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Allelopathic Effect of Organic Extract of Green Algal Weed *Pithophora oedogonia* (Mont.) Wittrock on Growth Promotion of *Nostoc* sp. Towards Agricultural Sustainability in Rice Field

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ABSTRACT

The present study on algal allelopathy was undertaken to determine the effect of green algal weed *Pithophora oedogonia* (Mont.) wittrock on the growth and development of a nitrogen fixing blue-green alga *Nostoc* sp. which co-exist together in nature. Fields under rice cultivation which are flooded with plenty of water serve as better site for the growth of *Nostoc* as well as of *Pithophora*, indicating mutualistic interaction between the cyanobacterium *Nostoc* and the algal weed *Pithophora*. In order to explore the possibility of enhancing the growth of the nitrogen fixer *Nostoc* vis-à-vis fertility of paddy field using the nuisance algal weed *Pithophora*, laboratory experiments were conducted in the author's laboratory. Interestingly it was noted that the methanolic extract of *Pithophora oedogonia* stimulated growth of the blue-green alga *Nostoc* linearly in BG-11 basal medium supplemented with algal extract under culture conditions as evidenced by visual appearance and the spectrophotometric analysis of chlorophyll 'a' content. The growth in *Nostoc* cultures in BG-11 growth medium enhanced with exogenous supply of algal extract. The exogenous supply of 20% *Pithophora* extract enhanced the growth of *Nostoc*, which maximized at 50% methanol extract. The promoting response may be due to the allelochemicals present in the algal weed that enhance the biochemical and enzymatic activity leading to growth and establishment of blue green alga.

Keywords Allelopathic effect, *Pithophora oedogonia*, Algal extract, *Nostoc* sp.

INTRODUCTION

In the recent era, the food production has been increasing the need of human life, but the quality of food should also be maintained is a great task among scientist due to enhance use of chemical fertilizers which showed health hazards. In this direction allelopathy has emerged an alternative approach to solve the problems of agriculture that enhanced sustainable crop yield by stabilizing the fertility of soil through application of algae. *Pithophora* grows in tropical and subtropical habitats. It occurs as a free floating and/or attached mass to the substratum in fresh water ponds and lakes. It grows vigorously and establishes as a dominant alga in its habitat and behaves as an algal weed causing nuisance. The thallus is green, filamentous, branched, non-mucilaginous, cylindri-

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cal or slightly swollen greenish cells with parietal reticulate chloroplast and appears rough as well as cottony in habit.

Allelopathy is an important applied aspect of algal biology which refers to the biological phenomenon by which an organism produces one or more biochemicals in the environment that influence the growth, survival and reproduction of other organisms in the vicinity of that organism. These biochemicals are also known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. Molish (1934) coined the term "Allelopathy"from two Greek words "Allelo" and "pathy" meaning mutual harm, through a natural phenomenonin which the inhibitory substances released by a plant inhibits the growth of other plant sharing the same habitat. But later on redefined allelopathy as both stimulatory and inhibitory effects of one plant upon another includes microorganisms (Rice 1984). Allelochemicals are infact secondary metabolites, which are not required for metabolism (i.e., growth, development and reproduction) of the organism that produces them. International Allelopathy Society in 1996 formulated allelopathy as any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of agricultural and biological systems and its mechanism of action affect the physiological process of plants such as in photosynthesis, enzyme activity and membrane transport process in various ways (Wang *et al.* 2016). Allelopathic interactions are an important factor in determining species distribution and abundance within plant communities or structuring of plant communities and are also thought to be important in the success of many invasive plants. Plants may have various complicated relations with their neighbors, including competition, inhibition, stimulation and interdependence. Some plants grow with many other species together, while others grow in a monoculture community and prevent other plants from thriving in their vicinity. In the nature, various phenomenon of allelopathy is widespread, such as decomposing residues of leaves and stems from *Helianthus tuberosus* showed phytotoxic potential and suppress the radicle growth of numbers of angiospermic plants (Franco *et al.* 2010). The recent finding also showed that microalgal growth was inhibited by marigold leaf extract of different concentrations (Wongsnansilp *et al.* 2022). Allelopathic substances isolated from different macrophytes are suggested to have growth inhibiting effects on phytoplankton. Furthermore, allelopathic potential of allelochemical present in rice varieties inhibit the growth of microbial population in aquatic environment (Sujinah *et al.* 2023).The allelochemicals may be some phenolic compounds, terpenes (Ladhari *et al.* 2020) and various other physico-chemicals of bioactive in nature (Singh and Choudhary 2010).

In the field of research involving bioactive substances of plant origin, greater interest has now arisen in algae. *Pithophora oedogonia*, a cladophoralean alga profusely grows in freshwater bodies and behaves as an algal weed causing great nuisance to aquatic biota and to the human life.

Allelopathic interactions are especially common in fully aquatic species, such as submersed macrophytes or benthic algae and cyanobacteria. The Blue Green Algae are distinguishable from other algae by their structural and functional peculiarities. These organisms are oxygen-evolving prokaryotes and are known as cyanobacteria. Generally cyanobacteria have the pigments chlorophyll 'a'carotenes and phycobillins. The absorption of light energy by cyanobacteria is based upon the occurrence of one or two forms of chlorophyll together with carotenes and phycobilins. Chlorophylls are the pivotal photosynthetic pigments. The allelopathic activity of the green alga *Pithophora*, a nuisance weed was studied on a cyanobacterium *Nostoc* (order Nostacales), which is filamentous and heterocystous in nature. *Nostoc* can be found in soil, on moist rocks, at the bottom of lakes and springs (both fresh and saltwater) and cultivated rice fields usually in the form of colonies composed of filaments of moniliform cells in a gelatinous sheath. *Nostoc* filaments bear terminally differentiated cells known as heterocyst which help in the fixation of atmospheric nitrogen and enhanced fertility to the paddy soil. Sometimes the filamentous green alga *Pithophora* is also found in the vicinity of *Nostoc* indicating allelopathic interactions. The aim of this study was to investigate the allelopathic effect of algal weed *Pithophora* extract on the growth

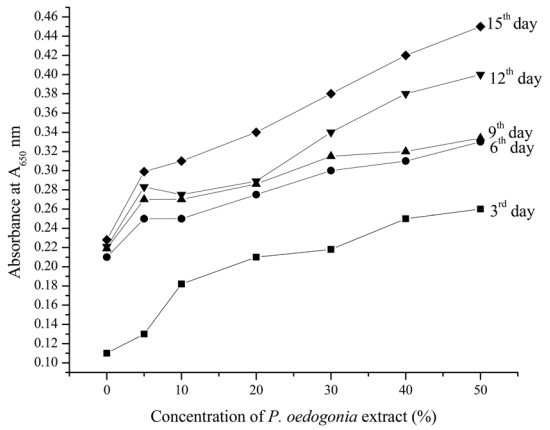

Fig. 1. Growth curve of blue-green alga *Nostoc* sp. in response to various concentrations of *Pithophora oedogonia* supplemented with BG-11 basal medium (pH 7.8).

and development of *Nostoc* to evaluate as to whether the former nuisance alga can be utilized to promote the growth of latter and thereby enhanced nitrogen fixation vis-à-vis increased rice yield.

MATERIALS AND METHODS

Nostoc strain

The blue-green alga was collected from the agriculture farm located inside the University Campus and identified as *Nostoc* with the help of available Books/ Monographs (Desikachary 1959, Gupta 2005).

Culture medium and growth conditions

The stock culture was maintained on agar slants (1% v/v) in the thermostatically controlled culture chamber maintained at 25 ± 1°C and provided with continuous illumination of 1600 lux light intensity. Culture of *Nostoc* sp. was grown in BG-11 nutrient medium and was used as the basal medium in all experiments.

Treatment and growth of *Nostoc*

Equal amounts of the actively growing cultures of blue-green alga *Nostoc* were treated over night with 5%, 10%, 20%, 30%, 40% and 50% methanolic extract of *Pithophora oedogonia* prepared in BG-11 basal medium. For control, *Nostoc* was inoculated in BG-11 medium devoid of *Pithophora* extract. Triplicates were maintained for reproducibility.

The cultures were maintained at 25 ± 1°C under fluorescent illumination of 16 k lux light intensity. The pH of the medium was adjusted to 7.8. Clumping of cells was reduced by gentle shaking of the flasks manually twice daily. The alga was subcultured for ready availability and the purity of cultures was checked microscopically regularly. Three to fifteen day old cultures of *Nostoc* in the exponential growth phase were harvested and used for the allelopathic assay.

Growth measurments

Optical density method

Equal amounts (3 ml) of *Nostoc* culture suspensions were drawn from the treated as well as control samples and absorbance was measured at 650 nm spectrophotometrically at 3 days interval till 15th day of growth**.**

Chlorophyll 'a' estimation method

Chlorophyll 'a' is the universal photosynthetic pigment present in all eukaryotic and prokaryotic algae (cyanobacteria) and is thus a reliable and commonly used proxy for the determination of algal growth. Accordingly, the assessment of growth of *Nostoc* was effected by measuring the amount of chlorophyll 'a' in treated and untreated (control) *Nostoc* with a view to evaluating the allelopathic effect of *P. oedogonia.*

3 ml of culture suspension of *Nostoc* sp. was harvested by centrifugation (5000 rpm, 10 min) and the resulting pellet was suspended in 95% methanol. After thorough mixing, the suspension was kept at 4°C for 24 hrs. Thereafter, it was centrifuged and the absorbance of cell-free methanolic extract was recorded at 663 nm against methanol as a blank using spectrophotometer and quantification of chl 'a' (µg ml^{-1}) was done (Mackinney 1941) and represented by : Chla (µg ml^{-1}) =12.7 × O.D.$_{663}$ × V / l.

Where 12.7 is molar extinction coefficient of chl 'a'. The volume of alcohol represent as V and l represent path length of spectrophotometer.

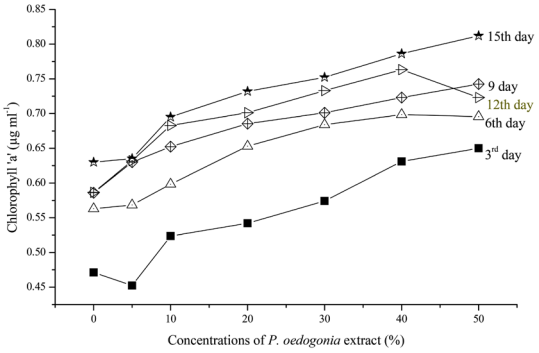

Fig. 2. Growth (in terms of chlorophyll 'a'content) of *Nostoc* sp. in response to various concentrations of *Pithophora oedogonia* supplemented with BG-11 basal medium (pH 7.8).

RESULTS AND DISCUSSION

Nutrient concentrations play a key role in regulating cyanobacterial growth and bloom formation in aquatic habitats. Besides nitrogen fixing ability, several strains of blue-green algae are known to possess promising pharmaceuticals potential. Studies investigating the response of varying concentrations of extract of nuisance algal weed *Pithophora* is therefore of fundamental importance for understanding the growth and development of *Nostoc*, a potential nitrogen fixer inhabiting Indian paddy fields.

Growth measurement of *Nostoc* was done directly through optical density absorbance of culture suspension (Fig.1), and indirectly through chlorophyll 'a' estimation (Fig. 2). It was noted that all the applied extract concentrations induced markedly enhanced growth of BGA in BG-11 basal nutrient medium maximum level being 50% *Pithophora* extract. Also, the maximum chlorophyll 'a'content was encountered with 50% concentration of *P. oedogonia* extract. An abrupt drop in chlorophyll 'a' content with 5% extract of *Pithophora* was recorded, but at concentrations above the amount of chl 'a' increased 3 days after treatment.

The growth retarding allelopathic activity of plants, has already been found in various plants but Berglund in 1969 found the two water soluble fractions from *Enteromorpha linza*, which were auto stimulatory to its growth, growth promoters thus could affect algal succession causing stimulated growth of algae to have advantage in competition. In this direction, the growth promoting effect of Leaf Aqueous Extracts of *Datura stramonium* on Leaf Chlorophyll Content, Shoot and Root Elongation of *Cenchrus ciliaris* (Elisante *et al*. 2013) and algal weed *Pithophora* on rice seedling growth (Singh and Chaudhary 2011) have already been evaluated. Surprisingly there is a aquatic blue green algae of non heterocystus species of *Oscollatoria* forms a tremendous bloom in laboratory condition by application of *Pithophora* extract (Singh 2022). It is highly desirable to identify auto stimulatory fractions as well in the fractions which stimulate the growth of other algae so the appkication of allelopathic phenomenon in crop production can be enhanced in near future.

The present work encountered growth stimulation effect of *Pithophora oedogonia* organic extract in the blue-green alga *Nostoc* sp. under laboratory culture conditions and can thus be used as a potential growth promoting allelopathic agent resulting into an increase in heterocyst frequency in the trichomes and in turn enhancement of nitrogen fixing ability of the cyanobacterium *Nostoc* vis-à-vis fertility of paddy fields.

The real interpretation of the finding of this study lies in the amazing prospect of the nuisance algal weed *Pithophora oedogonia* which grows luxuriantly as thick coarse dense mats clogging the municipal water supply, causing hindrance in the drainage system and recreation in the reservoirs, eutrophication in ponds, lakes and river. Harvesting of this trouble some green alga from the aquatic environment would not only mitigate the above problems, but could also be fruitfully utilized as potential growth promoter of cyanobacterial strains endowed with nitrogen fixing ability as biofertilizers and production of novel biomolecules of industrial potentialities including pharmaceuticals.

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