Effect of *Spirulina* Suspension on Germination, Growth and Yield of *Phaseolus lunatus* L. (Lima Bean)

Khaing Khaing[1], Khin Soe Aye[2], Cherry Soe[3]

Abstract

The effect of *Spirulina* on germination, growth and yield of *Phaseolus lunatus* L. (Lima bean) was studied by using *Spirulina* suspension under the laboratory and plot experiment. Laboratory experiment was carried out at Department of Botany, Pakokku University, during June to August 2017. The seed of lima bean were treated with different concentration of *Spirulina* suspension ($1gl^{-1}$, $2gl^{-1}$, $3gl^{-1}$, $4gl^{-1}$ and $5gl^{-1}$) and assigned as T1, T2, T3, T4 and T5 by using Randomized Complete Block Design (RCBD) with five replication. Among them, 4 gl^{-1} of *Spirulina* suspension treatment showed the best germination percentage, shoot and root length of lima bean. Based on this fact, plot experiment of this lima bean were grown with two different concentration of *Spirulina* suspension ($3gl^{-1}$ and 4 gl^{-1}). These experiments were conducted at Mindan Village, Myaung Township during October to January 2018. $4gl^{-1}$ of *Spirulina* suspension treated on lima bean had better plant height, total leaf area, total dry matter, yield and yield component characters than the control. According on these results, *Spirulina* biomass could give a potential algal biofertilizer. Thus *Spirulina* biofertilizer has an effective role in germination, growth and yield of lima bean.

Keyword: *Spirulina*, biofertilizer, Lima bean, germination, growth and yield

Introduction

Lima bean originated from Mexico and Peru. Once domesticated, it was spread throughout the Americas and imported to the Pacific Islands and Philippines. It later spread to the South-East Asia while the slave trade resulted in the introduction the lima bean to Western and Central Africa. Lima bean is the main pulses in wet region of tropical Africa and is widely grown in India and Myanmar. It is now cultivated throughout the warmer parts of the world. The largest share of exports goes to China and Malaysia was major important of Myanmar lima bean (Vander Maesen, 1992). In recent decade, agricultural scientists and farmers were interested in natural and biofertilizers to substitute the chemical fertilizers. The main sources of biofertilizer were bacteria, fungi, cyanobacteria (blue green algae) and other macro and micro algae. They are known to deliver a number of benefits including plant nutrition, nitrogen fixation, disease resistance and tolerance to adverse soil and climate condition. Biofertilize is living fertilizer compound of microbial inoculants or groups of microorganisms which are able to fix atmospheric nitrogen or solubilize phosphorous decompose organic materials or oxidize sulphur in the soil. On application, it enhanced the growth of plants, increased in yield and was able to improve soil fertility and reduced pollution (Nguvu, 2009).

Spirulina is an edible micro-organism which naturally grows in alkaline lakes of tropical and sub-tropical regions. *Spirulina* lakes are found in Peru, Chile, Myanmar, Australia and stretch across the Sahara and East Africa. It was eaten widely by the Aztecs of Mexico up till the 16th century and the Africans in the vicinity of lakes Chap up till now. *Spriulina* of which the most popular known species are *Spirulina platensis* and *Spirulina maxima*, a group of cyanobacteria characterized by loosely spiral-shaped

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trichome arranged in an open helix enclosed in a thin mucilaginous sheath (Castenholz & Waterbury, 1989).

In Myanmar, the Ministry of Industry is running a project under the direction of Dr Min Thein to exploit the natural production of *Spirulina* in 4 lakes. These lakes are a series of volcanic creater lakes with bicarbonate and carbonate salts and Ye Kharr is a shallow lake with its salt content mainly as sulphate. Than Tun (1959) studied on the inter-relationships between photosynthesis and nitrogen fixation in a blue-green alga. Kyaw Soe Naing and Win Naing Oo (2008) had studied the effect of *Spirulina* on the germination and growth of three important oil crops and cereal crops.

The aims of this study areto investigate the effect of *Spirulina* biofectilizer on improvement of the germination of lima bean and to analyze the effect of different concentration of *Spirulina* suspension on growth and yield of lima bean in plot culture.

Materials and Methods

In the present study, laboratory experiment was conducted at Department of Botany, Pakokku University, during June to August 2017. The *Spirulina* powder from Myanmar Pharmaceutical Factory, Yekharr, Sagaing Region was used. Lima bean seeds were obtained from Myanmar Agricultural Service, Pakokku. The plastic petridishes 16 cm in diameter and 4.5 cm in height were used. In this experiment, *Spirulina* powder was weighed according to w/w ratio and different concentration such as (1 g/l, 2 g/l, 3 g/l, 4 g/l and 5 g/l) by using digital balance. The various weight and *Spirulina* powders were dissolved in pure water for about 24 hours. And then, different concentrations of *Spirulina* suspension were obtained. The seeds of lima bean were soaked in different concentration of *Spirulina* suspension for 12 hours. Then, control was soaked in the pure water.

After treatment, twenty five lima bean seeds were placed on tissue paper in each petridish according to different treatment and control. The petridishes were covered with lids to prevent from drying, and placed at room temperature (18º-23ºC) in natural condition. The experimental design wasarranged in Randomized Complete Block Design (RCBD) with five replications. Twenty milliter of water was added to each petridish to get moisture. Each petridish was regularly water with 10 ml once a day. The percentage of germinating seeds was counted on 2 DAS. The shoot and root length (cm) were measured in 7 DAS by using ruler. Percentage germination was recorded for every 24 h after the treatment up to 72 h. Percentage of germination was measured according Achakzai (2009) and it was expressed in terms of percentage (%).

$$\text{Germination percent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Fig. 1 Experimental layout in RCBD design

Plot Experiment

The plot experiment was conducted at Mindan Village, Myaung Township during October to January 2018. The wild grasses were cut and the land was ploughed to clear the the root-stocks and to clean the land a week before the experiment was started. This experiment used lima bean seed. Randomized Complete Block Design (RCBD) was used with five replications. Each plot was 2.7 m × 3.5 m in size. It was consist of six rows per plot with spacing adjacent rows of 0.45 m and adjacent plants of 0.15 m. The outermost rows were bordered, the second inner rows were sampled and the two innermost rows were harvested. Lima bean seeds were

soaked in different concentrations of *Spirulina* suspension in water for 12 hours. The different treatments were Control, T_3 ($3gl^{-1}$) and T_4 ($4gl^{-1}$). The plant germinated wasthinned to one plant per stand at 14 DAS.

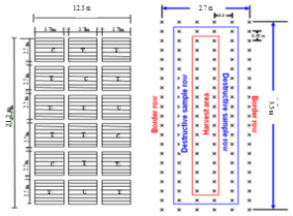

Fig. 2. Randomized Complete Block Design and experimental plot layout (Source: IRRI 1995)

Data Collection

Plant height (cm) at 2 weeks interval after planting or sowing and at maturity. This done by measuring the plant height of all plant at ground level to the tip of the highest leaves or apical leaves. Starting from 91 DAS three consecutive plants from one row of each plot were randomly collected biweekly. Thus leaf length, maximum width of four representative leaflets, stems and roots length were measured by a ruler. These bags were spread out in air dry until to get the constant dry weight. The leaf area was determined by using the formula leaf area (cm^2) = leaf length × leaf breath × Adjustment Factor (K). The proportion of biological yield represented by economic yield has been called the harvest index (HI). All these terms characterize the movement of dry matter to the harvest part of the plant (Gardner *et al*. 1985).

$$HI = \frac{\text{Economical yield}}{\text{Biological yield}}$$

Plant height, total leaf area and total plant dry matter (plot experiment) of lima bean were recorded and calculated by using students 't' test.

Results

Morphological Character of Lima Bean

Scientific Name - *Phaseolus lunatus* L.
English Name - Lima bean
Local Name - Pe ni gya
Family - Fabaceae

Small climbers or erect herbs, herbaceous bushes, annual, 1 m in height. Leaves trifoliolate, imparipinnate, alternate; stipules foliaceous, pulvinous; petiolate. Inflorescence axillary racemes, few to numerous flowered, 2-4 flowered at each node. Flowers bisexual, zygomorphic, hypogynous, white to yellow. Calyx 5, synsepalous, campanulate. Corolla 5, polypetalous, papilionaceous; standard ovate-orbicular the outermost large one; wings obliquely obovate; keels incurved, glabrous. Stamens 1+ (9), diadelphous, free, white; anther ovoid, dithecous, equal, basifixed. Ovary monocarpellary, superior, marginal placentation; style long; stigma simple. Fruit pod, linear-oblongoid, straight or curved, flattened. Seeds 3-6 seeds, obovoid, slightly compressed, white with red spot (Figure 3).

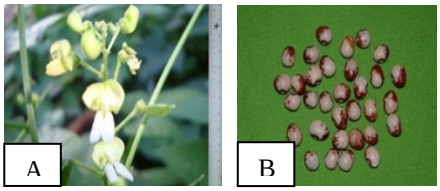

Fig. 3 A. Inflorescence of *Phaseolus lunatus* L.
B. Seed of *Phaseolus lunatus* L.

The Effect of *Spirulina* Suspension on Lima Bean in Laboratory Experiment

The result of the effect of the different concentration of ($1\ gl^{-1}$-$5gl^{-1}$) treatment of *Spirulina* suspension were tested as biofertilizer on the germination, shoot and root length were showing in Table 1. The highest germination percentage 92% and control

72 % was found in *Spirulina* suspension treatment 4 gl^{-1} on 2 DAS. The mean shoot length of 4 gl^{-1} treatment was 12.10 cm but control was 7.90 cm. The mean root length of 4 gl^{-1} treatment was 5.68 cm and that of control was 3.82 cm on 7 DAS (Table 2, Figure 5, 6).

Table 1. Effect of different percentage of *Spirulina* suspension on the germination of lima bean (Laboratory experiment)

Control and Treatment (gl^{-1})	Germination percentage (%)
C	72
T1	76
T2	80
T3	88
T4	**92**
T5	84

Fig. 4. Comparison on the effect of different concentrations of *Spirulina* suspension on mean germination percentage of lima bean

Table 2. Effect of different *Spirulina* suspension (gl^{-1}) on shoot and root length of lima bean at 7 DAS in Laboratory experiment

Spirulina Suspension Treatment (gl^{-1})	7DAS	
	Mean shoot length (cm) ± sd	Mean root length (cm)± sd
Control	7.90 ± 0.495	3.82 ± 0.516
T1	8.32 ± 0.454	4.12 ± 0.438
T2	9.26 ± 0.611	4.66 ± 0.660
T3	10.35 ± 0.259	5.25 ± 0.482
T4	**12.10 ± 0.837**	**5.68 ± 0.376**
T5	10.30 ± 0.155	5.16 ± 0.403

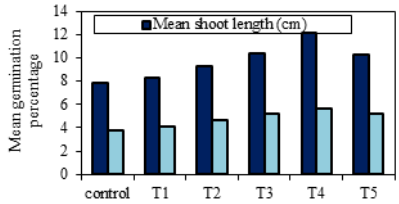

Fig. 5. Comparison on the effect of different concentration of *Spirulina* suspension on mean shoot and root length of lima bean

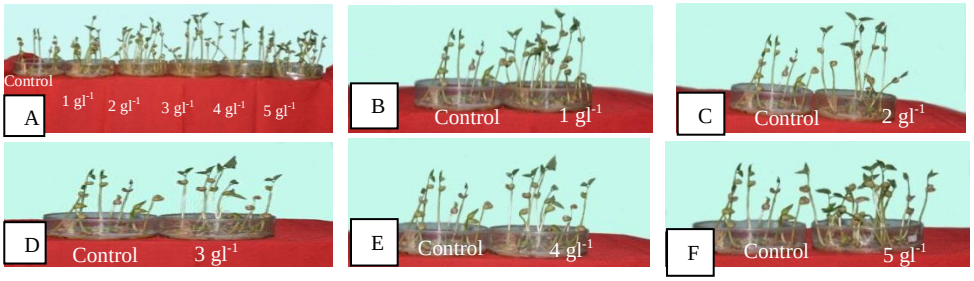

Figure 6 The effect of different concentrations of *Spirulina* suspension on germination of lima bean in laboratory experiment (7 DAS)

A. C and 1 to 5gl^{-1} of *Spirulina* suspension
B. C and 1gl^{-1} of *Spirulina* suspension
C. C and 2 gl^{-1} of *Spirulina* suspension
D. C and 3gl^{-1} of *Spirulina* suspension
E. C and 4 gl^{-1} of *Spirulina* suspension
F. C and 5 gl^{-1} of *Spirulina* suspension

The Effect of *Spirulina* Suspension on lima bean in Plot Experiment

The effect of different *Spirulina* suspension treatment on mean plant height of lima bean in plot experiment was showed in Table 3. The mean plant height (cm) treated with different concentrations (3gl^{-1} and 4gl^{-1}) of *Spiurlina* suspension. The highest mean value of plant height on 49DAS was 36.16 cm and control was 31.33 cm with *Spirulina*

suspension 4 gl^{-1}. The highest mean leaf area on 49 DAS was 38.35 cm^2 that of control was 28.01 cm^2 in *Spirulina* suspension 4gl^{-1} on 49 DAS (Table 4, Figure 7). And then number of pods per plant and no. of seeds per pod were maximum at T_4. The highest pods per plant was obtained in T_4 (47.29) and control (31.90). The maximum no. of seeds per pod was found in T_4 (5.70) and control (3.10). The optimum mean total plant dry weight (gm^{-2}) was found in T_4 (865.70 gm^{-2}) and control (763.60 gm^{-2}). The highest mean, dry weight of seeds was observed in T_4 (615.21 gm^{-2}) and control (509.36 gm^{-2}) (Table 5).

Table 3. The effect of different *Spirulina* suspension treatments on mean plant height (cm) of lima bean (Plot experiment)

Spirulina Suspension Treatment(gl^{-1})	Mean Plant height (cm) ± Standard deviation		
	21 DAS	35 DAS	49 DAS
Control	16.46 ± 0.730	22.56 ± 2.769	31.33 ± 3.053
T_3	18.94 ± 0.907	25.10 ± 1.568	33.40 ± 2.851
T_4	**19.54 ± 1.803**	**27.28 ± 1.845**	**36.16 ± 3.341**

Table 4. The effect of different *Spirulina* suspension treatments on mean leaf area (cm^2) of lima bean (Plot experiment)

Spirulina Suspension Treatment(gl^{-1})	Mean leaf area (cm^2) ± Standard deviation		
	21 DAS	35 DAS	49 DAS
Control	4.78 ± 0.677	16.20 ± 3.320	28.01 ± 3.669
T_3	6.92 ± 0.970	20.35 ± 7.187	**38.35 ± 11.490**
T_4	**7.62 ± 0.326**	**23.26 ± 4.928**	33.25 ± 9.609

Table 5. Effect of *Spirulina* suspension on yield and yield component of lima bean at 91 DAS (Plot Experiment)

Control & Treatment	No. of Pods $plant^{-1}$	No. of Seeds pod^{-1}	100 seeds Wt.(g)	TDM (gm^{-2})	HI	D.W of seeds (gm^{-2})
C (control)	31.90	3.10	29.45	763.60	0.62	509.36
T_3 (3 gl^{-1})	39.25	5.05	30.28	809.50	0.59	578.23
T_4 (4 gl^{-1})	47.29	5.70	34.71	865.70	0.53	615.21

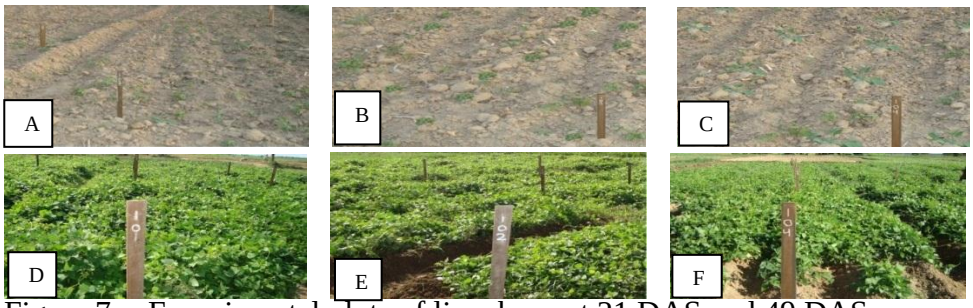

Figure 7. Experimental plots of lima bean at 21 DAS and 49 DAS

A. Experimental layout for lima bean at 21 DAS
B. Plot no. of T_3 (3 gl^{-1}) at 21DAS
C. Plot no. of T_4 (4 gl^{-1}) at 21 DAS
D. Experimental layout for lima bean at 49 DAS
E. Plot no. of T_3 (3 gl^{-1}) at 49 DAS
F. Plot no. of T_4 (4 gl^{-1}) at 49 DAS

Discussion and Conclusion

The effects of *Spirulina* suspension with different concentration on lima bean showed the best germination and seedling growth in 4 gl^{-1} treatment. Win Naing Oo (2008) reported that the effect of different presoaking period in *Spirulina* suspension (2gl^{-1}) was the best for germination and shoot growth of wheat.

The results of the present plot experiment indicated that the *Spirulina* suspension effect on the growth and yield of lima bean. All morphological characters increased with age except leaf character. In the present study, *Spirulina* suspension 4 gl^{-1} produced the maximum plant height, no. of pods per plant, no. of seeds per pod and 100 seed weight than control. Sandhu *et al.*, (1980) indicated that pods per plant, seeds per pod and 100 seed weight were found to be the important yield component characters for yield of mung bean. It was observed that the harvest index (HI) of

Spirulina suspension 4 gl^{-1} and control did not significantly differ from each other. Effect of *Spirulina* suspension 4 gl^{-1} on lima bean possessed lower the harvest index. This may be due to the highest amount of TDM. Gardner *et al.* (1985) stated that crop yield can be increased either by increasing the total dry mutter produced in the field or by increasing the proportion of economic yield (the harvest index) or both and there is potential for increasing yield by both methods. The result of the study agreed with thefindings. According to the results, 4 gl^{-1} produced the highest weight of total dry matter was 865.70 gm^{-2} that of control was 763.60 gm^{-2}. Similarly, 4gl^{-1} produced the maximum weight of seeds was 615.21 gm^{-2} and control was 509.36 gm^{-2}. Thus, 4gl^{-1} treatment were chosen for the field experiment.

These results recommended *Spirulina platensis* could be used as a successful biofertilizer. There wasan increase in lima bean seed germination, growth, yield and of seeds by using *Spirulina*. The quality crops could be produced by biofertilizer *Spirulina* as well as it is safer from the health point of view. Therefore, the use of the bio-fertilizer *Spirulina* may cover shortage in these nutrients. It can be concluded that T_4 would be optimum to produce higher yield of lima bean.

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