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A STUDY ON THE EFFECT OF SPIROGYRA SP. ON GERMINATION AND YIELD OF RICE (ORYZA SATIVA)

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ABSTRACT

Inoculation of soil with beneficial bacteria is a practice from time immemorial. Plant growth promoting algae inhabiting soil are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the near vicinity. An attempt was done to evaluate the effect of algae Spirogyra in the rice fields of Kuttanadu and Kole lands in Vembanadu wetland agroecosystem in Kerala. The extract of Spirogyra sp. inhibited the germination of rice plants and the effect was dose dependent. The germination percentage ranged from 90% in control to 43.5% in 5% extract. The germination index decreased with the increasing concentration of the extract. There was no discernable effect on the MGT and T50. However more detailed studies are needed to expose the real situation.

INTRODUCTION

Rice is one of the prominent food crops globally, and is the staple diet of nearly half of the human population of the world. The wet rice fields are described as "temporary aquatic environment" (Roger, 1996) which is influenced and maintained by farmer's activities. Paddy fields provide all necessary requirements for the growth of algae such as light, water and nutrients. Algae occur even at 20cm depth with pronounced effect on the surface soil layer (Goyal, 1996). Several studies have noted that the inoculation of farm soils with algae increases grain yields by 15-25% (Gurung and Prasad, 2005; Song et al., 2005). Kaushik (2007) reported that cyanobacteria excrete complex organic compounds that bind to the soil particle and improve the structure and permeability of soil. There are reports of chemical composition of green algae but their effect on rice plants are less investigated. Sterol and polysaccharide composition of some *Spirogyra* and *Mougeotia* species were investigated by Mitova et al. (1999) and Stefanov et al. (1996). Singh and Chaudhary (2011) have reported the allelopathic effect of alga *Pithophora oedogonia* on *Oryza sativa. Bioactivity* of the green filamentous algae Spirogyra, Chara and Cladophora on bacteria and fungi were demonstrated by Patil et al. (2011) and *Ansari* et al. (2012). The present study is an attempt to evaluate the effect of alga Spirogyra in the rice fields of Kuttanadu and *Kole* lands in *Vembanadu* wetland *agroecosystem* in *Kerala.*

MATERIALS AND METHODS

Preparation of algal extract

The algal scums of *Spirogyra sp.* were collected from the paddy fields in Kuttanadu – the rice bowl of Kerala. The samples were washed repeatedly to remove sediment and other organisms if any. These samples were air dried at 260C - 320C in the laboratory for seven days to ensure that the moisture content is <10%. The moisture content of the air dry sample was determined in terms of % weight loss when dried at 1050C. The air dried samples were stored in polythene bags in desiccator. The samples were crushed in a glass mortar, and extracted for eight hours with 90% ethanol in soxhlet apparatus. The extracts were evaporated in rotary vaccum evaporator and dried in vaccum desiccator. Five grams of the residue was dissolved in 100ml of 1% acetone.

Seed Treatment

The seeds of the *Oryza sativa* (cultivar '*Uma*') were procured from farmers and were soaked in a graded dilution series (5%, 2.5%, 1.25%, and 0.625%) of the extract of *Spirogyra sp*. Four replicates of ten seeds each were exposed to the extract for 12 hours. Control set were maintained in distilled water, and the solvent of extraction i.e. 1% acetone.

Seed Germination Test

The treated seeds including the control were sown in clean washed soil taken in petridish. Germination was evaluated by counting the number of germinated seeds at 24 hours interval over a period of seven days. The length of seedling was measured on the 7th day. The data of the treatments were corrected for the solvent control applying the Abbott's equation.

Germination percentage was calculated using the following formula:

$$\text{Germination percentage} = \left(\frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}}\right) \times 100$$

The germination index (GI) was calculated by applying the formula (AOSA, 1983):

$$GI = \frac{\text{No.of germinated seed}}{\text{Days of first count}} + - - + \frac{\text{No.of germinated seed}}{\text{Days of final count}}$$

Mean germination time (MGT) was calculated based on (Ellis and Roberts, 1981)

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

Where n is the number of seeds, which germinated on day D, and D is number of days counted from the beginning of germination. The Vigour Index was calculated using Abdul-baki and Anderson (1973).

Vigour Index = Seedling length (cm) × Germination percentageThe time to 50% germination (T_{50}) was calculated according to Coolbear *et al*. (1984) modified by Farooq *et al*. (2005).

$$T_{50} = ti + \frac{\left\{\left(\frac{N}{2}\right) - ni\right\}(ti - tj)}{ni - nj}$$

Where N is the final number of germination and ni, nj cumulative number of seeds germinated by adjacent counts at times ti and tj when ni<N/2<nj.

The data on germination percentage and vigour index were evaluated statistically by ANOVA followed by Tukey multiple comparison using the software KyPlot. The effective concentration of the extract of Spirogyra sp. that inhibited germination of seeds (EC50) was computed using the software 'R' (version 3.1.0.).

Evaluation of yield

The growth of the treated seedlings was evaluated by pot experiments. Soil was collected from the farm, washed and filled in eight pots. Healthy and even sized seedlings of seeds treated with 5% extract were planted in four pots at the rate of four seedlings per pot. The rest of the four pots were similarly planted with the seedlings of the untreated water control. The pots were exposed to sunlight and watered regularly in the field conditions. Fertilization was done as per the farmer practice by applying urea and NPK mixture at the time of tillering, and before the time of panicle initiation. Growth of the plants was monitored till the harvest of the crop.

The effect of the extract was evaluated in terms of the biomass of grain produced, and percentage of empty grains upon harvest. The grain yield was determined in terms of the weight of the air dried filled grains of moisture content <10%. The percentage of

empty grain was calculated by counting the number of empty grains, and total number of grains from the subsamples of the produce of each treatment. Percentage of empty grain was calculated by using the formula:

$$\% \text{ of Empty grains} = \left(\frac{\text{Number of Empty grains}}{\text{Total Number of grains}}\right) \times 100$$

The significance of difference in biomass and percentage of empty grains between treated and control plants were evaluated by Student's-t test.

RESULTS

The results of the germination study revealed that the extract of *Spirogyra sp.* inhibited the germination of rice plants and the effect was dose dependent. As the concentration of algal extract increased the number of seeds germinated decreased (Fig. 1). The germination percentage ranged from 90% in control to 43.5% in 5% extract. The germination index decreased with the increasing concentration of the extract. There was no discernable effect on the MGT and T50. However the ANOVA - Tukey comparison revealed that there is no significant difference in germination % of control, 0.625% and 1.25% Spirogyra extract. The analysis of vigour index revealed that the control has significantly higher value than those treated at 0.625% extract. Further significant reduction of vigour index occurs at > 2.5% (Table 1).

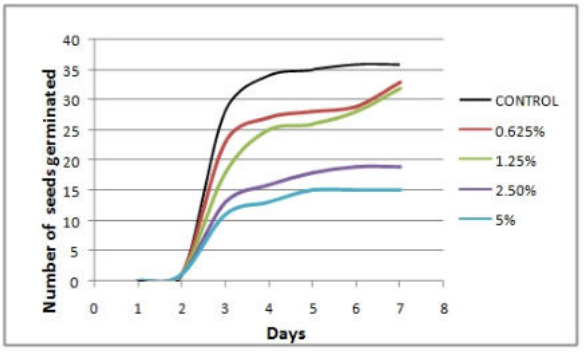

Fig.1 Effect of Spirogyra sp. extract on seed germination

Treatments	Germination %	GI	MGT (day)	VI	T50 (day)
Control	90 a	11.16	3.33	1623 a	2.64
0.625%	88 a	9.29	3.77	1459 b	2.76
1.25%	86 a	8.85	3.73	1418 b	2.94
2.50%	53.5 b	5.47	3.33	819 c	2.80
5%	43.5 b	4.57	3.00	642 d	2.77

* Figures not sharing the same letters in the same column differ significantly at P < 0.05

Table 1 Effect of Spirogyra sp. extract on germination of rice cultivar '*Uma*'

The Effective Concentration (EC50) of the extract that inhibited 50% seed germination was estimated as 3.07 % (95% confidence level 2.135 – 4.006). The seeds treated with 5% *Spirogyra* extract was grown to the seed stage and harvested along with control plants. The treatment of seeds with 5% extract of *Spirogyra sp.* did not visibly affect the growth of the plants. However the biomass of the filled grain was 38% lower in treated plants compared to control. The difference was significant (P<0.01). The proportion of empty grains in the treated plants was 26% higher than the control with a significance level P <0.01(Table 2).

S.I No.	Biomass of grain/pot (g)		% of empty grain	
	Control	Treated	Control	Treated
1	19.1	10.6	29	38
2	18.5	10.5	33	38
3	19.5	13.1	33	41
4	19.6	13.2	32	43
Mean	19.18	11.85	31.75	40
Variance	0.249	2.257	3.583	6.00
P value	0.0008*	0.0018*		

Table 2. Effect of 5% extract of Spirogyra sp. on grain production (Pot experiment) and results of student's t-test

Fig.2. Pot experiment to study the effect of Spirogyra extract on rice yield

Discussion

The present experiment clearly showed that Spirogyra sp. can negatively affect the seed germination, seedling vigour and the yield of rice. Yousif et al. (2015) reported that the alcoholic extracts of Spirogyra sp. contain terpenoid, flavonoids, phenols, saponins and alkaloids. They have observed antimicrobial and antifungal activity for this extract. Water extracts of Spirogyra jugalis has stimulatory effect on seed germination, root and shoot development in tomato (Mahadik and Jadhav, 2015). According to Brahmbhatt and Kalasuriya (2015) Spirogyra species can promote growth of Medicago sativa better than Oscillatoria species but need more study for formulation as biofertiliser.

The results of the germination study revealed that the extract of Spirogyra sp. inhibited seed germination and reduced seedling vigour. As the concentration of algal extract increased the number of germinated seeds decreased. The vigour index also decreased significantly even at 0.625% of extract. The growth of the treated seedlings was evaluated by pot experiments. The seeds treated with 5% extract of Spirogyra was grown to the seed stage and harvested along with control plants. The treatment of seeds with 5% extract of Spirogyra sp. did not visibly affect the growth of the plants. The yield of grains was significantly reduced in treated plants. The biomass of the filled grain was 38% lower in treated plants, and the proportion of empty grains was higher by 26% than the control plants. The results clearly showed that Spirogyra sp. can negatively affect the seed germination, seedling vigour, and the yield of rice. So it may be assumed that the Spirogyra bloom observed in this study contains water soluble metabolites which may negatively affect the rice plants. There are earlier reports that certain species of Spirogyra can stimulate seed germination. It may be assumed that effect of the metabolites of

Spirogyra may vary with the species.

Probably the effect of the metabolites of Spirogyra varies with the species. Some of the metabolites reported in the alcoholic extracts of Spirogyra sp. (Shatha et al., 2015) have allelopathic property. So it may be assumed that the Spirogyra bloom observed in this study contains water soluble metabolites which may negatively affect the rice plants. There is a further need to identify the bioactive compounds involved and their impact on rice crop.

CONCLUSION

The benefit of cyanobacterial biofertilizers is promising due to its capacity to secrete bioactive substances like auxin, gibberellins, cytokinins, vitamins, polypeptide,amino acid, which promotes plant growth and development. Better growth and germination of seeds of many crop plants after treating them with algal cultures or their extracts are noted.

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