

Effect of *Spirulina* on The Germination and Growth of *Cicer arietinum* L.

Thet Naing Htwe¹, Moat War Dine Naw², Min Thein³

Abstract

Spirulina was used as a biofertilizer in cultivation of chick pea seeds. These chick pea seeds were treated with 2%, 4%, 6%, 8%, and 10% of *Spirulina* powder (w / w) and experimentally studied in laboratory, pot and plot culture. Germination percentage of chick pea was observed that *Spirulina* 2% in laboratory, pot and plot culture was the best. On the shoot growth in pot and plot culture, *Spirulina* 6% treatment was better than other percentages and control during third week. Protein content of chick pea desi type cultivated in control and *Spirulina* treatment were 13.03% and 18.80%, showing a 45% significant increase with treatment over control. It is obvious that Myanmar *Spirulina* actually promotes the germination, the growth and protein content of chick pea. It can be pointed out that this increase in protein content of chick pea, due to prebiotic effect of *Spirulina* on the root nodule system, that increase the nutrients value is a major mile stone in the use of microalgae biotechnology in agriculture. Further study with other legumes should also be investigated extensively to solve the potential problem of food in recent year.

Key words: *Spirulina*, chick pea, protein

Introduction

The nutritive values of chick pea are varied. The average nutritional composition of chick pea seed is as follows; moisture 9.8%, protein 17.1%, fat 5.3%, carbohydrate 61.2%, crude fibre 3.9% and ash 2.7% (Bahl 2003). For good field of legumes, fertilizer is important. Biofertilizers are microorganisms that enrich the nutrient quality of soil. It has long been known for good biofertilizer agents.

This investigation has shown that mixing *Spirulina* powder and chick pea seeds can enhance the germination and growth of the seedlings. The aim of this study is to know the effectiveness of various treatments of *Spirulina* powder (0%, 2%, 4%, 6%, 8% and 10%) on chick pea plant. The chick peas were experimentally studied with laboratory, pot and plot culture with desi type. In this study, the effect of *Spirulina* on the germination, growth and protein contents of chick pea were conducted. The results showed 6% *Spirulina* treatment is the best for cultivation of chick pea.

1. Demonstrator, Department of Botany, Yenanchaung Degree College

2. Assistant Lecturer, Department of Botany, Kalay University

3. General Manager, Myanmar Pharmaceutical Factory (MPF), Yekharr, Sagaing

Materials and Methods

In the present works, laboratory culture and pot culture experiments were conducted at Botany Department, Mandalay University, Mandalay. And plot culture experiment has been carried out at Myanmar Pharmaceutical Factory (MPF), Yekharr, Sagaing. *Spirulina* biomass (Lot No. S - 324) from MPF was used in this study as shown in appendix I. Chick pea seeds were obtained from Ministry of Agriculture and Irrigation, Mandalay. In this experiment triplicate treatments and control were repeated three times at the same condition.

Laboratory Culture

Before testing, petridishes were autoclaved for 45 minutes at temperature 100°C, 15 psi. Then different percentages of *Spirulina* (2%, 4%, 6%, 8% and 10%) biomass and 2 g of chick pea seeds were selected and weighed according to w / w ratio by using analytical balance. Before sowing, ten seeds were mixed with different percentages of *Spirulina* biomass and soaked in water for one hour. This seeds were put on blotting paper in each petridish according to biomass treatment percentage and control. And then, these petridishes were placed at room temperature (ranging from 27° C - 32° C) and natural condition, 1 cc of water was added to each petridish to get moisture. Each petridish was regularly watered with 1 cc once a day. After 7 DAS, the germination percentages were calculated.

Pot Culture

The soil (4 kg) was prepared in the earthen pots, which has 12' 3" in diameter and 10" in height. Different percentages of *Spirulina* biomass and 2 g of chick pea seeds were weighed by analytical balance (w / w). Before sowing, this seeds were mixed with different percentages of *Spirulina* biomass and soaked in water for one hour. This seeds were sown at the loam soil in each pot according to biomass treatment percentages and control. This seeds were planted in the depth of 2 - 3 cm approximately in the pots. And then, these pots were placed under natural condition. Each pot was daily watered with 1 liter. The germinating seeds were counted and shoot height and root lengths were measured by ruler. After 30 days, the fresh and dry weights of the whole plant (25 plants) were weighed by using analytical balance.

Plot Culture

The experiment was done during February to April, 2008 and utilized a randomized complete blocks design (RCBD). The soil was prepared in the plot, which was 0.6 m × 1.5 m. There were six rows with 0.3 m inter-row spacing in each plot. 5 g of chick pea seeds and different percentages (2%, 4%, 6%, 8% and 10%) of *Spirulina* biomass were weighed by analytical balance (w / w). Before being sown, these seeds were mixed with different percentages of *Spirulina* biomass and soaked in water for one hour. This seeds were sown in each loam soil row according to biomass treatment percentages and control. These plots were regularly watered. After germination, the mean numbers of seedlings were calculated and the height of each shoot and root length were measured with a ruler. And then, the fresh and dry weights of the whole plant were marked by weighing with analytical balance at 30 days after sowing (DAS).

Determination of Protein - Digestion, Distillation, Titration, Calculation of Protein

Determination of Moisture

Statistical Data Analysis

Germination percentages (laboratory, pot and plot culture) of chick pea were counted. Shoot heights, root lengths of chick pea in pot and plot culture collected from different percentages of *Spirulina* were calculated. Comparison between the mean of the treatment percentages and of the control was also made. The fresh and dry weights and root length of chick pea were weighed, measured and expressed the mean (25 seedlings for each). Untreatment controls were used for a comparison.

Results

Germination and growth of Chick Pea in Laboratory, Pot and Plot Culture

Chick pea (desi type) was tested by *Spirulina*. The effect of *Spirulina* percentages on germination and growth of chick pea in laboratory, pot and plot culture are presented in Table 1.1 to 1.7. It was found that 2% *Spirulina* treatment was the best for germination of chick pea in all culture methods. In the laboratory culture, the desi type chick pea seeds germinated 63.33% at control and 93.33% at 2% *Spirulina* treatment on the third day.

The germination percentages were calculated two weeks after sowing (WAS) in pot and plot culture. The germination percentage of desi type chick pea in pot culture was 77.77% at control and 91.66% at *Spirulina* treatment. The mean shoot height of chick pea at different percentages and control was mentioned in Table 1.3 and 1.4. In the first and second week, the shoot heights of chick pea were better in 2% *Spirulina* treatment than control and other treatment *Spirulina* percent. However, in the third week 6% *Spirulina* treatment was the best on the growth of shoot. The mean shoot height in control was 7.60 cm, but it was 11.26 cm in the treatment. The shoot heights of chick pea in pot and plot culture were the same. Mean fresh and dry weights of the whole plant of chick pea in pot and plot culture are shown in Table 1.5 and 1.6. Comparative study with respect to control, showed with application of 6% *Spirulina*, the fresh weight of chick pea was 3.95 g / plant in pot culture (Table 1.6). The fresh weight of chick pea in plot culture was 1.59 g / plant. Mean root length of chick pea in pot and plot culture are shown in Table 1.7. It was found that the mean root length of chick pea in pot and plot culture were 13.27 and 11.7 cm at control and 22.90 and 22.54 cm at 6% *Spirulina* treatment.

Protein Contents of Chick pea in Pot Culture

In desi type, the protein content of *Spirulina* 6% treatment was found to be 18.80% and 13.02% at control in pot culture. The moisture content of desi type chick pea was recorded (Table 1.8).

Table 1.1 Mean germination percentages of chick pea seeds at 7 DAS
(Lab culture)

Control and treatment	First Day	Second Day	Third Day
Control	33.33%	53.33%	63.33 %
2%	63.33%	80.00%	93.33%
4%	56.66%	76.66%	86.66 %
6%	50.00%	73.33%	83.33 %
8%	46.66%	66.66%	76.66 %
10%	43.33%	56.66%	73.33 %

Table 1.2 Mean **germination** percentages of chick pea seeds at 14 DAS (Pot and Plot culture)

Control and treatment	14 DAS (Pot)	14 DAS (Plot)
Control	77.77 %	45.33%
2%	91.66 %	86.66%
4%	88.88 %	72.00%
6%	88.88 %	70.66%
8%	83.33 %	60.00%
10%	80.55 %	56.00%

Table 1.3 Effect of *Spirulina* biomass on the **shoot height** of chick pea at 30 DAS (Pot culture)

Control and treatment	Mean shoot height (cm) \pm sd of chick pea		
	First Week	Second Week	Third Week
Control	2.44 \pm 0.85	4.38 \pm 1.36	7.60 \pm 2.16
2%	4.22 \pm 0.86	6.34 \pm 1.04	10.30 \pm 2.19
4%	3.96 \pm 1.05	6.11 \pm 1.57	10.54 \pm 2.45
6%	3.92 \pm 1.22	6.06 \pm 0.76	11.26 \pm 1.91
8%	3.63 \pm 1.45	5.95 \pm 1.11	10.22 \pm 1.49
10%	3.45 \pm 1.01	5.42 \pm 1.00	9.85 \pm 2.17

Table 1.4 Effect of *Spirulina* biomass on the **shoot height** of chick pea at 30 DAS (Plot culture)

Control and treatment	Mean shoot height (cm) \pm sd of chick pea		
	First Week	Second Week	Third Week
Control	2.28 \pm 0.53	4.53 \pm 3.00	7.51 \pm 2.93
2%	3.67 \pm 0.84	6.58 \pm 3.33	8.30 \pm 2.33
4%	3.60 \pm 1.01	7.36 \pm 3.24	9.17 \pm 2.44
6%	3.52 \pm 1.20	8.88 \pm 2.76	11.04 \pm 2.58
8%	3.16 \pm 1.34	6.60 \pm 3.55	10.16 \pm 3.60
10%	3.04 \pm 0.99	7.69 \pm 2.83	9.88 \pm 3.87

Table 1.5 Mean fresh and dry weight of the whole plant of chick pea at 30 DAS (Pot culture)

Control and treatment	30 Days	
	Fresh weight (g / p)	Dry weight (g / p)
Control	1.28 ± 0.29	0.32 ± 0.13
2%	1.91 ± 0.30	0.47 ± 0.18
4%	2.73 ± 0.27	0.68 ± 0.21
6%	3.95 ± 0.24	0.93 ± 0.24
8%	2.93 ± 0.34	0.72 ± 0.23
10%	2.55 ± 0.37	0.63 ± 0.22

Table 1.6 Mean fresh and dry weight of the whole plant of chick pea at 30 DAS (Plot culture)

Control and treatment	30 Days	
	Fresh weight (g / p)	Dry weight (g / p)
Control	0.79 ± 0.34	0.19 ± 0.12
2%	1.46 ± 0.41	0.36 ± 0.19
4%	1.48 ± 0.33	0.37 ± 0.18
6%	1.59 ± 0.40	0.42 ± 0.21
8%	1.18 ± 0.36	0.29 ± 0.12
10%	1.08 ± 0.30	0.27 ± 0.14

Table 1.7 Mean root length of chick pea at 30 DAS (Pot and plot culture)

Control and treatment	Pot culture (cm)	Plot culture (cm)
Control	13.27 ± 1.54	11.70 ± 0.95
2%	16.52 ± 1.81	15.41 ± 1.21
4%	18.67 ± 0.80	17.15 ± 0.91
6%	22.90 ± 2.49	22.54 ± 2.14
8%	20.19 ± 1.73	19.88 ± 2.18
10%	18.31 ± 1.09	19.38 ± 0.82

Table 1.8 Protein and moisture content of chick pea (Pot culture)

Control and treatment	Desi type (Pot)	
	Protein (%)	Moisture (%)
Control	13.02	24.46
6%	18.80	30.69

**Effect of different percentages of *Spirulina* on the growth of Chick pea**

Discussion and Conclusion

Chickpea is an important winter grain legume crop in Myanmar and is cultivated as a second crop after rice (Bahl, 2003). It is one of the best legumes for human consumption as seeds are very nutritious. The seeds of chick pea were consumed by all classes of people in Myanmar. Not only the seeds but all other parts of the plant are said to be rich in protein. There are two types of chickpeas – desi and kabuli. In this study, the desi type was investigated, by mixing the seeds with *Spirulina* powder (2% to 10% treatment).

In the present study, the best germination percentage was 2% treatment and other percentages were better than control in this type. The whole plant of fresh and dry weight of 6% treatment was better than control and other percentages of *Spirulina* in pot and plot culture. The protein content of *Spirulina* 6% treatment in chick pea was 25.59% higher than control. Present studies on chick pea observed that the protein content of *Spirulina* 6% treatment was higher than the results obtaining by previous researchers.

Germination is very important in plant cultivation. This research emphasized on the effect of *Spirulina* on the germination and growth of chickpea. The results showed that *Spirulina* stimulates the germination of chick pea. The germination rate was found to be w / w respectively decreasing with increasing percentage of *Spirulina*. It showed that *Spirulina* contains much gibberellin like substances, which promotes the germination of seeds. However, the high level of gibberellin or plant hormone can also reduce germination rate. Some plant hormones enhance the shoot system and some promote the root system of plants. In this research several different percentages of *Spirulina* were mixed with chick pea seeds and cultivated in laboratory, pot and plot. The length of shoot and root, and the number of root hairs were increased by using *Spirulina*. The shoot system of chick pea was the best in *Spirulina* 6% treatment. According to these results, *Spirulina* probably contain some plant hormone like substances. *Spirulina* 6% treatment can promote the root development of chick pea.

The pulses are used as staple food to supply some or all of their protein requirements in most tropical countries. Chick pea is the second most important pulse crop in the world. Nutritionally, pulses are 2 to 3 times richer in protein than cereal crops. Nitrogen is very important element for formation of protein in crops. Nitrogen fixation is a well known legume characteristic. *Rhizobium*, beneficial bacteria species lives symbiotically in root nodules of leguminous plants and is able to fix atmospheric nitrogen. *Spirulina* promotes the growth of *Rhizobium*, which fix nitrogen for plants. For this reason, the protein contents of chick pea increased up to 45% by using *Spirulina*. The utilization of *Spirulina* as natural biofertilizer is not harmful to both living things and environments. It can be concluded that small amount of *Spirulina* can promote germination, growth and protein content of chick pea. The other benefit obtained from using *Spirulina* in bean cultivation and can be improve the soil fertility of the fields.

Acknowledgements

My deepest gratitude also goes to my supervisor Part-time Professor Dr. Min Thein, General Manager, Myanmar Pharmaceutical Factory (MPF), Yekharr, Sagaing, for his supervision, departmental facilities, valuable guidance, advice and encouragement for the success of this research. Invaluable appreciations are tendered to my supervisor, Dr. Moat War Dine Naw, Assistant Lecturer, Department of Botany, Kalay University for her guidance, understanding and cooperation throughout this research work.

I also specially wish to express my heart-felt thanks to U Tin Aung, Principle of Yenanchaung Degree College and Daw Khin Htwe, Professor, Botany Department, Yenanchaung Degree College for their kind permission and encouragement.

References

- Bahl P.N, 2003. *Pulses Training Manual*, Improved grain legume production Technologies Project TCP/MYA/0166 (A), FAO.
- Castenholz, R.W., And Waterbury, J.B. 1989. Oxygenic photosynthetic bacteria. Section 19, In: Staley, J.T., Bryant, M.P., Penning, N., Holt, J.G., Eds. *Bergey's Manual of Systematic Bacteriology*. Vol. 3, Williams and Wilkins Co, Baltimore, USA.
- Ghosh S.P., 1998. *Biotechnology and its Application in Horticulture*.
- Min Thein, 1987. *Laboratory examination of Spirulina samples from Burma and a study of Spirulina production and use*. UNIDO / UNDP / BUR / 85 / 018 in Netherlands, USA and Mexico.
- Purseglove J.W, 1968. *Tropical crops*. Dicotyledonous I. Longman, Green and Co. Ltd.
- Pyone Yi, 2007. *Morphology, Nutritional Value and Production of Pulses in Central Myanmar*. PhD Thesis, Department of Botany, Mandalay University.
- Sai Aung Hsan, & Sai Long Kyio Hsan, 2000. *Biochemical Genetics and Biostatistics*. Lecture Notes for M. Sc. Final Part 1 students in Department of Botany of Mandalay University, Mandalay.
- Sirenko L.A. & Pulz O., 2001. *International Symposium on Micro algae and Seaweed Products in Plant/Soil-System*. Mosonmagyer Ovar, Hungary, June 20-22.
- Venkataraman G.S., 1981. *Blue-green algae for rice production a manual for it promotion*. FAO soils Bulletin UN.
- Vincent E., Rubatzky M. Y., 1983. *World Vegetables (Principles, Production and Nutritive Values)*. International Thomson Publishing, New York.