



Effect of Cyanobacteria and Filamentous Green Algae on Growth of Rice and Maize

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ABSTRACT

In this study the effect of Cyanobacteria (Nostoc commune, Cyndrospermum muscicola, Oscillatoria curviceps) and green algae (Spirogyra sp and Microspora floccosa) on growth and yield of rice (MEGHA 1) and maize (Local Yellow variety) were evaluated. Plant parameters such as shoot height, number of tillers and number of panicle, root length, grain yield and dry weight were recorded for rice while for maize, height of the plant, length, dry weight and number of leaves were recorded. On comparing the germination percentage of seeds in Cyanobacteria and algal extract, it was observed that Spirogyra sp. inhibited the germination of rice seeds. However for maize seeds, all treatments increased the germination percentage. Mixed application of green algae and Cyanobacteria (T9) lead to an increase in growth independent of chemical fertilizers and gave the same results as that with full dose of NPK (T2) for both rice and maize.

Keywords: Cyanobacteria, green algae, germination, plant parameters

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INTRODUCTION

Rice and maize are the two important crops in the hilly state of Meghalaya. However a wide gap is available between plant nutrients removal by crops and nutrients replenishment in this region. On top of that, the soils of northeastern regions are acidic and greatly deficient in phosphorus, thus importance of fertilizers with enhancement ratio of N, P and K are done frequently. Such enhancement of single element has led to accelerated exhaustion of other major and minor nutrients, therefore use of microbial consortia or biofertilizer that can promote the growth of crop are deploys during the recent years. In recent years, emphasized of crop production using biofertilizers as an alternative to chemical fertilizers have been carried out.

De (1939) was the first to recognize the importance of Cyanobacteria as a great agricultural potential and its contribution to the process of biological nitrogen fixation. Such ability of Cyanobacteria to fix the atmospheric nitrogen is of economic importance [12]. Considering the global energy crisis, supplementary sources of nitrogen can be achieved through the use of biofertilizers such as those of Cyanobacteria and *Azolla* [3]. Increased growth of plants by cultivation of Cyanobacteria and green algae with rice, barley, oats, tomato, radish, cotton, sugarcane, maize, chilli, mustard, willow as biofertilizer have been observed by many researchers [5, 8, 10, 11, 15].

In spite of recent research and development on use of Cyanobacteria, information about the influence of single species on development of plant species are still limited and such studies on green algae are rare. Our research was performed to assess the ability of some selected Cyanobacteria and green algae as inoculant for pot experiment in order to study the growth and yield of rice and maize.

MATERIAL AND METHODS

Germination

The algal scum collected/cultured were washed thoroughly and air dried for 15 days in lab conditions and then grounded to fine powder. Algal powder was soaked in distilled water for 24 hours in the dark. The mixture was filtered and stored at 4°C in the dark. Three replicates of ten seeds were soaked in the extract for 24 hours. Controlled sets were maintained in distilled water. The treated seeds were sowed in sterilised soil in pots.

Germination percentage was calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{No. of seeds sown}}{\text{No. of seeds germinated}} \times 100$$

Experimental design and treatments

The pot culture experiment was set up to study the effect of different Cyanobacteria and green filamentous algae on yield of crop plants. For the experimental set up, two crops were selected viz., rice (Megha 1) and maize (local yellow variety) with varying proportions of Cyanobacteria and algae in net house of Botany department, NEHU. Soil was collected from rice field adjacent to NEHU campus and was used as medium for raising the crops. Soil was autoclaved for sterilisation to minimize contamination and then poured in plastic pots of 12" diameter. Cyanobacteria strains selected for the experiment were *Nostoc commune*, *Cylindrospermum muscicola*, *Oscillatoria curviceps* and two green filamentous algae *Spirogyra* sp and *Microspora floccosa*.

The experiment was set up in three replicates and according to the use of single, two and three strain combination of algae; full dose of NPK fertilisers and control treatments (Table 1). Fertilizers added was according to the recommended rate which was 80:60:40 NPK kg ha⁻¹ for rice crop and 80:40:40 NPK kg ha⁻¹ for wheat. The rice and maize seeds were soaked in treatments with inoculums in separate containers except for the treatments with full dose NPK.

Three seeds were sown in each pots and irrigation was given after sowing. Suspension of selected species of Cyanobacteria and algae was applied 15 days after sowing. Soil cores were removed near the root region and culture suspension were poured into the pit and covered with soil. The application of second inoculum was given 30 days after sowing and the final application was given after 120 days.

Plant parameters

At the time of plant growth for rice, shoot height, number of tillers and number of panicle were recorded. Plant height was measured from the base of the plant to the tip of the primary. At the time of harvest, all the characters above were measured including the root length, grain yield and dry weight of the plant was recorded.

At the same time for maize, height of the plant from the base of the plant to the highest point of the arch of the uppermost leaf whose tip is pointing downwards was recorded. During harvest, the above characters along with root length, dry weight, number of cobs and leaves were recorded.

Table 1: Different treatments and their composition

Sl. no.	Treatments	Composition
1	T1	uninoculated bare soil
2	T2	uninoculated, full dose NPK
3	T3	<i>Spirogyra</i> sp
4	T4	<i>Microspora tumidola</i>
5	T5	T3 + T4
6	T6	<i>Oscillatoria curviceps</i>
7	T7	<i>Nostoc commune</i>
8	T8	<i>Cylindrospermum muscicola</i>
9	T9	T6 + T7 + T8
10	T10	mixed algae & 1/4NPK

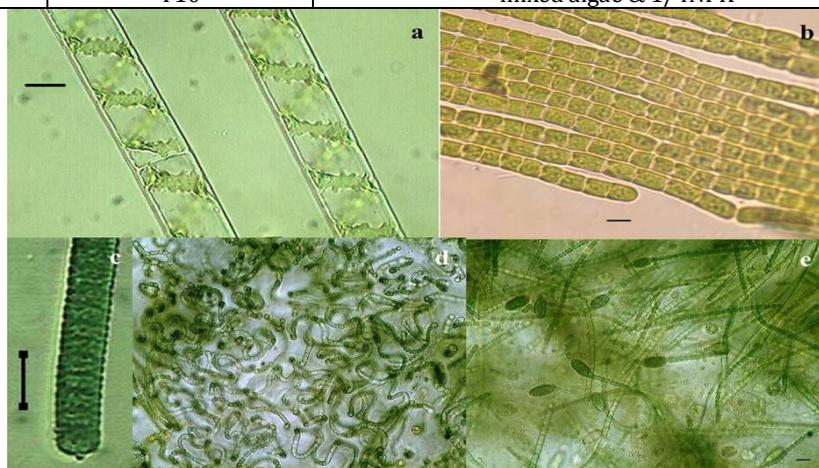


Figure 1: Green algae - a. *Spirogyra* sp, b. *Microspora tumidola*, Cyanobacteria - c. *Oscillatoria curviceps*, d. *Nostoc commune*, e. *Cylindrospermum mucicola*.

Statistical analysis

Data were subjected to ANOVA in accordance with experimental design and values were calculated at P level of 0.05%. Significance of variation was determined between T2 and the rest of treatments in order to observe the efficiency of use of Cyanobacteria and algae.

RESULTS

Germination

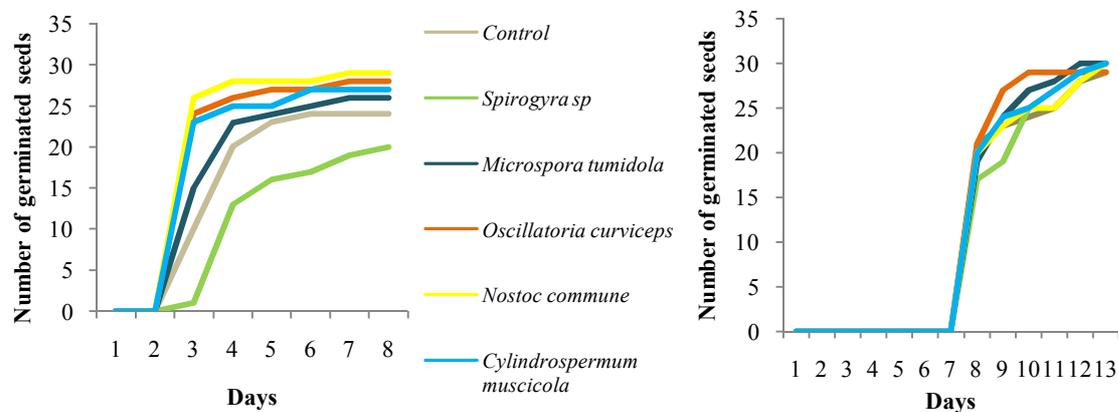


Figure 1: Effect of Cyanobacteria and algal extract on (a) rice and (b) maize seeds.

The result of germination study revealed that *Spirogyra sp.* inhibited the germination of rice seeds. The number of seeds germinated was even lower than control. Seed germination in *Nostoc commune* was highest followed by *Oscillatoria curviceps*, *Cylindrospermum muscicola* (Figure 1). On comparing rice and maize seeds, significant difference was observed in the germination percentage between them.

For rice seeds, germination percentage ranged from 65.21% in *Spirogyra sp* to 95.05% in *Nostoc commune*. We also observed that pre-soaking of seeds in Cyanobacteria culture enhanced the germination of rice seeds. Significant difference was observed between *Spirogyra sp* and Cyanobacterial inoculum. However for maize seeds, all treatments increased the germination percentage which ranged from 96.67% in control and *Spirogyra* to 100% in the rest of the Cyanobacteria and algal inoculum used in treatments. No significant difference was observed between the treatments (Figure 2).

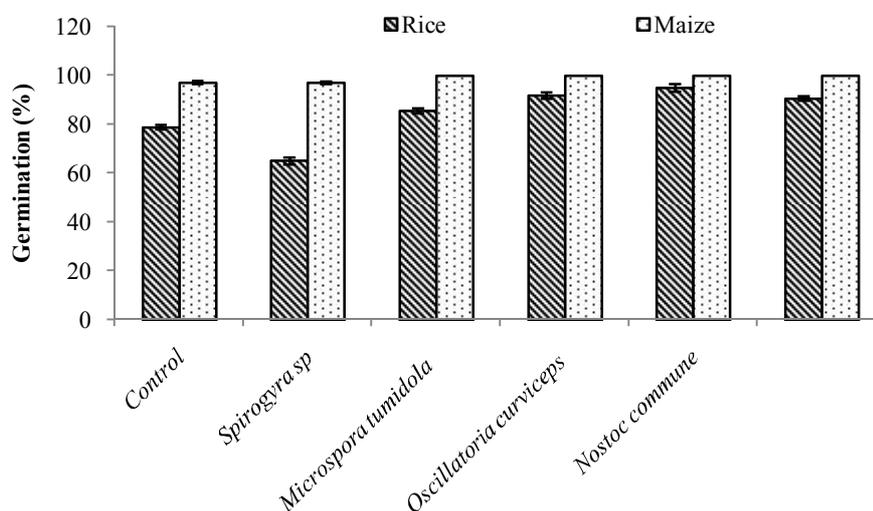


Figure 2: Germination percentage of rice and maize seeds.

Co-cultivation of Cyanobacteria and algae with rice

Shoot length of the rice plant from the base to the tip of the longest leaf of the plant was measured after 15, 30 and 120 days of plant growth. After 15 days, maximum shoot length was observed in T7 (23.25 cm) and minimum in T3 (12.85 cm) respectively. On the contrary, maximum shoot length after the 30 days was noticed in T10 (32.71 cm) and minimum in T3 (21.74 cm). The maximum plant height for 90

and was observed in T9 (102.10 cm) and minimum in T1 (64.93 cm). After 120 days, maximum shoot length was observed in T7 (126.79 cm) and minimum was observed in T1 (90.15 cm) (Figure 3).

At the time of harvest, total plant height (shoot and root length) was measured. The maximum shoot length and root length was observed in T7 with 131.33 cm and 31.66 cm respectively. The minimum shoot length and root length was observed in T1 with 92.33 cm and 19.66 cm respectively. At the time of harvest, total plant height (shoot and root length) was measured. The maximum shoot length and root length was observed in T7 with 131.33 cm and 31.66 cm respectively. The minimum shoot length and root length was observed in T1 with 92.33 cm and 19.66 cm respectively (Figure 4).

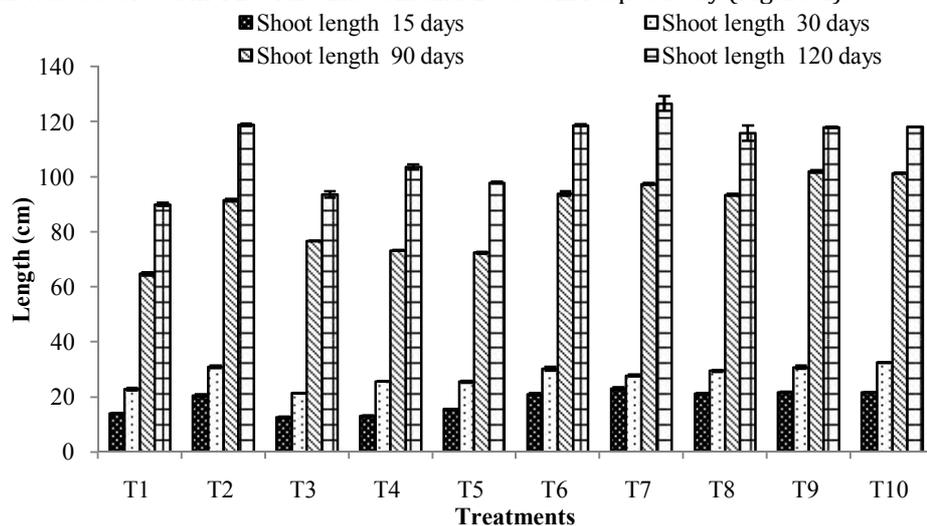


Figure 3: Shoot length after 15, 30, 90 and 120 days for rice

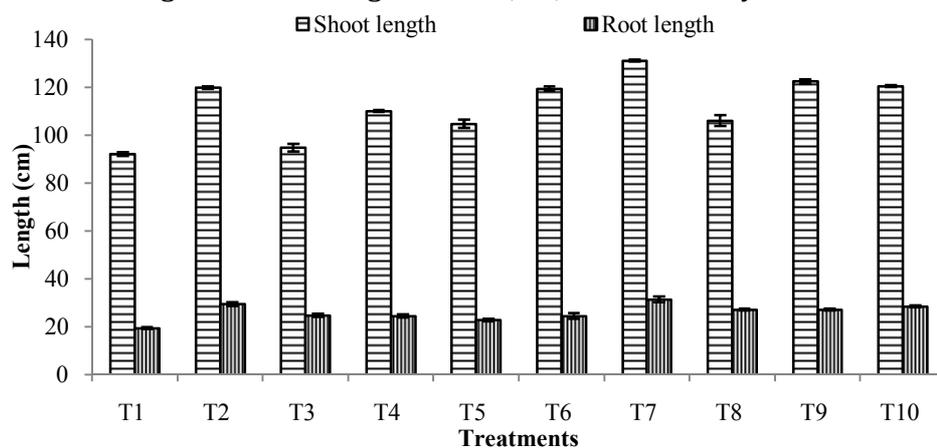


Figure 4: Shoot length and root length of rice at the time of harvest.

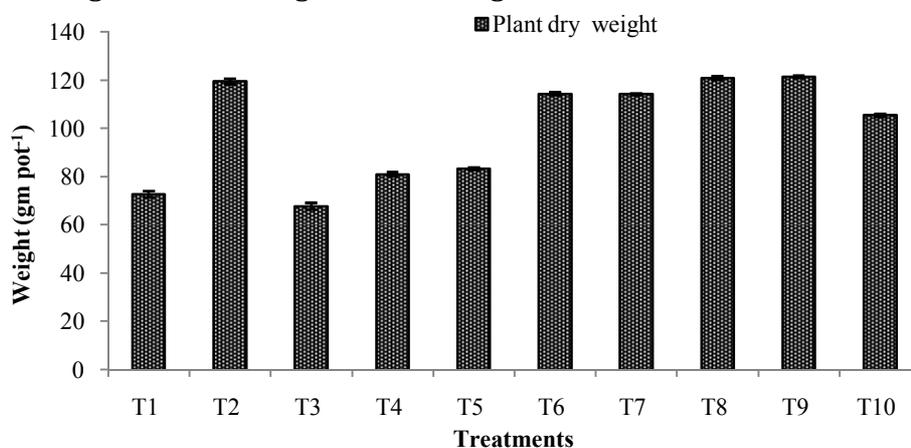


Figure 5: Plant dry weight of rice at the time of harvest.

Plant dry weight recorded at the time of after harvest was observed in the ranged from 67.78 - 121.55 gm pot⁻¹. Treatment T9 (mixed algae) was recorded with the highest plant dry weight with among all

treatment followed by T2 (uninoculated, full dose NPK) while the lowest was observed in treatments inoculated with *Spirogyra* sp (Figure 5).

The maximum number of tiller per plant was recorded in T9 and T10 with 8.33 while the minimum was recorded in T3 with 4.33. In contrary, the maximum number of panicle was recorded in T8 treatment with 7.33 and minimum in T1, T3 and T4 with 3.66 (Figure 6). Maximum weight of 100 grains was recorded in T10 treatments with 33.96 g while the minimum weight was recorded in T1 with 16.11 g (Figure 7).

All other characters plant dry weight, grain weight, number of tillers and panicles showed significant variations between T2 (full dose NPK) and T1 to T5 (T1-control, T3 - *Spirogyra* sp, T4- *Microspora tumidola*, T5- mixed green algae). However significant difference of observed between T2 and T6, T7 or T8 (T6-*Oscillatoria curviceps*, T7- *Nostoc commune*, T8- *Cylindrospermum muscicola*) is because the plant characters inoculated with Cyanobacteria are much higher than that to T2.

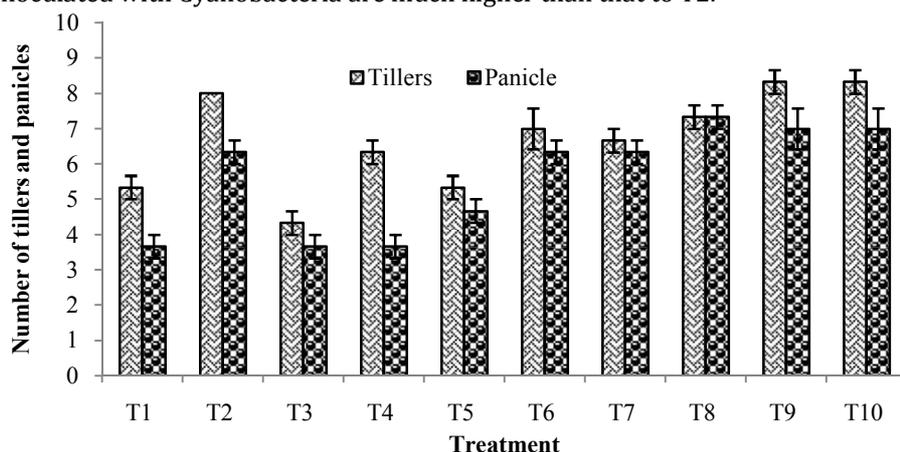


Figure 6: Number of tillers and panicles of rice at the time of harvest.

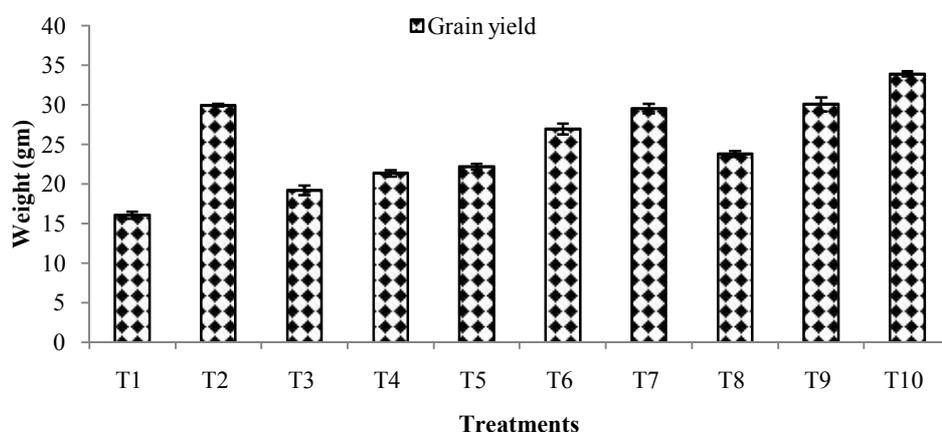


Figure 7: Grain yield of rice at the time of harvest.

Co-cultivation of Cyanobacteria and algae with maize

Growth pattern of maize during the 30, 45 and 70 days was observed for shoot length and recorded from the base of the plant to the highest point of the arch of the uppermost leaf whose tip is pointing downwards. After 30 days growth of maize, maximum shoot length was observed in T5 with 36.82 cm and minimum in T1 with 21.62 cm. After 45 and 70 days, maximum length was noticed in T7 and T9 with 79.33 cm and 180 cm and minimum in T1 with 57.82 cm and 120 cm respectively (Figure 8).

At the time of harvest, maximum shoot length was recorded in T2 (uninoculated, full dose NPK) with 190.33 cm and minimum in T1 with 141.33 cm. On comparing root length, maximum was recorded in T2 with 98.66 cm and minimum in T3 with 48.33 cm (Figure 9).

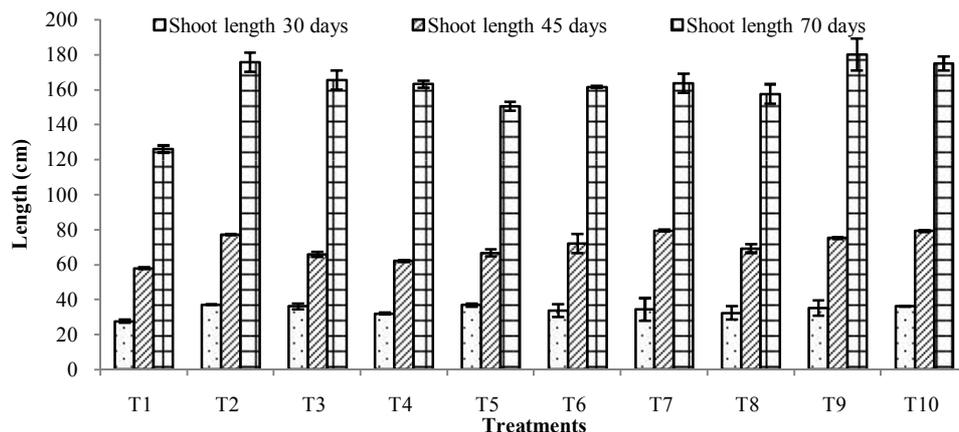


Figure 8: Shoot length after 30 and 70 days of maize in different treatments.

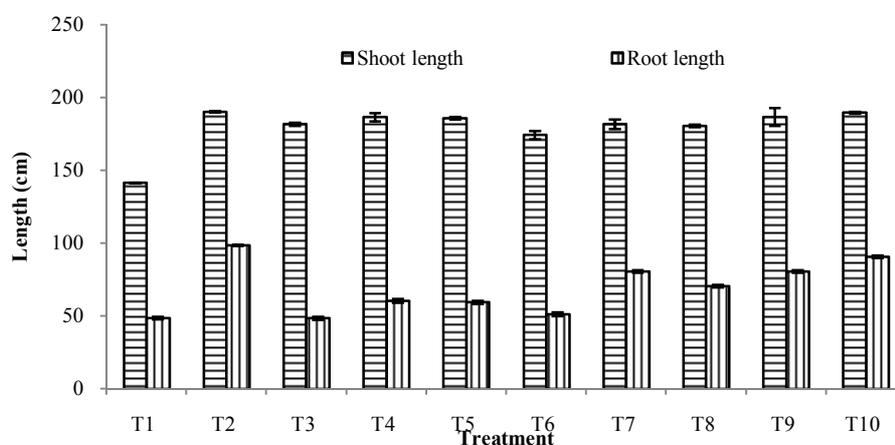


Figure 9: Shoot length and root length of maize in different treatments at the time of harvest (120 days)

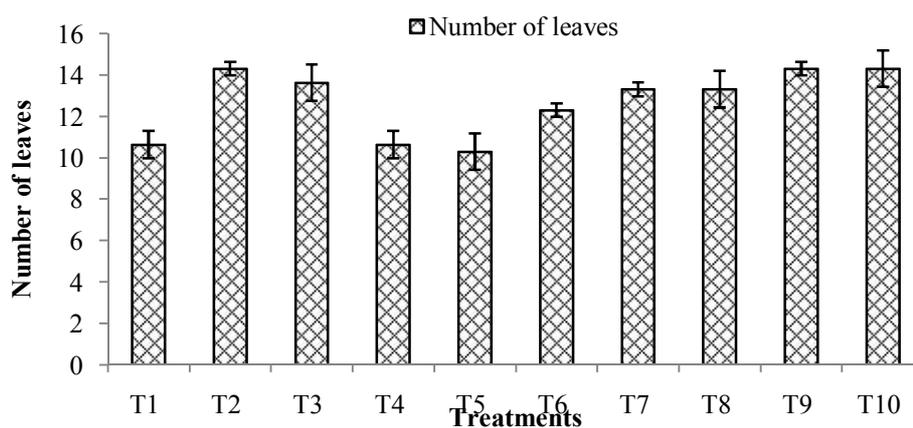


Figure 10: Number of leaves in maize at the time of harvest (120 days) in different treatments.

Finally at the time of harvest, number of leaves was maximum in T10 with 14.33 and the minimum was recorded in T6 with 8.33 (Figure 10). Plant dry weight recorded at the time of harvest was observed in the range of 58.85 - 109.55 gm pot⁻¹. Maximum plant dry weight was recorded in T2 (full dose NPK) and minimum in control. When comparing the treatments inoculated with algae the highest plant dry weight was noticed in T10 with 96.24 gm pot⁻¹ and lowest in 61.70 gm pot⁻¹ in T5 (Figure 11).

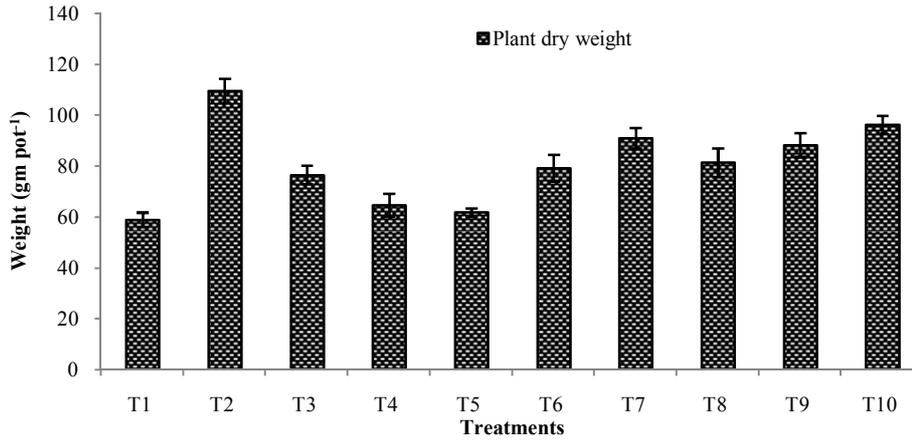


Figure 11 : Plant dry weight of maize at the time of harvest

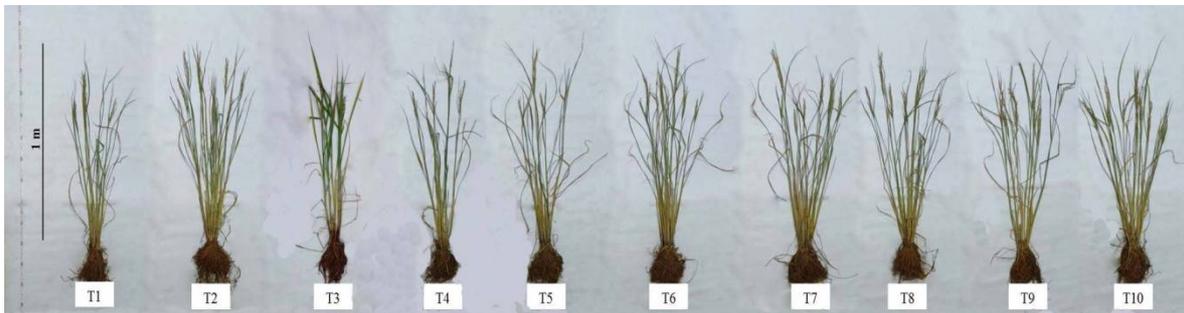


Figure 12 : Effect of different treatments on rice growth at the time of harvest.



Figure 13 : Effect of different treatments on maize growth at the time of harvest (120 days).

Table 2: Plant parameters in different treatments for rice

Treatments*	Shoot length at the time of growth				At the time of harvest					
	15 days (cm)	30 days (cm)	90 days (cm)	120 days (cm)	Shoot length (cm)	Root length (cm)	No. of tillers	No. of panicle	Plant dry weight	Grains (g ⁻¹ pot)
T1	14.25±0.02	23.05±0.84	79.93±0.57	90.15±1.01	92.33±1.52	19.66±0.57	5.33±0.57	3.66±0.57	72.81±2.38	16.11±0.62
T2	20.72±0.84	31.22±0.78	111.60±1.47	118.99±0.77	120.05±0.98	29.66±1.52	8.04±0.00	6.33±0.57	119.55±1.94	30.11±0.19
T3	12.85±0.25	21.74±0.01	86.82±1.19	93.72±1.97	95.06±2.64	25.08±0.98	4.33±0.57	3.66±0.57	67.78±2.41	19.26±0.86
T4	13.36±0.28	25.82±0.06	97.48±1.49	103.71±1.50	110.33±0.57	24.66±1.15	6.33±0.57	3.66±0.57	81.07±1.41	21.38±0.55
T5	15.82±0.21	25.75±0.58	92.65±1.56	98.04±0.55	105.11±2.87	23.12±0.97	5.33±0.57	4.66±0.57	83.44±0.93	22.22±0.47
T6	21.21±0.79	30.38±1.19	113.11±2.34	118.83±0.56	119.66±1.52	24.66±2.08	7.03±0.95	6.33±0.57	114.35±1.11	26.99±0.93
T7	23.25±0.74	28.06±0.70	120.06±1.01	126.79±4.50	111.33±0.57	31.66±2.08	6.66±0.57	6.33±0.57	114.26±0.55	29.59±0.86
T8	21.54±0.15	29.72±0.56	105.55±4.22	116.04±4.79	106.33±4.04	27.33±0.57	7.33±0.57	7.33±0.57	120.95±1.31	23.83±0.53
T9	21.83±0.26	31.05±0.87	110.10±1.90	118.16±0.25	122.66±1.52	27.33±0.57	8.33±0.57	7.03±0.97	121.55±0.91	30.10±1.24
T10	21.82±0.06	32.71±0.07	105.909±6.58	118.38±0.06	120.66±0.57	28.66±0.57	8.33±0.33	7.03±0.78	105.45±1.14	33.96±0.41

Table 3: Plant parameters in different treatments for maize

Treatments*	Shoot length at the time of growth			At the time of harvest			
	30 days (cm)	45 days (cm)	70 days (cm)	Total no. of leaves	Shoot length (cm)	Root length (cm)	Plant dry weight
T1	27.62±1.60	57.82±1.32	126±2.82	10.66±1.15	141.33±0.57	48.66±1.52	58.85±4.90
T2	37.10±0.32	77.10±0.32	175.50±7.77	14.33±0.57	190.33±0.57	98.66±0.57	109.55±8.31
T3	36.15±1.83	65.85±2.26	165.50±7.77	13.66±1.52	181.66±2.08	48.33±2.08	76.47±6.20
T4	32.12±0.94	62.05±0.87	163.12±2.82	10.66±1.15	186.66±4.93	60.33±2.08	64.57±7.79
T5	36.82±1.31	66.67±1.10	150.50±3.53	10.33±1.52	185.66±1.52	59.33±1.52	61.70±2.80
T6	33.65±3.14	72.10±5.19	161.50±0.70	12.33±0.57	174.33±4.93	51.33±2.08	79.21±9.02
T7	34.38±1.97	79.33±9.11	163.50±7.77	13.33±0.57	181.66±5.50	80.66±1.52	90.93±7.14
T8	32.38±0.84	69.20±5.33	157.50±7.77	13.33±1.52	180.66±1.52	70.66±1.52	81.29±9.66
T9	35.27±6.19	75.27±6.19	180.22±12.72	14.33±0.57	186.66±10.69	80.66±1.52	88.24±8.13
T10	36.05±4.93	79.26±0.39	175.12±5.65	14.33±1.52	189.66±1.15	90.66±1.52	96.24±6.08

*(Treatments: T1- control, T2 - full dose NPK, T3 - *Spirogyra* sp, T4 - *Microspora tumidola*, T5 - mixed green algae, T6 - *Oscillatoria curviceps*, T7 - *Nostoc commune*, T8 - *Cylindrospermum muscicola*, T9 - mixed Cyanobacteria, T10 - mixed algae and ¼ NPK)

Table 4: One-way ANOVA of plant parameters in different treatments for rice.

Treatments**	Shoot length at the time of growth				At the time of harvest					
	15 days (cm)	30 days (cm)	90 days (cm)	120 days (cm)	Shoot length (cm)	Root length (cm)	No. of tillers	No. of panicle	Plant dry weight	Grains (g ⁻¹ pot)
T2:T1	0.008*	0.009*	0.0002*	0.03*	1.25E-05*	0.0004*	0.001*	0.004*	1.24E-05*	0.001*
T2:T3	0.006*	0.003*	0.0002*	0.02*	0.0001*	0.01*	0.0003*	0.004*	8.53E-06*	0.003*
T2:T4	0.007*	0.01*	0.003*	0.04*	0.0001*	0.01*	0.007*	0.004*	1.23E-05*	0.002*
T2:T5	0.01*	0.01*	0.04*	0.04*	0.001*	0.003*	0.001*	0.02*	8.41E-06*	0.002*
T2:T6	0.61	0.49	0.08	0.27	0.76	0.02*	0.15	0.98	0.06	0.04*
T2:T7	0.08	0.06	0.01*	0.005*	0.004*	0.25	0.01*	0.99	0.01*	0.58
T2:T8	0.31	0.15	0.03*	0.05*	0.004*	0.06	0.11	0.10	0.36	0.004*
T2:T9	0.21	0.85	0.23	0.87	0.06	0.06	0.37	0.37	0.18	0.92
T2:T10	0.20	0.11	0.21	0.23	0.37	0.34	0.37	0.37	0.12	0.12

*P<0.05

Table 5: One-way ANOVA of plant parameters in different treatments for maize.

Treatments**	Shoot length at the time of growth			At the time of harvest			
	30 days (cm)	45 days (cm)	70 days (cm)	No. of leaves	Shoot length (cm)	Root length (cm)	Plant dry weight
T2:T1	0.01*	0.002*	0.01*	0.007*	0.0001*	0.0009*	0.0008*
T2:T3	0.54	0.02*	0.32	0.51	0.004*	0.001*	0.005*
T2:T4	0.01*	0.001*	0.16	0.007*	0.30	0.001*	0.002*
T2:T5	0.79	0.006*	0.05*	0.01*	0.02*	0.001*	0.0007*
T2:T6	0.26	0.30	0.12	0.01*	0.001*	0.001*	0.01*
T2:T7	0.02*	0.01*	0.03*	0.10	0.0003*	0.007*	0.04
T2:T8	0.01*	0.02*	0.04*	0.34	0.006*	0.003*	0.01*
T2:T9	0.01*	0.01*	0.01*	0.98	0.96	0.007*	0.03
T2:T10	0.73	0.02*	0.11	0.97	0.69	0.06	0.08

*P<0.05

** (Treatments: T1 - control, T2 - full dose NPK, T3 - *Spirogyra* sp, T4 - *Microspora tumidola*, T5 - mixed green algae, T6 - *Oscillatoria curviceps*, T7 - *Nostoc commune*, T8 - *Cylindrospermum muscicola*, T9 - mixed Cyanobacteria, T10 - mixed algae and ¼ NPK)

DISCUSSION

Organic fertilizer and biofertilizer are beneficial because they limit the application of chemical fertilizers which have been already known to cause a variety of harm to the environment. Cyanobacteria and green algae play an important role in maintenance of soil fertility by providing fixed nitrogen.

Mixed application of green algae and Cyanobacteria (T9) lead to an increase in growth independent of chemical fertilizers and give the same result as that with full dose of NPK (T2) for both rice and maize. Biofertilization with proper use of Cyanobacteria and green algae will greatly reduce the use of fertilizers by almost 50% without compromising the growth and biomass of crop plants. This observation is in line with studies by [5] who concluded that *Microcystis aeruginosa*, *Anabaena* sp. and *Chlorella* sp significantly increased seed germination and growth of corn seedlings while also intensifying metabolic processes. This also agreed with our results where vegetative growth improved in maize plants with inoculated

treatments as full dose NPK. Algal biofertilizer in different combination with organic and inorganic fertilizers showed positive growth on different growth parameters of *Vigna radiata*. Increased growth and physiological performance was observed with application of selected strains of *Microcystis aeruginosa*, *Anabaena* sp. and *Chlorella* sp without significant effect on growth and biomass of willow [6]. Further, they reported marked reduction almost one-two in the doses of chemical fertiliser.

Inhibition of germination of rice with *Spirogyra* extract was reported [13]. In our study increased seed germination and growth has been observed only with *Microspora* inoculum and not with *Spirogyra* sp. However, high seeds germination of jowar, mothbean and sesamum with *Spirogyra* extract was reported [7]. Stimulatory effects of *Spirogyra jugalis* extracts on seeds germination and plant growth of tomato plants was also observed [9]. In the present study, germination of maize seeds was independent of any inoculation type. But seed germination and seedling growth of treated maize seeds in *Spirogyra* extract was comparatively better than that of rice. Significant increase in germination and seedling growth of alfalfa in *Spirogyra* extract was studied [2]. Furthermore, they reported a significant increase in number of leaves and plant height in soil inoculated with *Oscillatoria* compared to those treated with *Spirogyra*.

In the present study, no significant variation was observed in the height of rice plants when treated with T6 (*Nostoc muscorum*) and T9 (mixed Cyanobacteria) when compared with T2 (uninoculated, full dose NPK). But in maize, vegetative growth i.e. the height, number of leaves and dry weight increased with T9 (mixed Cyanobacteria) and T10 (Cyanobacteria and algae + ¼ NPK) inoculated in the soil. This has also been confirmed by many researchers. Significant increase in growth parameters of wheat, sorghum, maize and lentil with the use of *Nostoc muscorum* as biofertilizer compared to control was observed [1], while high grain yield of wheat was observed when treated with *Nostoc* sp (single species inoculation) [8]. Increased in shoot length was observed but leaf area of maize remained unaffected when inoculated with *Nostoc muscorum* or *Nostoc rivulare*, which showed the same results as those when treated with chemical fertilizers [14]. From the present experiment, it could be said that supplementation of Cyanobacteria with small quantity of fertilizer will result in high yield and therefore can greatly reduce the use of chemical fertilizers.

CONCLUSION

Depletion of soil fertility, high fertilizer use and environmental pollution are major concern to agricultural crop productivity. Thus, the use of biofertilizer can prove a suitable supplement to the chemical fertilizers. Mixed application of green algae and Cyanobacteria lead to an increase in growth independent of chemical fertilizers and give the same result as that with full dose of NPK for both rice and maize. Yield in rice could be monitored through pot experiment but yield in maize was observed to be dependent on other factors like soil surface. Thus application of Cyanobacteria and algal strain in fields could minimize the use of chemical fertilizer.

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Effect of Cyanobacteria and Filamentous Green Algae on Growth of Rice and Maize

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ABSTRACT

In this study the effect of Cyanobacteria (Nostoc commune, Cyndrospermum muscicola, Oscillatoria curviceps) and green algae (Spirogyra sp and Microspora floccosa) on growth and yield of rice (MEGHA 1) and maize (Local Yellow variety) were evaluated. Plant parameters such as shoot height, number of tillers and number of panicle, root length, grain yield and dry weight were recorded for rice while for maize, height of the plant, length, dry weight and number of leaves were recorded. On comparing the germination percentage of seeds in Cyanobacteria and algal extract, it was observed that Spirogyra sp. inhibited the germination of rice seeds. However for maize seeds, all treatments increased the germination percentage. Mixed application of green algae and Cyanobacteria (T9) lead to an increase in growth independent of chemical fertilizers and gave the same results as that with full dose of NPK (T2) for both rice and maize.

Keywords: Cyanobacteria, green algae, germination, plant parameters

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INTRODUCTION

Rice and maize are the two important crops in the hilly state of Meghalaya. However a wide gap is available between plant nutrients removal by crops and nutrients replenishment in this region. On top of that, the soils of northeastern regions are acidic and greatly deficient in phosphorus, thus importance of fertilizers with enhancement ratio of N, P and K are done frequently. Such enhancement of single element has led to accelerated exhaustion of other major and minor nutrients, therefore use of microbial consortia or biofertilizer that can promote the growth of crop are deploys during the recent years. In recent years, emphasized of crop production using biofertilizers as an alternative to chemical fertilizers have been carried out.

De (1939) was the first to recognize the importance of Cyanobacteria as a great agricultural potential and its contribution to the process of biological nitrogen fixation. Such ability of Cyanobacteria to fix the atmospheric nitrogen is of economic importance [12]. Considering the global energy crisis, supplementary sources of nitrogen can be achieved through the use of biofertilizers such as those of Cyanobacteria and *Azolla* [3]. Increased growth of plants by cultivation of Cyanobacteria and green algae with rice, barley, oats, tomato, radish, cotton, sugarcane, maize, chilli, mustard, willow as biofertilizer have been observed by many researchers [5, 8, 10, 11, 15].

In spite of recent research and development on use of Cyanobacteria, information about the influence of single species on development of plant species are still limited and such studies on green algae are rare. Our research was performed to assess the ability of some selected Cyanobacteria and green algae as inoculant for pot experiment in order to study the growth and yield of rice and maize.

MATERIAL AND METHODS

Germination

The algal scum collected/cultured were washed thoroughly and air dried for 15 days in lab conditions and then grounded to fine powder. Algal powder was soaked in distilled water for 24 hours in the dark. The mixture was filtered and stored at 4°C in the dark. Three replicates of ten seeds were soaked in the extract for 24 hours. Controlled sets were maintained in distilled water. The treated seeds were sowed in sterilised soil in pots.

Germination percentage was calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{No. of seeds sown}}{\text{No. of seeds germinated}} \times 100$$

Experimental design and treatments

The pot culture experiment was set up to study the effect of different Cyanobacteria and green filamentous algae on yield of crop plants. For the experimental set up, two crops were selected viz., rice (Megha 1) and maize (local yellow variety) with varying proportions of Cyanobacteria and algae in net house of Botany department, NEHU. Soil was collected from rice field adjacent to NEHU campus and was used as medium for raising the crops. Soil was autoclaved for sterilisation to minimize contamination and then poured in plastic pots of 12" diameter. Cyanobacteria strains selected for the experiment were *Nostoc commune*, *Cylindrospermum muscicola*, *Oscillatoria curviceps* and two green filamentous algae *Spirogyra* sp and *Microspora floccosa*.

The experiment was set up in three replicates and according to the use of single, two and three strain combination of algae; full dose of NPK fertilisers and control treatments (Table 1). Fertilizers added was according to the recommended rate which was 80:60:40 NPK kg ha⁻¹ for rice crop and 80:40:40 NPK kg ha⁻¹ for wheat. The rice and maize seeds were soaked in treatments with inoculums in separate containers except for the treatments with full dose NPK.

Three seeds were sown in each pots and irrigation was given after sowing. Suspension of selected species of Cyanobacteria and algae was applied 15 days after sowing. Soil cores were removed near the root region and culture suspension were poured into the pit and covered with soil. The application of second inoculum was given 30 days after sowing and the final application was given after 120 days.

Plant parameters

At the time of plant growth for rice, shoot height, number of tillers and number of panicle were recorded. Plant height was measured from the base of the plant to the tip of the primary. At the time of harvest, all the characters above were measured including the root length, grain yield and dry weight of the plant was recorded.

At the same time for maize, height of the plant from the base of the plant to the highest point of the arch of the uppermost leaf whose tip is pointing downwards was recorded. During harvest, the above characters along with root length, dry weight, number of cobs and leaves were recorded.

Table 1: Different treatments and their composition

Sl. no.	Treatments	Composition
1	T1	uninoculated bare soil
2	T2	uninoculated, full dose NPK
3	T3	<i>Spirogyra</i> sp
4	T4	<i>Microspora tumidola</i>
5	T5	T3 + T4
6	T6	<i>Oscillatoria curviceps</i>
7	T7	<i>Nostoc commune</i>
8	T8	<i>Cylindrospermum muscicola</i>
9	T9	T6 + T7 + T8
10	T10	mixed algae & 1/4NPK

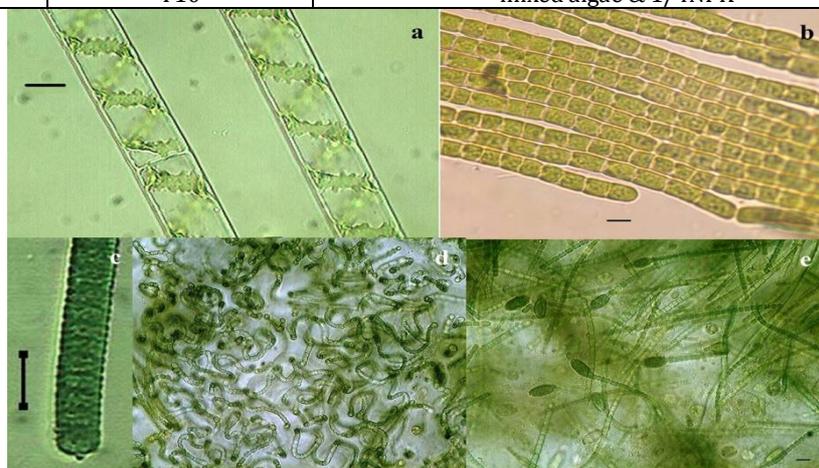


Figure 1: Green algae - a. *Spirogyra* sp, b. *Microspora tumidola*, Cyanobacteria - c. *Oscillatoria curviceps*, d. *Nostoc commune*, e. *Cylindrospermum mucicola*.

Statistical analysis

Data were subjected to ANOVA in accordance with experimental design and values were calculated at P level of 0.05%. Significance of variation was determined between T2 and the rest of treatments in order to observe the efficiency of use of Cyanobacteria and algae.

RESULTS

Germination

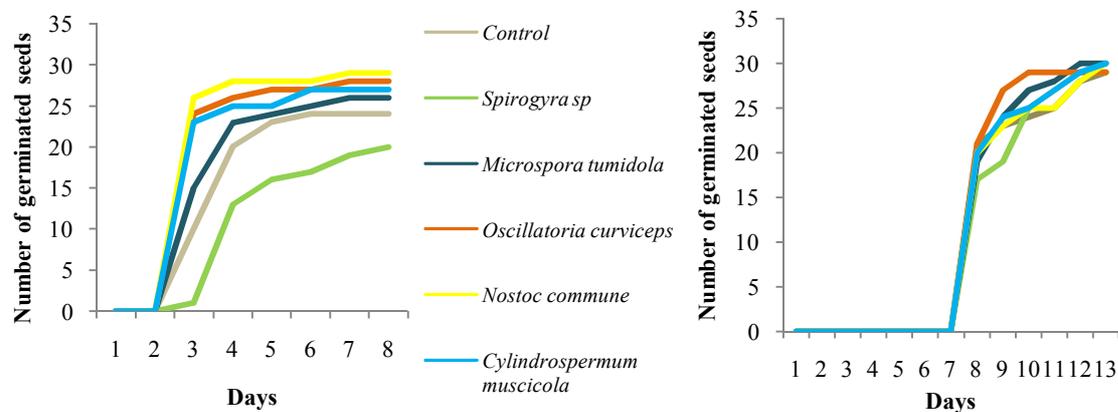


Figure 1: Effect of Cyanobacteria and algal extract on (a) rice and (b) maize seeds.

The result of germination study revealed that *Spirogyra sp.* inhibited the germination of rice seeds. The number of seeds germinated was even lower than control. Seed germination in *Nostoc commune* was highest followed by *Oscillatoria curviceps*, *Cylindrospermum muscicola* (Figure 1). On comparing rice and maize seeds, significant difference was observed in the germination percentage between them.

For rice seeds, germination percentage ranged from 65.21% in *Spirogyra sp* to 95.05% in *Nostoc commune*. We also observed that pre-soaking of seeds in Cyanobacteria culture enhanced the germination of rice seeds. Significant difference was observed between *Spirogyra sp* and Cyanobacterial inoculum. However for maize seeds, all treatments increased the germination percentage which ranged from 96.67% in control and *Spirogyra* to 100% in the rest of the Cyanobacteria and algal inoculum used in treatments. No significant difference was observed between the treatments (Figure 2).

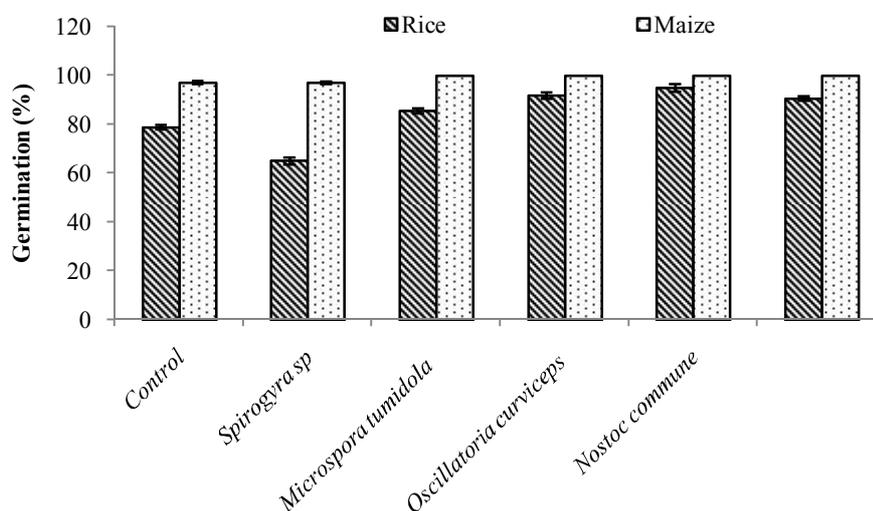


Figure 2: Germination percentage of rice and maize seeds.

Co-cultivation of Cyanobacteria and algae with rice

Shoot length of the rice plant from the base to the tip of the longest leaf of the plant was measured after 15, 30 and 120 days of plant growth. After 15 days, maximum shoot length was observed in T7 (23.25 cm) and minimum in T3 (12.85 cm) respectively. On the contrary, maximum shoot length after the 30 days was noticed in T10 (32.71 cm) and minimum in T3 (21.74 cm). The maximum plant height for 90

and was observed in T9 (102.10 cm) and minimum in T1 (64.93 cm). After 120 days, maximum shoot length was observed in T7 (126.79 cm) and minimum was observed in T1 (90.15 cm) (Figure 3).

At the time of harvest, total plant height (shoot and root length) was measured. The maximum shoot length and root length was observed in T7 with 131.33 cm and 31.66 cm respectively. The minimum shoot length and root length was observed in T1 with 92.33 cm and 19.66 cm respectively. At the time of harvest, total plant height (shoot and root length) was measured. The maximum shoot length and root length was observed in T7 with 131.33 cm and 31.66 cm respectively. The minimum shoot length and root length was observed in T1 with 92.33 cm and 19.66 cm respectively (Figure 4).

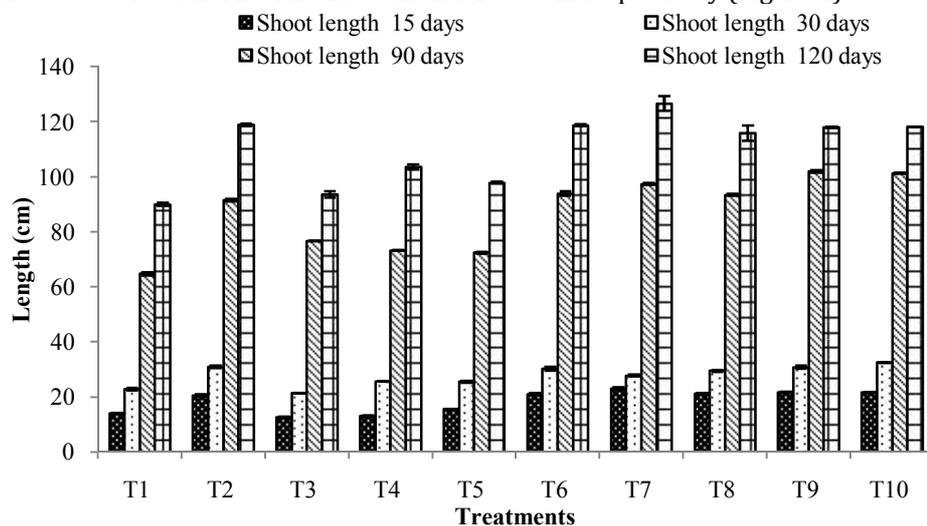


Figure 3: Shoot length after 15, 30, 90 and 120 days for rice

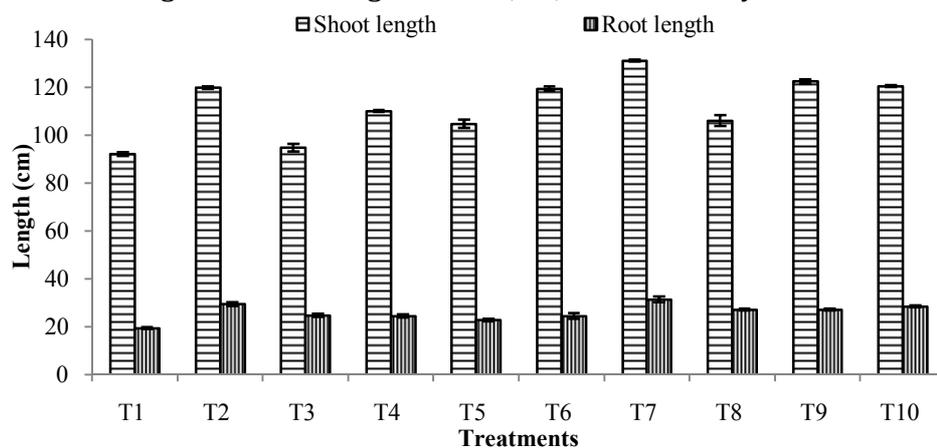


Figure 4: Shoot length and root length of rice at the time of harvest.

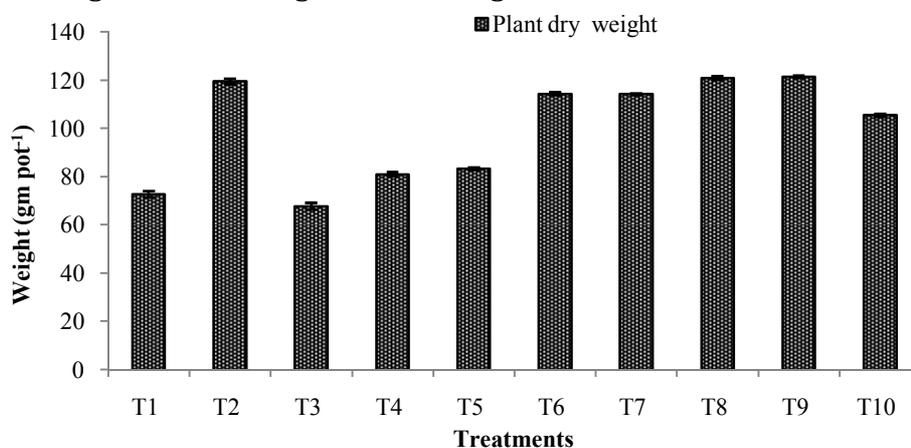


Figure 5: Plant dry weight of rice at the time of harvest.

Plant dry weight recorded at the time of after harvest was observed in the ranged from 67.78 - 121.55 gm pot⁻¹. Treatment T9 (mixed algae) was recorded with the highest plant dry weight with among all

treatment followed by T2 (uninoculated, full dose NPK) while the lowest was observed in treatments inoculated with *Spirogyra* sp (Figure 5).

The maximum number of tiller per plant was recorded in T9 and T10 with 8.33 while the minimum was recorded in T3 with 4.33. In contrary, the maximum number of panicle was recorded in T8 treatment with 7.33 and minimum in T1, T3 and T4 with 3.66 (Figure 6). Maximum weight of 100 grains was recorded in T10 treatments with 33.96 g while the minimum weight was recorded in T1 with 16.11 g (Figure 7).

All other characters plant dry weight, grain weight, number of tillers and panicles showed significant variations between T2 (full dose NPK) and T1 to T5 (T1-control, T3 - *Spirogyra* sp, T4- *Microspora tumidola*, T5- mixed green algae). However significant difference of observed between T2 and T6, T7 or T8 (T6-*Oscillatoria curviceps*, T7- *Nostoc commune*, T8- *Cylindrospermum muscicola*) is because the plant characters inoculated with Cyanobacteria are much higher than that to T2.

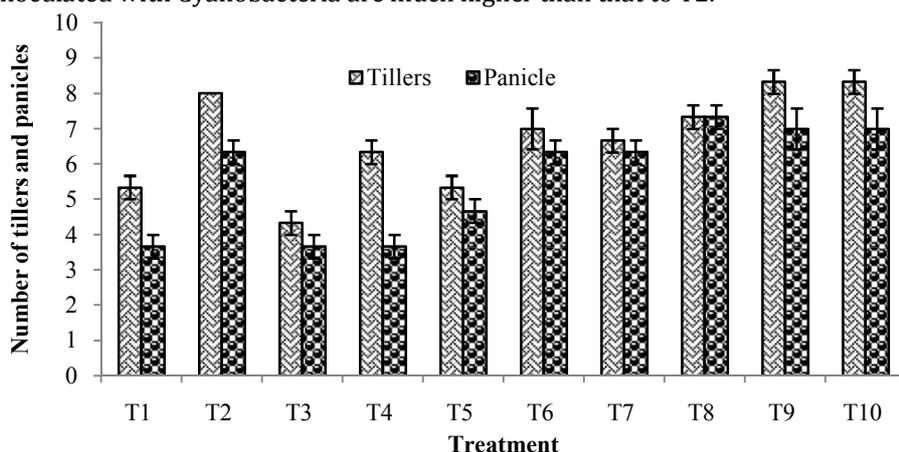


Figure 6: Number of tillers and panicles of rice at the time of harvest.

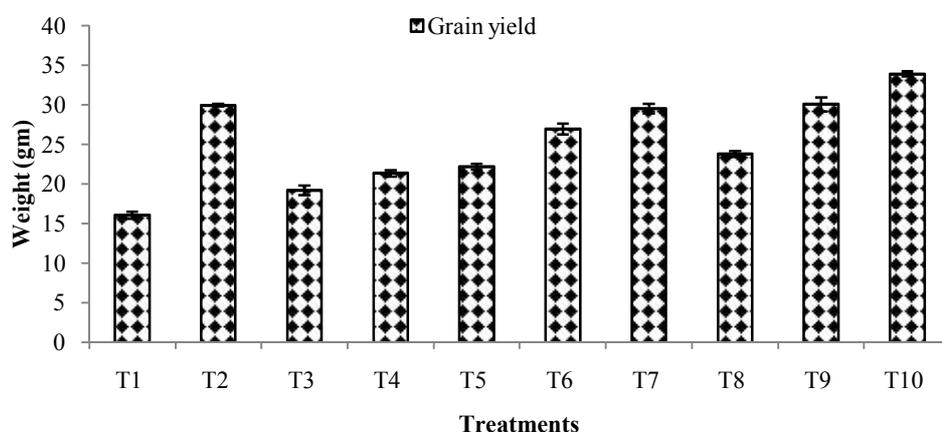


Figure 7: Grain yield of rice at the time of harvest.

Co-cultivation of Cyanobacteria and algae with maize

Growth pattern of maize during the 30, 45 and 70 days was observed for shoot length and recorded from the base of the plant to the highest point of the arch of the uppermost leaf whose tip is pointing downwards. After 30 days growth of maize, maximum shoot length was observed in T5 with 36.82 cm and minimum in T1 with 21.62 cm. After 45 and 70 days, maximum length was noticed in T7 and T9 with 79.33 cm and 180 cm and minimum in T1 with 57.82 cm and 120 cm respectively (Figure 8).

At the time of harvest, maximum shoot length was recorded in T2 (uninoculated, full dose NPK) with 190.33 cm and minimum in T1 with 141.33 cm. On comparing root length, maximum was recorded in T2 with 98.66 cm and minimum in T3 with 48.33 cm (Figure 9).

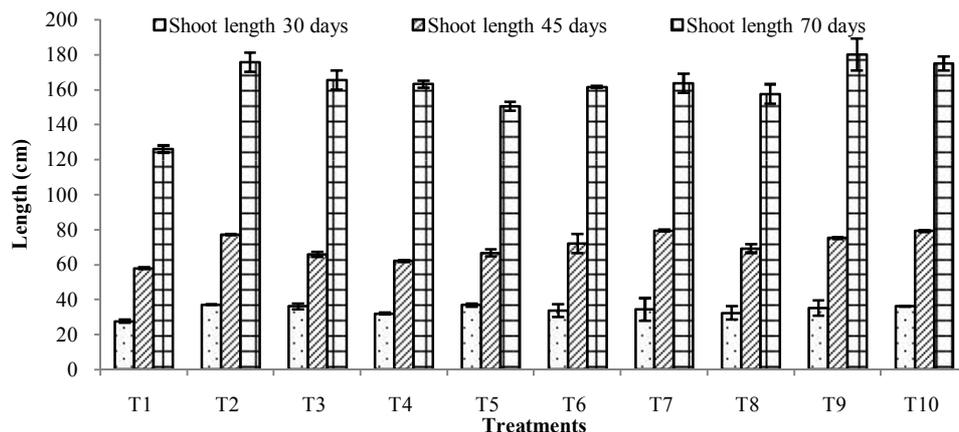


Figure 8: Shoot length after 30 and 70 days of maize in different treatments.

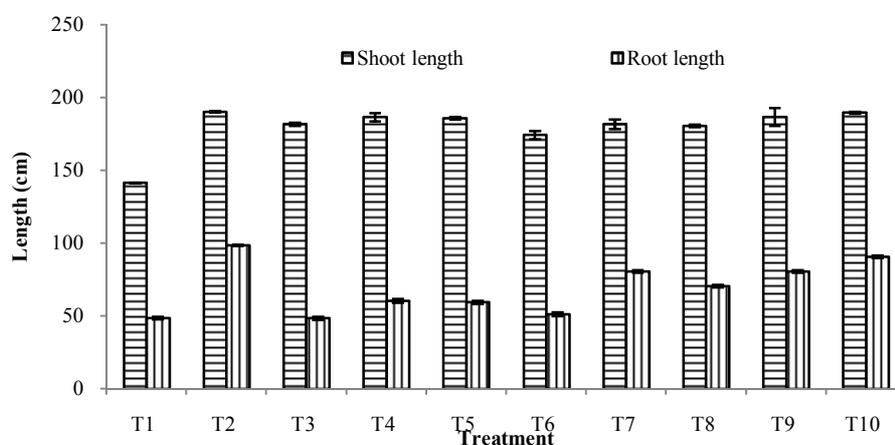


Figure 9: Shoot length and root length of maize in different treatments at the time of harvest (120 days)

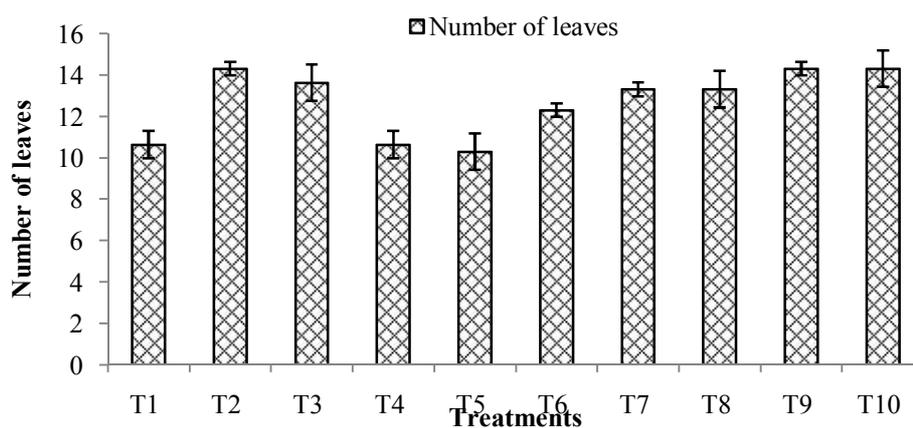


Figure 10: Number of leaves in maize at the time of harvest (120 days) in different treatments.

Finally at the time of harvest, number of leaves was maximum in T10 with 14.33 and the minimum was recorded in T6 with 8.33 (Figure 10). Plant dry weight recorded at the time of harvest was observed in the range of 58.85 - 109.55 gm pot⁻¹. Maximum plant dry weight was recorded in T2 (full dose NPK) and minimum in control. When comparing the treatments inoculated with algae the highest plant dry weight was noticed in T10 with 96.24 gm pot⁻¹ and lowest in 61.70 gm pot⁻¹ in T5 (Figure 11).

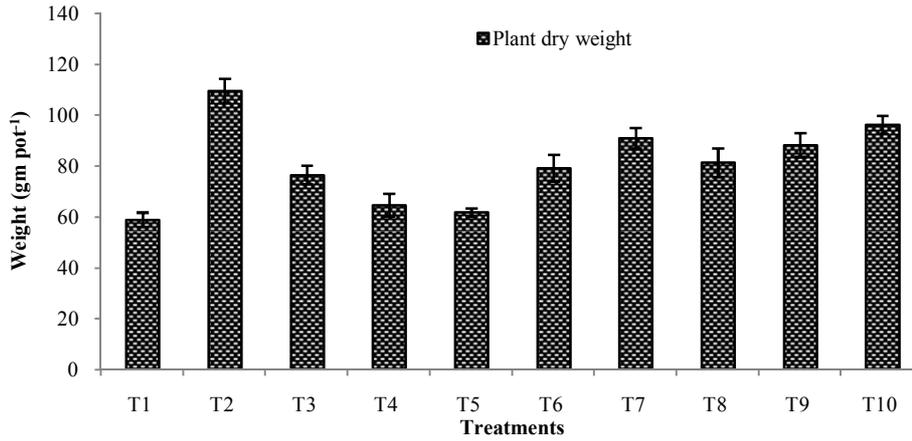


Figure 11 : Plant dry weight of maize at the time of harvest



Figure 12 : Effect of different treatments on rice growth at the time of harvest.



Figure 13 : Effect of different treatments on maize growth at the time of harvest (120 days).

Table 2: Plant parameters in different treatments for rice

Treatments*	Shoot length at the time of growth				At the time of harvest					
	15 days (cm)	30 days (cm)	90 days (cm)	120 days (cm)	Shoot length (cm)	Root length (cm)	No. of tillers	No. of panicle	Plant dry weight	Grains (g ⁻¹ pot)
T1	14.25±0.02	23.05±0.84	79.93±0.57	90.15±1.01	92.33±1.52	19.66±0.57	5.33±0.57	3.66±0.57	72.81±2.38	16.11±0.62
T2	20.72±0.84	31.22±0.78	111.60±1.47	118.99±0.77	120.05±0.98	29.66±1.52	8.04±0.00	6.33±0.57	119.55±1.94	30.11±0.19
T3	12.85±0.25	21.74±0.01	86.82±1.19	93.72±1.97	95.06±2.64	25.08±0.98	4.33±0.57	3.66±0.57	67.78±2.41	19.26±0.86
T4	13.36±0.28	25.82±0.06	97.48±1.49	103.71±1.50	110.33±0.57	24.66±1.15	6.33±0.57	3.66±0.57	81.07±1.41	21.38±0.55
T5	15.82±0.21	25.75±0.58	92.65±1.56	98.04±0.55	105.11±2.87	23.12±0.97	5.33±0.57	4.66±0.57	83.44±0.93	22.22±0.47
T6	21.21±0.79	30.38±1.19	113.11±2.34	118.83±0.56	119.66±1.52	24.66±2.08	7.03±0.95	6.33±0.57	114.35±1.11	26.99±0.93
T7	23.25±0.74	28.06±0.70	120.06±1.01	126.79±4.50	111.33±0.57	31.66±2.08	6.66±0.57	6.33±0.57	114.26±0.55	29.59±0.86
T8	21.54±0.15	29.72±0.56	105.55±4.22	116.04±4.79	106.33±4.04	27.33±0.57	7.33±0.57	7.33±0.57	120.95±1.31	23.83±0.53
T9	21.83±0.26	31.05±0.87	110.10±1.90	118.16±0.25	122.66±1.52	27.33±0.57	8.33±0.57	7.03±0.97	121.55±0.91	30.10±1.24
T10	21.82±0.06	32.71±0.07	105.909±6.58	118.38±0.06	120.66±0.57	28.66±0.57	8.33±0.33	7.03±0.78	105.45±1.14	33.96±0.41

Table 3: Plant parameters in different treatments for maize

Treatments*	Shoot length at the time of growth			At the time of harvest			
	30 days (cm)	45 days (cm)	70 days (cm)	Total no. of leaves	Shoot length (cm)	Root length (cm)	Plant dry weight
T1	27.62±1.60	57.82±1.32	126±2.82	10.66±1.15	141.33±0.57	48.66±1.52	58.85±4.90
T2	37.10±0.32	77.10±0.32	175.50±7.77	14.33±0.57	190.33±0.57	98.66±0.57	109.55±8.31
T3	36.15±1.83	65.85±2.26	165.50±7.77	13.66±1.52	181.66±2.08	48.33±2.08	76.47±6.20
T4	32.12±0.94	62.05±0.87	163.12±2.82	10.66±1.15	186.66±4.93	60.33±2.08	64.57±7.79
T5	36.82±1.31	66.67±1.10	150.50±3.53	10.33±1.52	185.66±1.52	59.33±1.52	61.70±2.80
T6	33.65±3.14	72.10±5.19	161.50±0.70	12.33±0.57	174.33±4.93	51.33±2.08	79.21±9.02
T7	34.38±1.97	79.33±9.11	163.50±7.77	13.33±0.57	181.66±5.50	80.66±1.52	90.93±7.14
T8	32.38±0.84	69.20±5.33	157.50±7.77	13.33±1.52	180.66±1.52	70.66±1.52	81.29±9.66
T9	35.27±6.19	75.27±6.19	180.22±12.72	14.33±0.57	186.66±10.69	80.66±1.52	88.24±8.13
T10	36.05±4.93	79.26±0.39	175.12±5.65	14.33±1.52	189.66±1.15	90.66±1.52	96.24±6.08

*(Treatments: T1- control, T2 - full dose NPK, T3 - *Spirogyra* sp, T4 - *Microspora tumidola*, T5 - mixed green algae, T6 - *Oscillatoria curviceps*, T7 - *Nostoc commune*, T8 - *Cylindrospermum muscicola*, T9 - mixed Cyanobacteria, T10 - mixed algae and ¼ NPK)

Table 4: One-way ANOVA of plant parameters in different treatments for rice.

Treatments**	Shoot length at the time of growth				At the time of harvest					
	15 days (cm)	30 days (cm)	90 days (cm)	120 days (cm)	Shoot length (cm)	Root length (cm)	No. of tillers	No. of panicle	Plant dry weight	Grains (g ⁻¹ pot)
T2:T1	0.008*	0.009*	0.0002*	0.03*	1.25E-05*	0.0004*	0.001*	0.004*	1.24E-05*	0.001*
T2:T3	0.006*	0.003*	0.0002*	0.02*	0.0001*	0.01*	0.0003*	0.004*	8.53E-06*	0.003*
T2:T4	0.007*	0.01*	0.003*	0.04*	0.0001*	0.01*	0.007*	0.004*	1.23E-05*	0.002*
T2:T5	0.01*	0.01*	0.04*	0.04*	0.001*	0.003*	0.001*	0.02*	8.41E-06*	0.002*
T2:T6	0.61	0.49	0.08	0.27	0.76	0.02*	0.15	0.98	0.06	0.04*
T2:T7	0.08	0.06	0.01*	0.005*	0.004*	0.25	0.01*	0.99	0.01*	0.58
T2:T8	0.31	0.15	0.03*	0.05*	0.004*	0.06	0.11	0.10	0.36	0.004*
T2:T9	0.21	0.85	0.23	0.87	0.06	0.06	0.37	0.37	0.18	0.92
T2:T10	0.20	0.11	0.21	0.23	0.37	0.34	0.37	0.37	0.12	0.12

*P<0.05

Table 5: One-way ANOVA of plant parameters in different treatments for maize.

Treatments**	Shoot length at the time of growth			At the time of harvest			
	30 days (cm)	45 days (cm)	70 days (cm)	No. of leaves	Shoot length (cm)	Root length (cm)	Plant dry weight
T2:T1	0.01*	0.002*	0.01*	0.007*	0.0001*	0.0009*	0.0008*
T2:T3	0.54	0.02*	0.32	0.51	0.004*	0.001*	0.005*
T2:T4	0.01*	0.001*	0.16	0.007*	0.30	0.001*	0.002*
T2:T5	0.79	0.006*	0.05*	0.01*	0.02*	0.001*	0.0007*
T2:T6	0.26	0.30	0.12	0.01*	0.001*	0.001*	0.01*
T2:T7	0.02*	0.01*	0.03*	0.10	0.0003*	0.007*	0.04
T2:T8	0.01*	0.02*	0.04*	0.34	0.006*	0.003*	0.01*
T2:T9	0.01*	0.01*	0.01*	0.98	0.96	0.007*	0.03
T2:T10	0.73	0.02*	0.11	0.97	0.69	0.06	0.08

*P<0.05

** (Treatments: T1 - control, T2 - full dose NPK, T3 - *Spirogyra* sp, T4 - *Microspora tumidola*, T5 - mixed green algae, T6 - *Oscillatoria curviceps*, T7 - *Nostoc commune*, T8 - *Cylindrospermum muscicola*, T9 - mixed Cyanobacteria, T10 - mixed algae and ¼ NPK)

DISCUSSION

Organic fertilizer and biofertilizer are beneficial because they limit the application of chemical fertilizers which have been already known to cause a variety of harm to the environment. Cyanobacteria and green algae play an important role in maintenance of soil fertility by providing fixed nitrogen.

Mixed application of green algae and Cyanobacteria (T9) lead to an increase in growth independent of chemical fertilizers and give the same result as that with full dose of NPK (T2) for both rice and maize. Biofertilization with proper use of Cyanobacteria and green algae will greatly reduce the use of fertilizers by almost 50% without compromising the growth and biomass of crop plants. This observation is in line with studies by [5] who concluded that *Microcystis aeruginosa*, *Anabaena* sp. and *Chlorella* sp significantly increased seed germination and growth of corn seedlings while also intensifying metabolic processes. This also agreed with our results where vegetative growth improved in maize plants with inoculated

treatments as full dose NPK. Algal biofertilizer in different combination with organic and inorganic fertilizers showed positive growth on different growth parameters of *Vigna radiata*. Increased growth and physiological performance was observed with application of selected strains of *Microcystis aeruginosa*, *Anabaena* sp. and *Chlorella* sp without significant effect on growth and biomass of willow [6]. Further, they reported marked reduction almost one-two in the doses of chemical fertiliser.

Inhibition of germination of rice with *Spirogyra* extract was reported [13]. In our study increased seed germination and growth has been observed only with *Microspora* inoculum and not with *Spirogyra* sp. However, high seeds germination of jowar, mothbean and sesamum with *Spirogyra* extract was reported [7]. Stimulatory effects of *Spirogyra jugalis* extracts on seeds germination and plant growth of tomato plants was also observed [9]. In the present study, germination of maize seeds was independent of any inoculation type. But seed germination and seedling growth of treated maize seeds in *Spirogyra* extract was comparatively better than that of rice. Significant increase in germination and seedling growth of alfalfa in *Spirogyra* extract was studied [2]. Furthermore, they reported a significant increase in number of leaves and plant height in soil inoculated with *Oscillatoria* compared to those treated with *Spirogyra*.

In the present study, no significant variation was observed in the height of rice plants when treated with T6 (*Nostoc muscorum*) and T9 (mixed Cyanobacteria) when compared with T2 (uninoculated, full dose NPK). But in maize, vegetative growth i.e. the height, number of leaves and dry weight increased with T9 (mixed Cyanobacteria) and T10 (Cyanobacteria and algae + ¼ NPK) inoculated in the soil. This has also been confirmed by many researchers. Significant increase in growth parameters of wheat, sorghum, maize and lentil with the use of *Nostoc muscorum* as biofertilizer compared to control was observed [1], while high grain yield of wheat was observed when treated with *Nostoc* sp (single species inoculation) [8]. Increased in shoot length was observed but leaf area of maize remained unaffected when inoculated with *Nostoc muscorum* or *Nostoc rivulare*, which showed the same results as those when treated with chemical fertilizers [14]. From the present experiment, it could be said that supplementation of Cyanobacteria with small quantity of fertilizer will result in high yield and therefore can greatly reduce the use of chemical fertilizers.

CONCLUSION

Depletion of soil fertility, high fertilizer use and environmental pollution are major concern to agricultural crop productivity. Thus, the use of biofertilizer can prove a suitable supplement to the chemical fertilizers. Mixed application of green algae and Cyanobacteria lead to an increase in growth independent of chemical fertilizers and give the same result as that with full dose of NPK for both rice and maize. Yield in rice could be monitored through pot experiment but yield in maize was observed to be dependent on other factors like soil surface. Thus application of Cyanobacteria and algal strain in fields could minimize the use of chemical fertilizer.

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