Botany Research International 4 (2): 36-40, 2011
ISSN 2221-3635

Allelopathic Potential of Algal Weed *Pithophora oedogonia* (Mont.) ittrock on the Germination and Seedling Growth of *Oryza sativa* L.

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Abstract: The allelopathic potential of a methanolic extract from the algal weed *Pithophora oedogonia* (Mont.) Wittrock was evaluated on seed germination, seedling growth, shoot length and biomass of rice (*Oryza sativa* L.). In Lab. weed extract promoted the seed germination and seedling growth in rice and the promotory effect was proportional to the concentration of weed extract (5-30% methanolic extracts) administered. The field studies also revealed substantial increase in shoot length and biomass of rice crop with the concentrations as used in lab bioassay. The allelopathy may thus be exploited as the possible mechanism to trigger the biochemical and enzymatic activity in rice seeds leading to the enhanced seed germination, root and shoot growth and the biomass of rice crop.

Key words: Allelopathic effect · *Pithophora oedogonia* · *Oryza sativa* · Seed germination · Root and radicle growth

INTRODUCTION

Algae are an important component of aquatic ecosystem. They grow in pelagic and benthic environments of hydrosphere in various forms e. g. green algae [phyla Chlorophyta and Charophyta, some taxa of which behave as algal weeds in aquatic bodies] and release chemicals (allelochemicals) into the environment affecting the neighbouring species [1-2] Allelopathy is defined as the effect (beneficial or inhibitory) of one plant (including microorganisms) on the growth of another one through the release of chemical compounds into the environment. It plays major role in plant interactions and in the structuring the plant communities; plants may have various complex relations (competition, inhibition, stimulation and interdependence) with their neighbours. Some species grow with many other species together, while others grow in monoculture community and prevent other plants from thriving in their vicinity [3-4]. Plant tissues contain several substances causing germination and/or growth inhibition or stimulation; these substances are called allelochemicals and are secondary plant metabolites. Their effects can be long lasting [5, 6]. Numerous plants are known to cause inhibitory or stimulatory effects on seed germination and growth of the neighbouring or successional plants by releasing allelopathic substances as exudates from living plant tissues or decomposition of plant residues [1, 7-10]. The growth promoting substances are present in many plants and their uses in agriculture are known [11]. Algal weeds also release phycochemicals into soil and water, which adversely affect germination, growth and yield of the crops. Algae are highly heterogeneous and represent tremendous diversity in morphological and biochemical attributes [12]. Production of novel compounds by algae requires in-depth allelopathic study. *Pithophora oedogonia*, a green filamentous algal weed, abundantly grows in rice fields, water channels and reservoirs causing nuisance in water bodies (Fig. 1). This study, thus, aimed at exploring the allelopathic potential of the nuisance algal weed *Pithophora oedogonia* extract on rice crop.

Fig. 1: A part of filament of aquatic algal weed *Pithophora oedogonia*

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MATERIAL AND METHODS

Preparation of Algal Extract: The material was collected from the aquatic bodies inside B.H.U. Campus and was identified as *Pithophora oedogonia* (Mont.) Wittrock with the help of research papers and monographs/books [13]. The algal mat was washed under running tap water to remove epiphytes and associated debris and dried at 40° C in a hot air oven for 4-5 days. The dried material was crushed with the help of a pestle and mortar and kept in 90% methanol for 7-8 days. The extract was filtered through Whatman no. 1 filter paper to remove all unextractable matter. The filtrate was concentrated under reduced pressure by using a rotator evaporator. The methanolic extract was transferred to a hot air oven and was dried to a constant weight at 45°C. A part of the residue was used for phyco-constituents analysis, while the rest was used for bioassays. Osmotic potential was measured with an Osmometer which varied from 0.05 to 0.41 MPa. at pH 7.6.

Laboratory Bioassay: The seeds were surface sterilized with 5.25% (w/v) sodium hypochlorite solution for 15 min and rinsed thrice with distilled water. Three replicates, each containing 25 seeds were prepared for each treatment and placed on two-layer filter paper in sterilized 9 cm Petri dishes in a completely randomized design. The filter papers were then moistened with equal amount of varying concentrations of methanolic extract of Pithophora *oedogonia*. Distilled water was used in the control treatment. Seeds sown in the Petri dishes were incubated in a dark room maintained at 25°C. Germination was evaluated by counting the number of germinated seeds at 24h interval over a period of 5 days and was expressed as total per cent germination. Germination was ensured to have occurred only after the radicle had protruded beyond the seed coat by at least 1 mm. The sensitivity of the rice seed test helped establish the concentration (s) of *Pithophora* extract which were potentially active to seed germination in rice. The data were recorded by counting the number of germinated seeds and also by periodic measuring of radicle length. The analysis of variance of the data was accomplished on the basis of completely Randomized Design [14](Cochrane and Cox, 1963) and the significance compared at 5% error probability level. Percentage of seed germination and elongation of the radicle were calculated as under [15]

$$\text{Relative germination ratio (\%)} = \frac{\text{Mean germination of treated seeds}}{\text{Mean germination of control seeds}} \times 100$$

$$\text{Relative elongation ratio of root} = \frac{\text{Mean root length of treated seeds}}{\text{Mean root length of control seeds}} \times 100$$

Percentage of inhibition or stimulation of germination and radicle elongation was calculated as follows:

$$\text{Inhibition (-) or stimulation (+)} = \frac{\text{Germinated seeds in extracts} - \text{Germinated seeds in control}}{\text{Germinated seeds in control}} \times 100$$

Pot Culture: In pot culture, the pots (22 cm length and 27cm diameter) were filled-in with equal amount (3 kg) of garden soil and the overnight treated seeds with different concentrations of algal extract, after thorough washing, were sown in these pots. The seeds soaked with distilled water served as control. Triplicates were maintained for reproducibility.

The pots were kept in research plot located in the Botanical Garden of the University with an average temperature of 30°C and 60-70% relative humidity and were watered as and when required to avoid water stress. The observations on shoot length and root length were recorded on 5th, 10th and 15th day, while total dry weight of shoot (70°C Oven dry) was determined on the 15th day of sowing.

Statistical Analysis: Repeated measure of one way ANOVA was used to reveal the differences in the growth and radical elongation between the algal extract treated and control rice seeds maintained in triplicate (Table 1). The data were tested for normal distribution and homogeneity of variance. Probability less than 0.05 was considered significant. The Tukey's HSD post-hoc test was used to find out differences between the means. The statistical analysis was performed using the software SPSS 12.00 for window.

RESULTS AND DISCUSSION

Lab Bioassay: Treated *Oryza sativa* L. seeds germinated in Petri dishes revealed enhancement in germinability and radical elongation. The percentage of seed germination increased with increase in the concentration of algal extract administered. The stimulation in seed germination over control was found to be significant (p <0.05), revealing maximum (41.17%) stimulation with 30% extract and the minimum (11.7%) with 5% algal extract after a period of 120 h. The data also indicated that at 20 and 25% concentrations no marked variation in the stimulation of seed germination occurred (Fig. 2).

Table 1: Effect of methanolic extract of *Pithophora oedogonia* on radicle elongation in *Oryza sativa*

	Radicle length (mm) at different time intervals (h)				
Methanol Extract (%)	24 h	48 h	72 h	96 h	120 h
Control	2.2	3.0	5.8	9.5	12.5
5	3.6(+63.63)	5.8(+93.33)	9.0(+55.17)	11.3(+18.94)	14.0(+12.0)
10	3.8(+72.72)	5.8(+93.33)	9.7(+67.24)	12.8(+34.73)	14.6(+16.8)
15	3.8(+72.72)	6.0(+100.0)	9.8(+68.96)	12.8(+34.73)	14.8(+18.4)
20	4.3(+95.45)	6.8(+126.66)	11.0(+89.65)	13.0(+36.84)	16.3(+30.4)
25	4.9(+122.72)	7.0(+133.33)	11.7(+101.7)	14.2(+44.47)	18.3(+46.4)
30	5.2(+136.36)	7.3(+143.33)	11.8(+103.44)	15.3(+57.89)	19.2(+53.6)

Numerals outside the bracket represent radicle length (mm), while those inside brackets designated by '+' show % stimulation of radicle in the treated seeds.

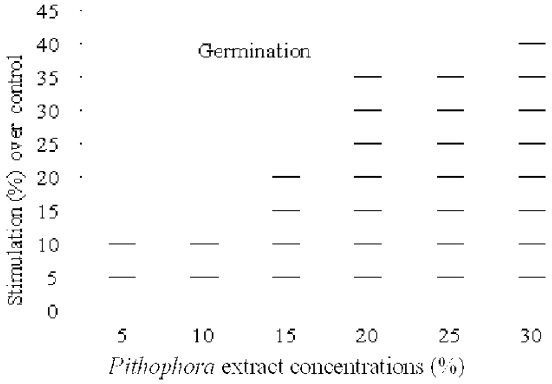

Fig. 2: Stimulatory effects of *Pithophora oedogonia extracts* on germination of rice seeds at 120 h after sowing at concentration extracts: 5%, 10%, 15%, 20%, 25% and 30% over control

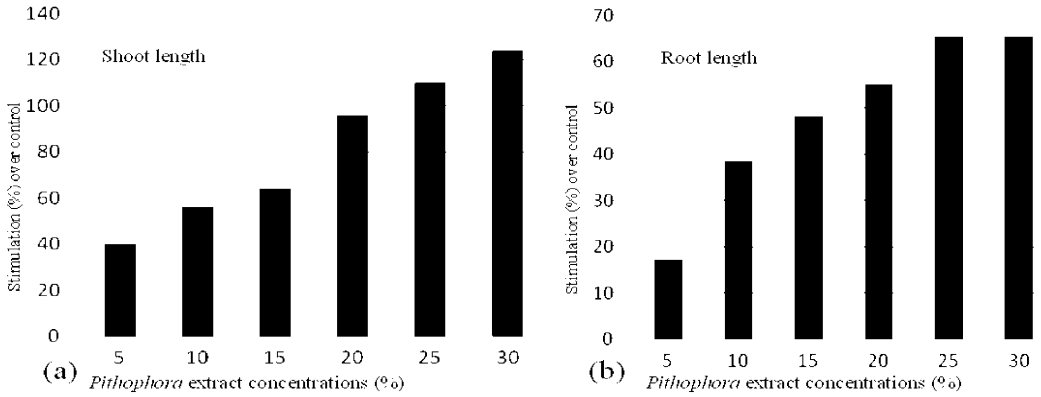

Fig. 3: Stimulatory effects of *Pithophora oedogonia extracts* on (a) shoot length and (b) root length of rice at 120 h after sowing at concentration extracts: 5%, 10%, 15%, 20%, 25% and 30% over control

Similar trend was also noticed in case of radical elongation. The extract concentration of 30% caused maximum radical elongation (143.33%) at 48 h and the minimum (53.6%) at 120 h as compared to control values (Table 1).

Pot Culture: Pot experiments were designed to contrast and verify the results obtained on seed germination and seedling establishment in laboratory bioassay. Pot experiments conducted to evaluate the response of algal weed *P. oedogonia* on seed germination were found to exhibit promotory effect in rice plant. Maximum stimulation, in contrast to control, in the shoot length (124%) and root length (65.51%) with 30% administered algal extract was noted after 120 h (Figs. 3a-b). The increment in total shoot dry weight was significant with all

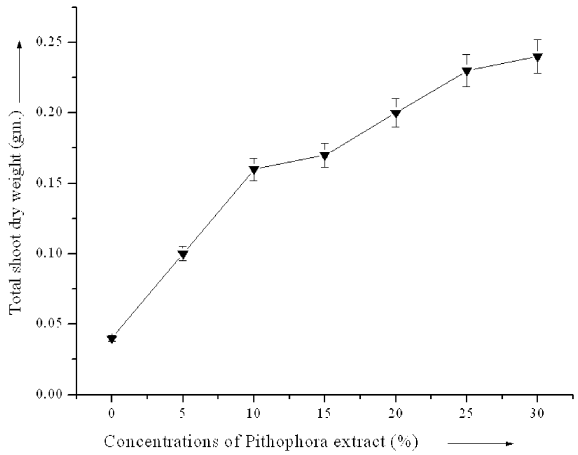

Fig. 4: Pot culture showing total shoot dry weight of rice plant on 15th day of exposure with different concentrations (%) of methanolic extract of *P. oedogonia*

the applied doses after 15 days of sowing. The increase in total shoot dry weight was considerable with 5% and 10% extract concentrations; the rate of increase, however, was slowed down with higher applied doses (Fig. 4). The concentration dependent response of *Oryza sativa* suggests that the extract of *P. oedogonia* contains growth promoting substance(s) or allelochemical(s).

The germination of seeds and radical growth in laboratory bioassay and root length, shoot length and total shoot dry weight under pot/field conditions were significantly stimulated by all the concentrations of methnolic extract indicate the presence of some growth stimulating allelochemicals in the algal weed *P. oedogonia*. Several studies accomplished in the past suggested that plant residues (of weeds) adversely affect the growth and development of crops through release of allelochemicals into the adjacent soil and aquatic environment [16-20]. *Polygonum hydropiper* L. has been reported to contains polygodial, which has piscicidal activity [21]. Recently, Batish *et al*. [22] reported that nettle-leaved goosefoot (*Chenopodium murale*) roots and their exudates exerted allelopathic effects on wheat by releasing water-soluble phenolic acids as putative allelochemicals in soil, while Gupta and Boswal [23] reported stimulatory effect of green algal extract on tube elongation and germination of pollens in *Zea mays*. The findings of the present study on methanolic extract of algal weed *P. oedogonia* stimulating seed germination and seedling growth in rice substantiates the earlier reports. These results are also in agreement with those of Shukla and Gupta [11], who reported stimulatory effect of *Pharmidium* extract on the germination of paddy seeds. Our findings based on laboratory bioassay and pot/field experiments indicate the presence of some water soluble plant growth promoting substances in *Pithophora* extract. Isolation, identification and characterization of the allelochemicals exhibiting stimulatory effect would help develop sustainable crop production. There is a further need to identify the allelochemicals produced by *Pithophora* and validate its allelopathic effect under actual field conditions to strengthen the viable poposition for its commercial exploitation.

ACKNOWLEDGEMENTS

The authors express sincere thanks to the UGC, New Delhi for providing financial assistance in the form of UGC Research Fellowship to APS and to the Head of the Department of Botany, for providing necessary facilities.

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