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Allelopathic effect of algal leachates on seed germination and seedling growth of Paddy (*In vitro*)

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Abstract

This experiment was carried out to understand the allelopathic response of tested BGA leachates on the germination and growth parameters of Paddy (var. PR 114) under lab conditions. In present investigation the main aim of *In vitro* study was to understand the allelopathic role of tested BGA strains as individual as well as in combination form on the growth parameters of Rice seedlings. Different concentrations of BGA leachate affected the growth of Rice seedlings at different rate during the observed time of periods (6^{th} DAS, 12^{th} DAS and 18^{th} DAS).

Introduction

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 (Stainer *et al.* 1979). Roger (1982), well studied the Rice and BGA relationship. He stated that besides increasing nitrogen fertility, BGA have benefitted to Rice plants by the production of growth-promoting substances. Francis (1878), first time reported about toxicity of cyanobacterial metabolites with regard to their effects on human and environmental health. He also stated about death of livestock in South Australia, after consumption of Cyanobacteria-contaminated drinking water from Lake Alexandria.

Kannaiyan *et al*. (1992), stated that Cyanobacterial metabolites encompass a wide range of chemical classes, particularly including a diversity of nitrogen rich alkaloids and peptides which have been suggested to both pose threats to human and environmental health worldwide, and equally hold considerable potential for development of pharmaceuticals and other biomedical applications. Sivonen *et al.* (1986), stated that the compounds produced by blue green algae specially *Aphanezomenson flosaquae*, *Nodularia squmigena*, *Microcystis* species and *Oscillatoria* species have been reported to be toxic, either to other plants or to their own population.

A limited literature was published on the effect of Cyanobacteria toxins on plants (Abe *et al.* 1996, Kurki-Helasmo and Meriluoto 1998, Codd *et al.* 1999, Saker *et al*. 2000, McElhiney *et al.* 2001). On the other hand, the occurrence of biologically active substances which promote the growth of algae and other plant organisms had been reported by many investigators (Gupta and Gupta 1970, Abdel-Wahab and Kobbia 1976, Jeannin *et al.* 1991, Cho *et al.* 1999, Hong *et al*. 1997, Shanab 2001). Recently the crude extracts of five freshwater Cyanobacteria were found to have auxin like activity on potato tissue cultures (Shanab *et al.* 2003). Besides increasing N_2 fertility, BGA have been assumed to benefit higher plants by producing growth promoting substances. This hypothesis is based on the additive affects of BGA inoculation in the presence of nitrogenous fertilizers. Host of these results have been obtained with Rice but similar results were observed also with vegetables such as radishes and tomatoes (Roger and Reynaud 1979).

This experiment is much important because very few studies have been done to understand the allelopathic response of algal leachates on the growth parameters (*In- vitro*) rather than their effect on productivity under field conditions especially with genera i.e., *Nostoc*, *Anabaena* and *Aulosira*, which are well known in respect to algalization in Rice agro-ecosystems.

Materials and Methods

Pure cultures of selected BGA genera were procured from National Facility for Utilization and Conservation of Blue Green Algae, IARI, New Delhi. The procured selected strains were again maintained in liquid BG-11 medium by following the described method to conduct further *In-vivo* and *in -vitro* studies. Procured cultures were incubated in a BOD growth chamber maintained at 25 ^{o}C and 2000-3000 lux light.

This study was based on laboratory bioassays which consisted five treatments i.e. leachates of *Nostoc* sp. (Treatment A), *Anabaena variabilis* (Treatment B), *Aulosira fertilissima* (Treatment C), Combination of these three strains (Treatment D) and Water Control (WC). These treatments were applied on seeds of *Oryza sativa* L. (var. PR 114) to test their allelopathic effect on seed germination and seedling growth of paddy. In nature, allelochemicals are released in to the environment in water soluble form, i.e., leachate, root exudates, volatiles and decomposition products from plant residues (Narwal and Tauro 1996). Therefore, distilled water was used as solvent for preparation of algal leachate by following the method of Hamdi *et al.* (2001).

The abbreviations used for denoting the leachates of selected BGA strains were as follows:

Treatments	leachate of BGA
A	*Nostoc* sp.
B	*Anabaena variabilis*
C	*Aulosira fertilissima*
D	Combination (A+B+C)
WC	Water control

Total three parameters were taken for recording the observations (i.e. germination (%), root length (cm), shoot length (cm) at regular intervals of 6th, 12th, 18th days after sowing (DAS).

Results and Discussion

By peruse of results (Figure 1, Table 1) it is clear that lower concentrations (25-50%) of all treatments, acted as stimulatory, on the other hand the higher concentrations (50-100%) of the same treatments were found to play its role as inhibitory on the growth parameters of Rice seedlings. From results it has been found that there is inverse relationship between concentration and observed growth parameters (seed germination, root length and shoot length), it means increase in concentration resulted as decrease in growth parameters. The inhibitory effect of algal leachate on the growth of Rice seedlings was progressively increased with increase of concentration. Data presented in Figure 1, shows that presoaked seeds of Rice in the low concentrations (25-50%) of *Nostoc* sp. leachate (treatment A) enhanced the germination percentage (maximum 77.33 %) which was followed by treatment D (68.67 %), treatment B (59.33%) and treatment C (55%) respectively as compared to the seeds presoaked in water control (64.67%).

Figure 1: Effect of different concentrations of algal leachates on germination of paddy grains.6th Day after sowing (DAS)

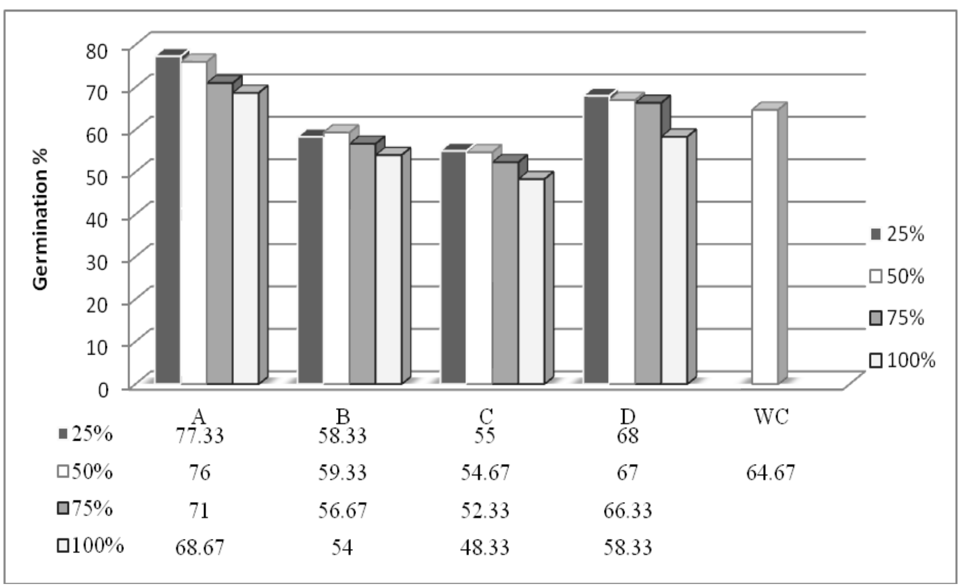

Table 1: Effect of different concentrations of treatments on the shoot and root length of paddy under lab conditions (mean ± SD)#

T	Con. (%)	Shoot length (cm)			Root length (cm)		
		6 DAS	12 DAS	18 DAS	6 DAS	12 DAS	18 DAS
A	25	2.54 ± 0.38	3.42 ± 0.26	4.37 ± 0.15	3.05 ± 0.03	3.54 ± 0.14	4.1 ± 0.09
	50	2.38 ± 1.94	3.66 ± 0.15	4.31 ± 0.1	3.26 ± 0.17	3.44 ± 0.17	3.91 ± 0.25
	75	1.94 ± 0.13	2.42 ± 0.04	2.28 ± 0.12	2.89 ± 0.17	3.24 ± 0.12	3.86 ± 0.37
	100	1.84 ± 0.07	2.11 ± 0.32	2.18 ± 0.45	2.61 ± 0.63	3.14 ± 0.63	3.16 ± 0.59
B	25	2.71 ± 0.19	3.51 ± 0.05	4.25 ± 0.36	2.41 ± 0.17	2.96 ± 0.14	3.34 ± 0.02
	50	2.59 ± 0.11	3.56 ± 0.14	4.09 ± 0.25	2.7 ± 0.29	3.22 ± 0.28	3.53 ± 0.17
	75	2.26 ± 0.19	2.59 ± 0.13	2.88 ± 0.13	2.04 ± 0.22	3.04 ± 0.12	3.23 ± 0.24
	100	2.22 ± 0.17	2.29 ± 0.14	2.64 ± 0.1	1.82 ± 0.11	1.82 ± 0.11	2.53 ± 0.32
C	25	3.51 ± 0.45	4.5 ± 0.15	5.48 ± 0.27	1.95 ± 0.68	2.4 ± 0.24	2.88 ± 0.07
	50	3.84 ± 0.03	4.74 ± 0.08	5.56 ± 0.16	1.54 ± 0.21	2.46 ± 0.18	2.81 ± 0.23
	75	3.72 ± 0.08	4.4 ± 0.03	5.22 ± 0.18	1.33 ± 0.15	1.64 ± 0.16	1.94 ± 0.12
	100	3.22 ± 0.2	24.34 ± 0.22	4.89 ± 0.16	1.18 ± 0.27	1.59 ± 0.29	1.75 ± 0.26
D	25	2.71 ± 0.33	3.07 ± 0.39	4.54 ± 0.19	2.37 ± 0.17	2.39 ± 0.12	3.18 ± 0.11
	50	2.56 ± 0.24	3.46 ± 0.09	4.22 ± 0.22	2.45 ± 0.15	2.45 ± 0.17	3.48 ± 0.25
	75	2.41 ± 0.26	2.64 ± 0.38	3.3 ± 0.11	2.25 ± 0.11	2.8 ± 0.11	2.71 ± 0.22
	100	2.33 ± 0.18	2.45 ± 0.16	3.13 ± 0.2	2.03 ± 0.14	2.45 ± 0.11	2.59 ± 0.06
Water Control		3.02 ± 0.18	3.29 ± 0.33	4.56 ± 0.7	2.48 ± 0.33	2.85 ± 0.25	2.98 ± 0.25
ANOVA (F value) df= 4, 12		42.12**	16.11**	11.66**	28.16**	7.26**	11.43**

T = Treatments; DAS = Days after sowing; Con. = Concentrations; **Significant at 5% and 1% level; # mean of three replicates

More direct evidence for hormonal effects has come primarily from treatments of Rice seedlings with algal cultures or their extracts. Presoaking of Rice seeds in BGA cultures or extracts has decreased losses from sulfate-reducing processes and this has been attributed to the enhancement of germination and a faster seedling growth due to algal exudates (Jaw and Roger 1977). At the end of 18^{th} DAS, maximum shoot length (5.56 cm) was observed in the seedlings of Rice, treated with treatment C (*Aulosira fertilissima*) at lower concentrations (25-50%) of leachate as compared to control (4.56 cm) and other treatments (Table 1). Presoaking of Rice seedlings in extracts of *Phormidium* (a non N_2 fixing BGA) has been shown to accelerate germination (Gupta and Lata 1964), promote the growth of roots and shoots (Gupta and Shukla 1969) and stimulate vegetative growth of the plants (Goryunovsa and Orleanski 1967, Gupta and Shukla 1969, Shukla and Gupta 1967).

Lower (25-50%) to higher (50-100%) concentrations of BGA leachate was found to act as positive to negative allelopathic on the root length of Rice seedlings. Maximum root length was observed in the seedlings treated with treatment A (*Nostoc* sp.) at lower concentrations (Table 1). Gupta and Shukla (1967), also observed that presoaking of Rice seedlings in BGA extracts promote the growth of root. Mishra and Kaushik (1989), observed similar positive indication for presence of auxin like substances in *Nostoc* sp., which may act as root promoting substances. The growth pattern of Rice seedlings treated with algal filtrate from *Aulosira fertilissima* resembled seedlings treated with gibberellic acid (Singh 1939). On the other hand, extracts of *Cylindrospermum muscicola* that have given a positive effect on root growth of Rice seedlings had an action similar to that produced by vitamin B12, which was found to be present in the algal cells (Venkataraman 1968, Venkataraman and Neelakanthan 1967).

It has also been established, that algal growth-promoting substances are beneficial to other crops besides Rice and that the production of such substances is not confined to BGA. Whether these substances are hormones, vitamins, amino acids or any other components is still unknown (Roger 1985). So more time and efforts are still due to understand the actual role these substances.

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