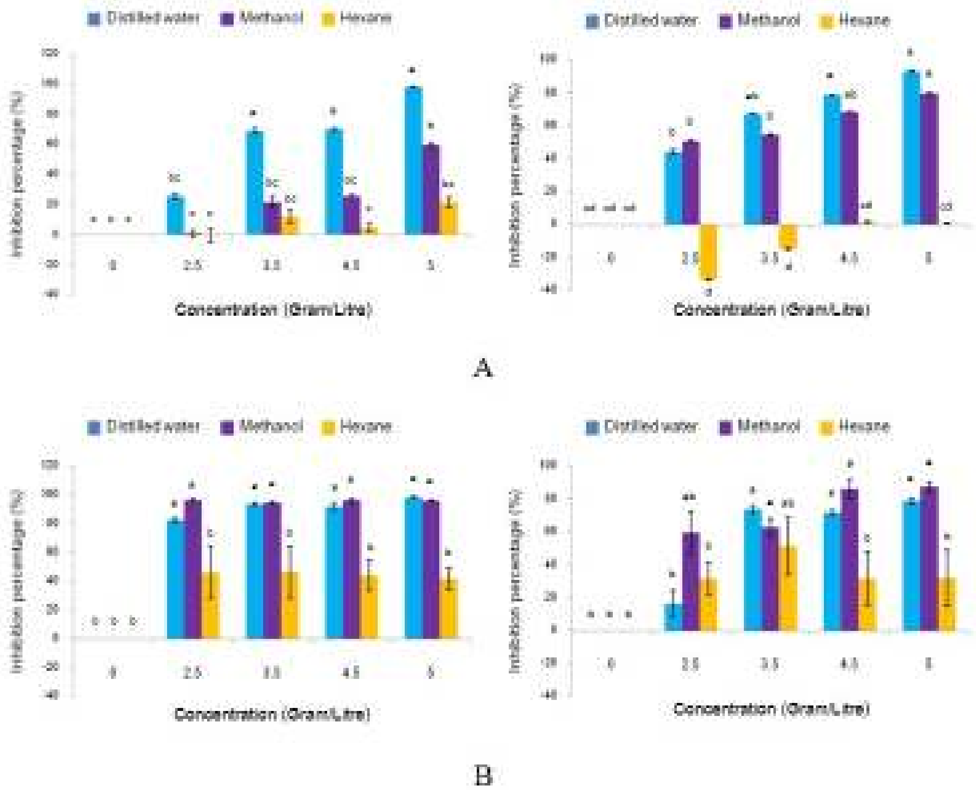

Fig. 3 Effect of the concentration of *S. maxima* and the solvent of extraction on the inhibition percentage of the root (left) and shoot (right) grown from the seeds treated at the radicle emergence stage in *V. radiata* (A) and *O. sativa* var. Japonica (B). Values are given as means ± SD of four experiments in each group. Values not sharing a common marking (a,b,c,…) differ significantly at $P<0.05$ (Tukey's test).

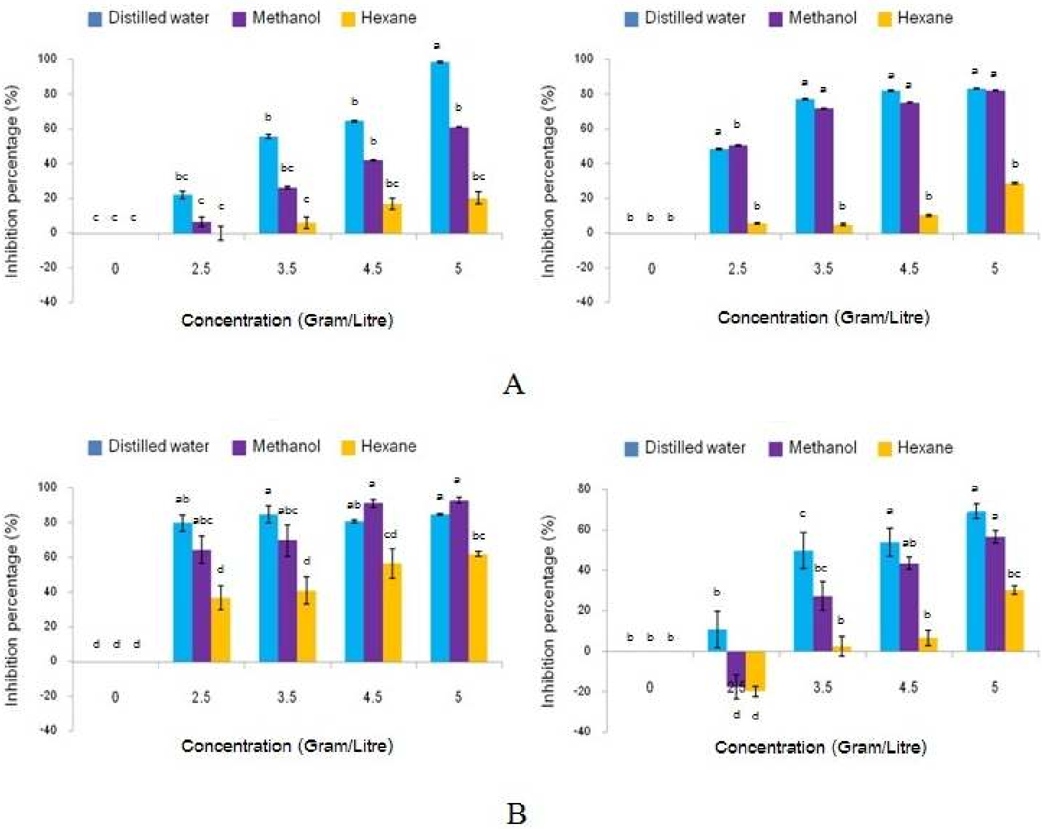

Fig. 4. Inhibition percentage during the growth stage of *Vigna radiate* (A) and *Oryza sativa* (B) on the root (left) and the shoot (right). Values are given as means ± SD of four experiments in each group. Values not sharing a common marking (a,b,c,…) differ significantly at $P<0.05$ (Tukey's test).

Based on the results, *S. maxima* extract had an effect on the growth stages of *V. radiata* and Japanese rice. The dry seed stage, the radicle emergence stage and the growth stage all gave different responses to *S. maxima* extract. This may be because during each growth stage the plant produced different hormone levels and the hormone from the *S. maxima* extract also caused different responses from *V. radiata* and Japanese rice seeds during these stages. Based on the Amin (2009) experiment, the extract from Spilurina was derived and the substances were isolated by high performance liquid chromatography (HPLC) technique, where phytohormones including indole acetic acid (IAA), gibberellic Acid (GA), benzyladenine (BA), abscisic acid (ABA), jasmonic acid (JA) and methyl jasmonic Acid (MeJA) were found in relatively high volume. The properties of these hormones control the growth and balance of plant hormones wherein the ABA can inhibit plants growth and make them resistant to stress conditions. In addition to the hormones, the substances from extract of algae such as sodium chloride, cyanide and dinitrophenol also have the capability to inhibit plant growth.

Further results showed that *V. radiata* and Japanese rice tested with *S. maxima* extract during these stages demonstrated inhibition of root and shoot length. According to Charoenying and his colleagues (2010), aqueous extract from *S. platensis* contained potential substances which can inhibit the shoot and root length of dicot plants. The C-phycocyanin from the algae can increase the inhibition of plant growth with increasing concentration. The extract caused the plant to have short roots with a dark or brown colour. This may be due to inhibitors of plant growth which restrict cell division and elongation (Rice, 1974).

Results showed that the extracts derived using different solvents had differing effects on *V. radiata* and Japanese rice growth. The distilled water and methanol extracts reduced root and shoot length of *V. radiata* and Japanese rice. Solvents with different polarities may contribute to this result as they extract different substances. Distilled water and methanol extracts are expected to contain polar substances. These substances may reduce the growth of seedling plants by inhibiting the cell division or cell enlargement in the meristematic areas of shoot and root plant and may also interfere with the rearrangement of microtubules during cell division (Singh et al. 2002). Consequently, the extract caused the plants to have shorter shoots and roots than the normal ones.

Our results corroborate with Sanevas (2000) who discovered that the blue-green algae had an inhibiting effect on the growth of weeds and seedlings. The extract using water as the solvent at a concentration of 0.1 g L^{-1} caused Jasmine rice (*Khao Dawk Mali* 105) to yield the least dry weight at 74.25 mg. The hexane extract induced root and shoot growth which may be due to the presence of . non-polar substances which can induce the growth of the plants.

Based on the results, the concentration of *S. maxima* extracts tested affected both *V. radiata* and Japanese rice growth. At a concentration of 2.5 g L^{-1}, the extract using hexane as solvent induced growth in both *V. radiata* and Japanese rice. This may be because the *S. maxima* extract helped to accumulate essential substances needed for plant growth, such as antioxidants. This is congruent with the work of Amin (2009) on the effects of *S. platensis* extract on the growth of *Sisymbrium irio* in a germ free system. This extract induced antioxidant accumulation, leading to increase in growth and cell division of callus. In addition, the extracts may affect the accumulation of the secondary metabolite of Japanese rice according to Ramachandra (1996). Phycocyanin in Spirulina caused the accumulation of secondary metabolites such as capsaicin and anthocyanin in *Capsicum frutescens* and *Daucus carota,* respectively. This finding is consistent with the work of Charoenying *et al*., (2010) on the allelopathic effects of *S. platensis* extract on germination and growth of monocots and dicots. Results showed that the hexane extract at a concentration of 500 ppm. provided the best result in inducing the root length of monocot plants.

CONCLUSION

Our research demonstrated substantial reduction of germination and growth of *V. radiata* at 5 g L^{-1} by the *S. maxima* methanol extract. The aqueous extract produced the highest reduction in germination of Japanese rice at 4.5 g L^{-1}. In addition, *S. maxima* extract caused different growth rates during the dry, radicle emergence and growth stages. Further results revealed that the inhibition percentage was positively correlated with the concentration of *S. maxima* extract.

Allelopathic effects of *S. maxima* extract on *V. radiata* and Japanese rice growth were demonstrated by the methanol and distilled water extracts which inhibited root and shoot growth, while the hexane extract tended to induce shoot length. The concentration levels tested using the three solvents had an effect on both *V. radiata* and Japanese rice growth. However, *S. maxima* hexane extract tended to induce shoot growth. The future prospects of the study aim to purify and identify the active component of the algae extract and also develop the appropriate formulation to control weeds.

The methanol and distilled water extracts of *S. maxima* contain alleochemicals that can interfere with the growth of rice and mung bean. This may be applied to the control of seed germination and seedling growth of weeds. Therefore, *S. maxima* extracts have the potential as a natural herbicide. Further work needs to be done to prove that this bioefficacy can be maintained under field environmental conditions.

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