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Research Paper

Comparative study of impact of Rhizobium, Phosphate Solubilizing Bacteria, Vermicompost and Urea fertilizer on growth parameters, Chlorophyll and Protein content of soybean (*Glycine max*)

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Abstract

A pot experiment was conducted to evaluate the impact of biofertilizer and chemical fertilizer on growth parameters, Chlorophyll and Protein content of soybean *(Glycine max).* Soybean seeds were treated separately with Rhizobium, Phosphate Solubilizing Bacteria, Vermicompost and Urea. Seven days and fifteen days old seedlings were used to assess shoot length, root length, fresh weight, dry weight, leaf perimeter and vigour index, Protein content and Chlorophyll content. The results showed that the all growth parameters get increased in plants treated with biofertilizers as compared to control. Protein content (35.89 mg/g) was higher in Phosphate Solubilizing Bacteria treated 7 days old seedlings while in 15 days seedlings Protein content (50.28 mg/gm) was higher in Vermicompost treated seedlings as compared to control. In 7 days old seedlings there was no increase in Chlorophyll content in all treatments while in 15 days Chlorophyll (a) (0.1639 mg/g) was highest in Urea treated seedlings as compared to control. Chlorophyll (b) content (2.4897 mg/g in 7 days, 2.4664 mg/g in 15 days) and total Chlorophyll content (2.5099 mg/g in 7 days, 2.6292mg/g in 15 days) was highest in Urea treated seedlings in 7 and 15 days old seedlings of soybean as compared to control. Highest germination percentage was observed in Phosphate Solubilizing Bacteria and Vermicompost. The present study suggest that the Vermicompost is the best suited for getting protein rich soybean.

Keywords: Biofertilizer, Chemical fertilizer, Vigour index, Protein.

Introduction

Soybean is one of the most important crops worldwide. Soybean grains are important as Protein meal and vegetable oil. The crop is grown on an estimated 6% of the world's arable land, and since the 1970s, the area in soybean production has the highest increase compared with other major crops [1].

With the increasing population, the cultivable land resource is shrinking day to day. To meet the food, fiber, fuel, fodder and other needs of the growing population, the productivity of agricultural land and soil health needs to be improved. In order to reap a better harvest, farmers inoculate the soil with fertilizers. Increasingly high inputs of chemical fertilizers during last 150 years have not only left soils degraded, polluted and less productive but have also posed severe health and environmental hazards. To overcome these problems Biofertilizers are preferred [2]. Biofertilizers are ready to use live formulation of beneficial microorganism which on application to soil mobilize the availability of nutrients by their biological activity and help build up the micro flora and in turn the soil health in general. Organic fertilizers (manure, compost, vermicompost) are also considered as Biofertilizers,

which return nutrients in available forms due to the interactions of micro-organisms or their association with plants [3]. The uses of biofertilizers make the ecosystem healthier. A small dose of biofertilizer is sufficient to produce significant results because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain [4].Nitrogen fertilizer can increase the production of total dry matter, which improves the potential of plant to produce more pods, seeds and ultimately grain yield [5], also Leaf Area Index, Chlorophyll pigments [6]. A positive correlation was found between the nitrogen and the Chlorophyll content of leaves [7].

Results of various researchers showed that, biological fertilizers have a significant role in improving yield of soybean. The main objective of this study was to assess the most suitable and effective biofertilizers for soybean (*Glycine max*).

Seeds of soybean were taken and treated separately with Rhizobium, PSB, Vermicompost and Urea fertilizer. Untreated seed served as control. Seven days and fifteen days old seedlings were considered:

Germination percentage estimation: This was done by S. Rehman method [8].

Length of Root and Shoot: These were measured by using the standard centimetre scale.

Vigour index: It was estimated by A.A. Abdul-Baki and J.D. Anderson method [9].

Fresh weight and Dry weight: They were measured by using electronic balance.

Leaf perimeter: It was measured through centimeter scale.

Protein estimation: It was estimated by using method given by E. Layne [10].

Chlorophyll estimation: Chlorophyll was estimated according to the method given by S. Sadasivam and A. Manickam [11].

Statistical analysis

Data regarding seven days and fifteen days old seedlings of various growth parameters and Chlorophyll content, Protein content were subjected to analysis of variance (ANOVA), p value and results are expressed in terms of mean ± standard deviation.

Results and Discussion

The germination percentage in control was 50% while in Rhizobium as well as Urea treated plant germination percentage was found to be 80% and germination of PSB and Vermicompost was about 86.66%.

As shown in table 1 The shoot length of seven day old untreated seedlings of soybean was 9.5±0.5 cm while the shoot length of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 13.83±0.76 cm, 13.67±0.76 cm, 11.57±0.81 cm and 11.5±0.5 cm respectively.

Increase in shoot length was observed in all treatments as compared to control. This increase was highly significant ($p<0.01$) in Rhizobium, PSB and Urea treatment, significant ($p<0.05$) in Vermicompost as compared to control (Figure 1). Calculated value (20.64) of F is greater than the table value (3.48), thus the difference in shoot length is significant among treatment used.

As shown in table 1 the shoot length of fifteen day old untreated seedlings of soybean was 19±1 cm while the shoot length of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 22.67±0.76 cm, 19.5±0.5 cm, 22.83±0.76 cm and 21.93±0.40 cm respectively.

Increase in shoot length was observed in all treatments as compare to control. This increase was highly significant ($p<0.01$) in Rhizobium, Vermicompost and Urea treatment as compare to control but it was non-significant in PSB treatment (Figure 1). Calculated value (19.02) of F is greater than the table value (3.48), thus the difference is significant in shoot length among treatment used.

Table 1: shows the effect of biofertilizers and chemical fertilizer on growth parameter of seven days and fifteen days old seedling of *Glycine max L*.

Parameters	Days	Control	Rhizobium	PSB	Vermicompost	Urea
Shoot length (in cm)	seven	9.5±0.5	13.83±0.76**	13.67±0.76**	11.57±0.81*	11.5±0.5**
	fifteen	19±1	22.67±0.76**	19.5±0.5 ns	22.83±0.76**	21.93±0.40**
Root length (in cm)	seven	2.67±0.28	4.9±0.51**	4.57±0.40**	5±0.86**	4±0.5**
	fifteen	7.8±0.76	11.4±1.03**	6.83±0.76 ns	12.13±0.32**	10.30±1.46*
Fresh weight (in gm)	seven	0.83±0.11	1.37±0.41*	1.09±0.26ns	0.677±0.073*	0.703±0.11 ns
	fifteen	1.573±0.32	1.867±0.12 ns	1.373±0.37 ns	1.95±0.13 ns	1.77±0.14 ns
Dry weight (in gm)	seven	0.43±0.03	0.523±0.05*	0.603±0.085*	0.597±0.068**	0.44±0.052 ns
	fifteen	0.77±0.04	1.047±0.13*	0.79±0.03 ns	1.153±0.11**	0.973±0.06**
Leaf perimeter (in cm)	seven	7.2±0.3	9.2±0.72**	9.4±0.36**	9.17±0.15**	8.13±0.6*
	fifteen	8.03±0.15	9.34±0.3**	9.57±0.5**	9.6±0.4**	9.29±0.3**
Vigour index	seven	608.33±38.18	1493.33±180.37**	1579.33±101.04 ns	1379.44±88.02 ns	1240±80**
	fifteen	1325±114.56	2794.3±199.46**	2280.7±109.20**	2922±203.36**	2578.3±148.35**

*Indicates p value < 0.05 and is significant as compared to control.
**Indicates p value < 0.01 and is highly significant as compared to control. ns indicates p>0.05 and is non significant as compared to control.

Table 2: shows the effect of chemical and biofertilizers on Chlorophyll (a), (b) and total Chlorophyll (mg/g) content in seven and fifteen days old seedlings of *Glycine max*

Treatment/Chlorophyll	Days	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	seven	0.0791±0.118	0.7741±0.51	0.8529±0.631
	fifteen	0.15315±0.115	1.6346±0.45	1.7861±0.341
Urea	seven	0.0214±0.005 ns	2.4897±0.030**	2.5099±0.026**
	fifteen	0.1639±0.011 ns	2.4664±0.039*	2.6292±0.027**
Rhizobium	seven	0.03365±0.021 ns	0.57548±0.11 ns	0.60887±0.13 ns
	fifteen	0.1223±0.032 ns	1.7515±0.019 ns	1.8729±0.032ns
PSB	seven	0.02065±0.007 ns	1.4274±0.012*	1.4481±0.009 ns
	fifteen	0.1265±0.046 ns	1.6988±0.004 ns	1.8245±0.0417ns
Vermicompost	seven	0.03525±0.004 ns	0.9583±0.036 ns	0.99303±0.033 ns
	fifteen	0.0237±0.002 ns	2.1603±0.021*	2.1830±0.022*

*Indicates p value < 0.05 and is significant as compared to control.
**Indicates p value < 0.01 and is highly significant, ***Indicates p value<0.001 and is extremely significant as compared to control.
ns indicates p>0.05 and is indicates non significant as compared to control**.**

Table 3: shows the effect of different fertilizers on Protein content (mg/gm) of seven and fifteen days old seedlings of *Glycine max.*

Treatment	Protein content in 7th day old seedlings	Protein content in 15th day old seedlings
Control	23.67±3.55	29.94±1.59
Urea	27.06±0.42 ns	42.17±4.65**
Rhizobium	30±1.67*	43±3.28**
PSB	35.89±5.39*	48.76±4.09***
Vermicompost	25.28±3.94 ns	50.28±2.55***

*Indicates p value < 0.05 and is significant as compared to control. **Indicates p value < 0.01 and is highly significant, ***Indicates p value<0.001 and is extremely significant as compared to control. ns indicates p>0.05 and is indicates non significant as compared to control.

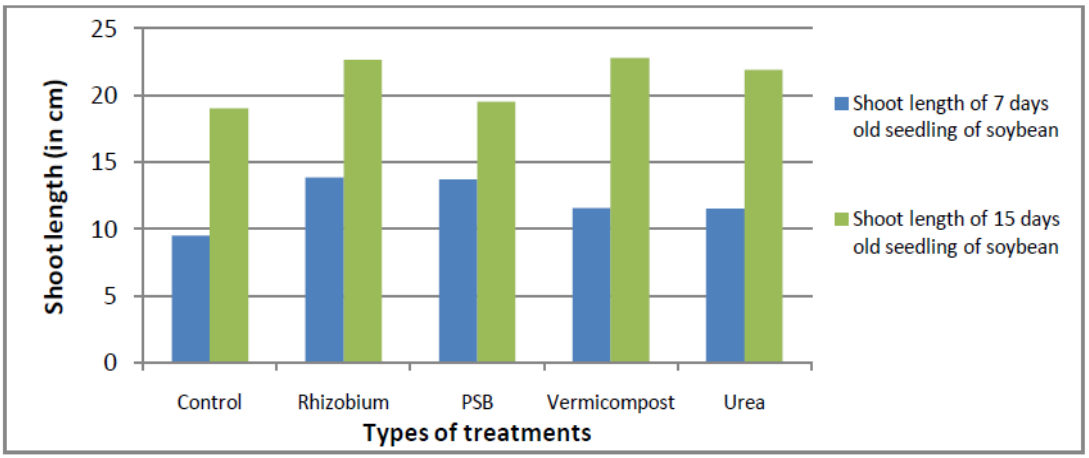

Figure 1: Effect on shoot length of soybean.

The results of present study shown that shoot length of seven days old seedlings increases in Rhizobium treatment as compare to control. Increase was significant in PSB, Rhizobium and Urea treatment as compare to control. In fifteen day old seedlings highest shoot length increase was observed in Vermicompost treatment as compare to control.

The root length of seven day old untreated seedlings of soybean was 2.67±0.28 cm while the root length of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 4.9±0.51 cm, 4.57±0.40 cm, 5±0.86 cm and 4±0.5 cm respectively.

Increase in root length was observed in all treatments as compared to control. This increase was highly significant ($p<0.01$) in Rhizobium, PSB, Vermicompost and Urea treatment as compared to control (Figure 2.). Calculated value (9.18) of F is greater than the table value (3.48), thus the difference is significant in root length of soybean among treatment used.

The root length of fifteen day old untreated seedlings of soybean was 7.8±0.76 cm while the root length of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 11.4±1.03 cm, 6.83±0.76 cm, 12.13±0.32 cm and 10.30±1.46 cm respectively.

Increase in root length was observed in all treatments as compare to control. This increase was highly significant ($p<0.01$) in Rhizobium, Vermicompost and significant ($p<0.05$) in Urea treatment as compare to control, while non-significant in PSB treatment (Figure 2.). Calculated value (17.37) of F is greater than the table value (3.48) so that the difference is significant in root length among treatment

used. Root length increase was found in Vermicompost treated seedlings of seven day old seedlings. In fifteen days old seedlings root length increase was observed in Vermicompost treated seedlings.

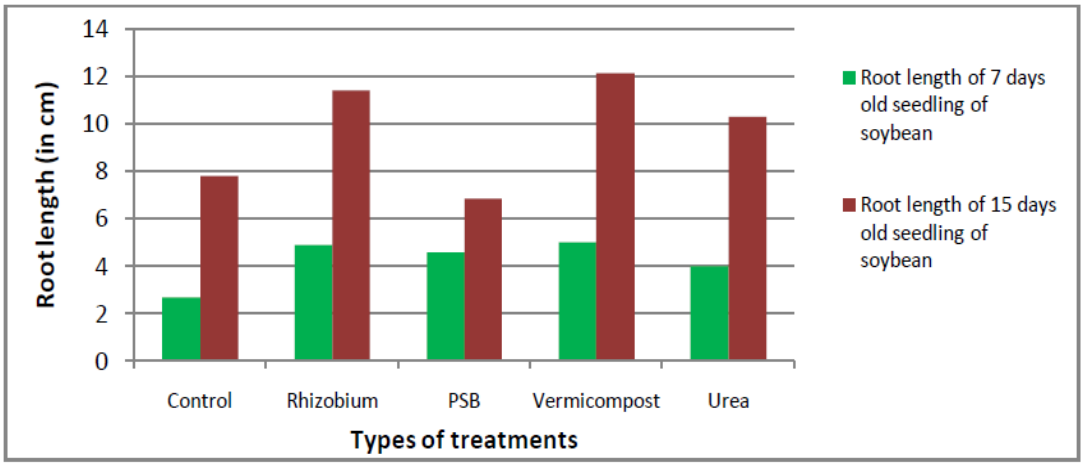

Figure 2: Effect on root length of soybean seedlings.

The fresh weight of seven day old untreated seedlings of soybean was 0.83±0.11 gm while the fresh weight of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 1.37±0.41 gm, 1.09±0.26 gm, 0.677±0.073 gm and 0.703±0.11 gm respectively.

Increase in fresh weight was observed in all treatments as compare to control. This increase was significant ($p<0.05$) in Rhizobium and Vermicompost while non significant in PSB and Urea as compared to control (Figure 3.). Calculated value (4.64) of F is greater than the table value (3.48), thus the difference is significant in fresh weight.

The fresh weight of fifteen day old untreated seedlings of soybean was 1.573±0.32 gm while the fresh weight of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 1.867±0.12 gm, 1.373±0.37 gm, 1.95±0.13 gm and 1.77±0.14 gm respectively.

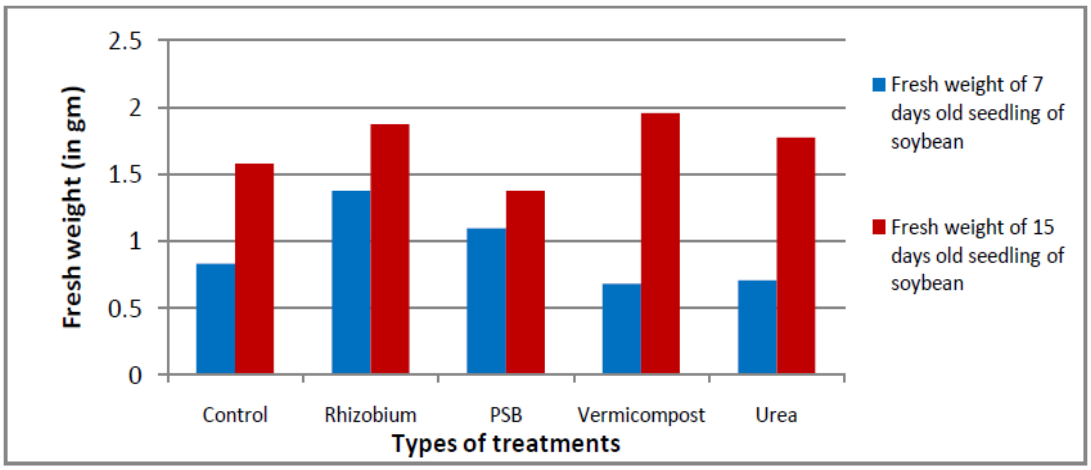

Figure 3: Effect on fresh weight of soybean

Increase in fresh weight was observed in all treatments as compare to control. This increase was non-significant in all treatments as compare to control. Calculated value (2.73) of F is lesser than the table value (3.48), thus the difference in fresh weight for four fertilizers treatment is insignificant.

In seven days old seedlings the fresh weight was higher in Rhizobium treatment as compared to control. Many scientists used various selected strains of phosphate solubilizers which increase the dry

matter, grain yield and 'P' uptake [12], PSB increased grain yield and nodulation [13]. The fresh weight of fifteen days old seedlings was higher in Vermicompost treatment as compared to control.

The dry weight of seven days old untreated seedlings was 0.43±0.03 gm while the dry weight of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 0.523±0.05 gm, 0.603±0.085 gm, 0.597±0.068 gm and 0.44±0.052 gm respectively.

Increase in dry weight was observed in all treatments as compare to control. This increase was significant ($p<0.05$) in Rhizobium, PSB treatment and highly significant ($p<0.01$) in Vermicompost as compare to control, while non-significant in Urea (Figure 4.). Calculated value (5.39) of F is greater than the table value (3.48), thus difference is significant in dry weight among treatment used.

The dry weight of fifteen day old untreated seedlings of soybean was 0.77±0.04 gm while the dry weight of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 1.047±0.13 gm, 0.79±0.03 gm, 1.153±0.11 gm and 0.973±0.06 gm respectively.

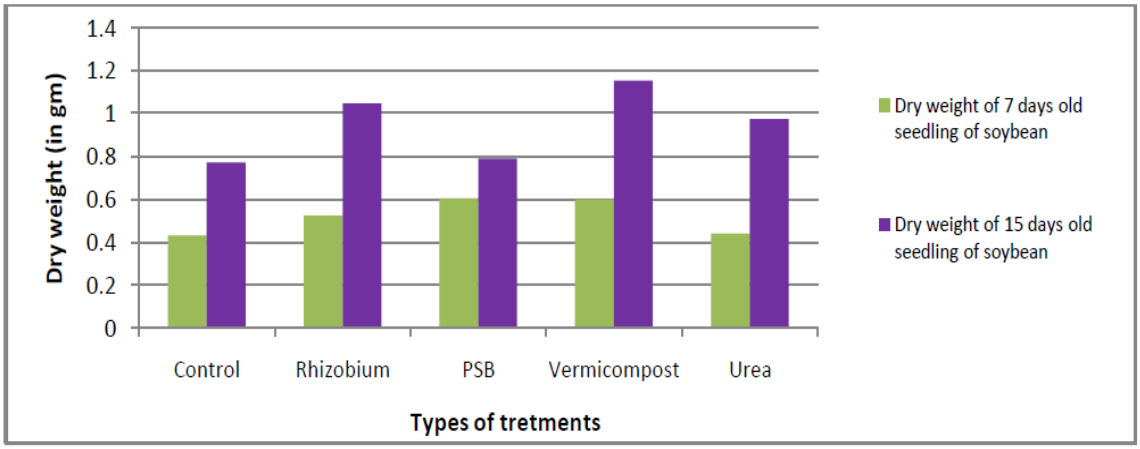

Figure 4: Effect on dry weight of soybean.

Increase in dry weight was observed in all treatments as compare to control. This increase was significant ($p<0.05$) in Rhizobium treatment and highly significant ($p<0.01$) in Vermicompost and Urea as compare to control, while non-significant in PSB (Figure 4.). Calculated value (10.83) of F is greater than the table value (3.48), thus there is significant difference in dry weight under the treatment of four fertilizers.

The dry weight of seven day old seedlings was higher in Vermicompost and PSB as compared to control while the dry weight of fifteen day old seedlings was higher in Vermicompost and Rhizobium as compared to control. A study also showed that the inoculation of *Azospirillum* increased fresh and dry weights of soybean which supports present study.

The leaf perimeter of seven days old seedlings of control was 7.2±0.3 cm while the leaf perimeter of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 9.2±0.72 cm, 9.4±0.36 cm, 9.17±0.15 cm and 8.13±0.60 cm respectively.

Increase in leaf perimeter was observed in all treatments as compare to control. This increase was highly significant ($p<0.01$) in Rhizobium, PSB and Vermicompost and significant ($p<0.05$) in Urea as compared to control (Figure 5.).

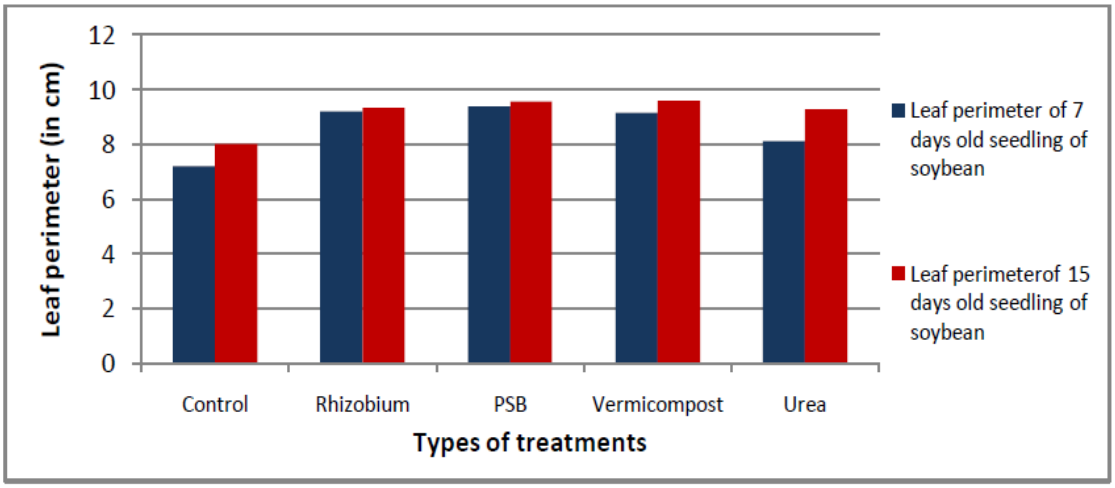

Figure 5: Effect of fertilizer on leaf perimeter of soybean

Calculated value (11.64) of F is greater than the table value (3.48), thus there is significant difference in the leaf perimeter of seedlings treated with four types of fertilizers.

The leaf perimeter of fifteen day old seedlings of control was 8.03±0.15 cm while the leaf perimeter of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 9.34±0.3 cm, 9.57±0.5 cm, 9.6±0.4 cm and 9.29±0.3 cm respectively.

Increase in leaf perimeter was observed in all treatments as compare to control. This increase was highly significant ($p<0.01$) in Rhizobium, PSB and Vermicompost and Urea as compare to control (Figure 5.). Calculated value (9.53) of F is greater than the table value (3.48), thus there is significant difference in the leaf perimeter of the four varieties of fertilizers.

In seven days old seedlings the leaf perimeter was higher in PSB treated seedlings as compared to control. In fifteen days old seedlings the leaf perimeter was higher Vermicompost treated seedlings as compared to control.

The vigour index of seven days old seedlings of untreated seedlings was 608.33±38.18, while the vigour index of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 1493.33±180.37, 1579.33±101.04, 1379.44±101.19 and 1240.4±80 respectively.

Increase in vigour index was observed in all treatments as compare to control. This increase was highly significant ($p<0.01$) in Rhizobium and Urea, was non-significant in PSB and Vermicompost as compare to control (Figure 6.).

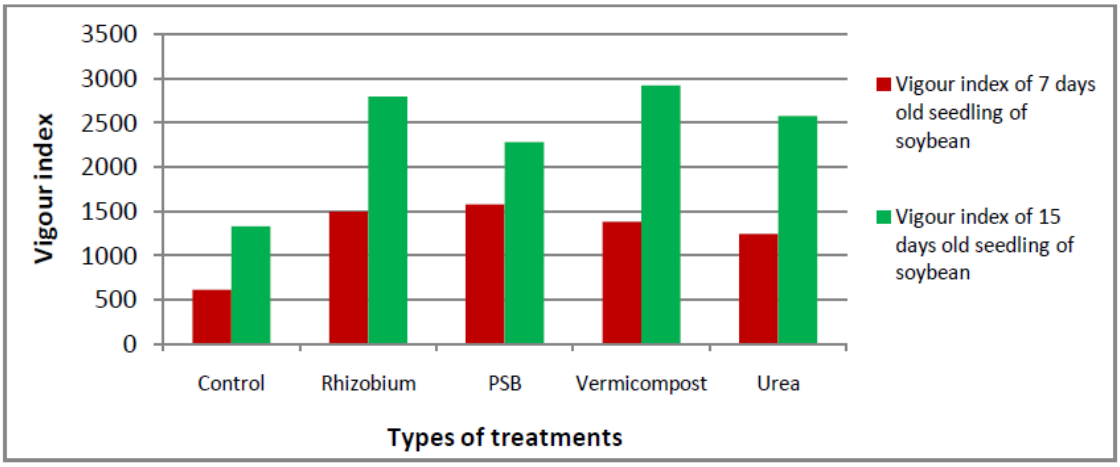

Figure 6: Effect on vigour index of soybean

The vigour index of fifteen day old seedlings of control was 1325±114.56, while the vigour index of seedlings treated with Rhizobium, PSB, Vermicompost and Urea were 2794.33±199.46, 2280.67±109.20, 2922±203.36 and 2578.3±148.35 respectively.

Increase in vigour index was observed in all treatments as compare to control. This increase was highly significant ($p<0.01$) in Rhizobium, PSB, Vermicompost and Urea treatment as compare to control (Figure 6.).

Vigour index in seven days old seedlings of soybean was higher in PSB as compared to control. A study showed that seed treatment with biofertilizers had their significant effect on microbial population in conjunction with P application in soy-bean field [14]. In fifteen days old seedlings vigour index was higher in Vermicompost as compared to control.

As shown in table 2 the Chlorophyll (a) content of seven day old untreated seedlings of soybean was 0.0791±0.118 mg/g while the Chlorophyll (a) of seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 0.0214±0.005 mg/g, 0.03365±0.021 mg/g, 0.02065±0.007 mg/g and 0.03525±0.004 mg/g respectively. Decrease in Chlorophyll (a) was observed in all treatments as compare to control. No significant change was observed in all treatments as compare to control (Figure 7.).

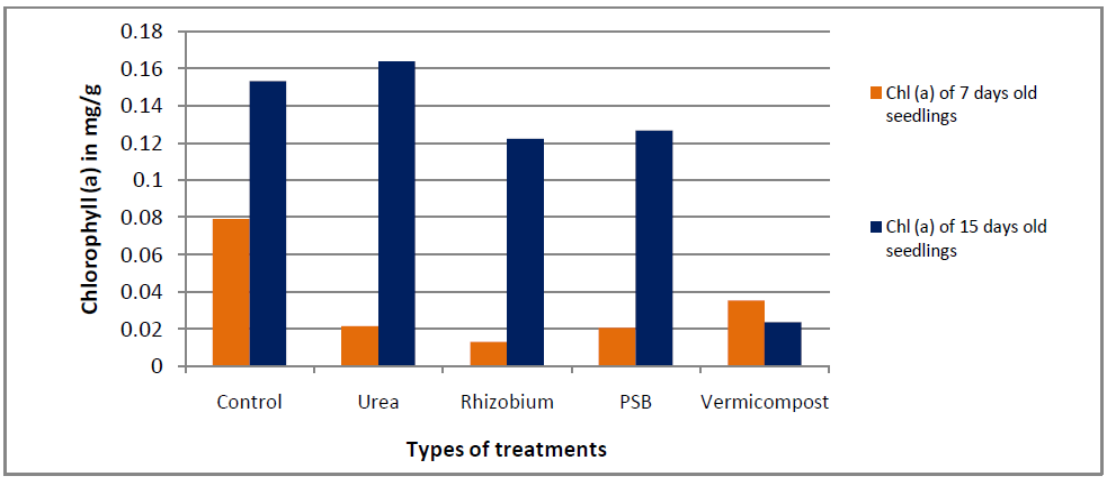

Figure 7: Effect on Chlorophyll (a) content of soybean

Calculated value (0.59) of F is lesser than the table value (3.48) so the difference in Chlorophyll a content of soybean for four fertilizers treatment is insignificant.

As shown in table 3 the Chlorophyll (a) content of fifteen day old untreated seedlings of soybean was 0.15315±0.115 mg/g while the Chlorophyll (a) of seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 0.1639±0.011 mg/g, 0.1223±0.032 mg/g, 0.1265±0.046 mg/g and 0.0237±0.002 respectively. Increase in Chlorophyll (a) was observed in Urea as compare to control while decrease was observed in Rhizobium, Vermicompost, Vermicompost and PSB treated seedlings as compare to control(Figure 7.). Change was insignificant in Urea, Rhizobium and PSB as compare to control.

Calculated value (2.75) of F is lesser than the table value (3.48), thus the difference in Chlorophyll (a) content for four fertilizers treatment is insignificant.

In seven days old seedlings Chlorophyll (a)content was not increased but in fifteen days old seedlings of soybean Chlorophyll (a) content was found higher in Urea treated seedlings as compared to control.

The Chlorophyll (b) content of seven day old untreated seedlings of soybean was 0.7741±0.51 mg/g while the Chlorophyll (b) of seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 2.4897±0.030 mg/g, 0.57548 ±0.11 mg/g, 1.4274±0.012 mg/g and 0.9583±0.036 mg/g respectively. Increase in Chlorophyll (b) was observed in Urea and PSB as compare to control. Change was highly

significant in Urea and significant change was observed in PSB as compare to control. While insignificant increase was observed in Vermicompost treated seedling and insignificant decrease was found in Rhizobium treated seedlings as compare to control (Figure 8.).

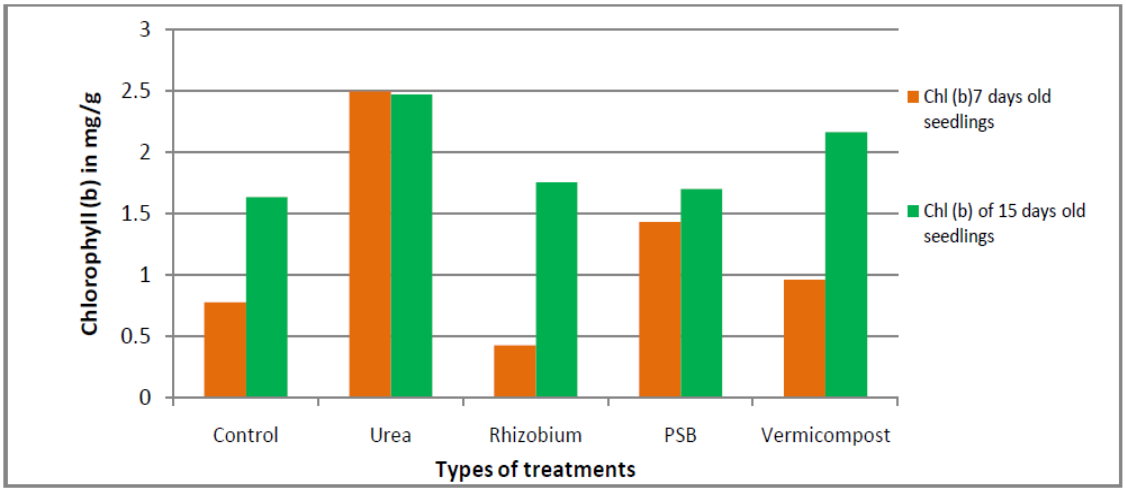

Figure 8: Effect on Chlorophyll (b) content of soybean

Calculated value (31.50) of F is greater than the table value (3.48) so the difference is significant in Chlorophyll (b) content in different fertilizer treatment.

The Chlorophyll (b) content of fifteen days old untreated seedlings of soybean was 1.6346±0.45 mg/g while the Chlorophyll (b) of seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 2.4664±0.039 mg/g, 1.7515±0.019 mg/g, 1.6988±0.004 mg/g and 2.1603±0.021 mg/g respectively. Increase in Chlorophyll (b) was observed in all treatments of biofertilizers. Significant increase was observed in Vermicompost and Urea treated seedlings as compare to control (Figure 8.).

Calculated value (9.11) of F is greater than the table value (3.48) we conclude that the difference is significant in Chlorophyll (b) content in different fertilizer treatment.

In seven days and fifteen days old seedlings of soybean Chlorophyll (b) content was found higher in Urea treated seedlings as compared to control.

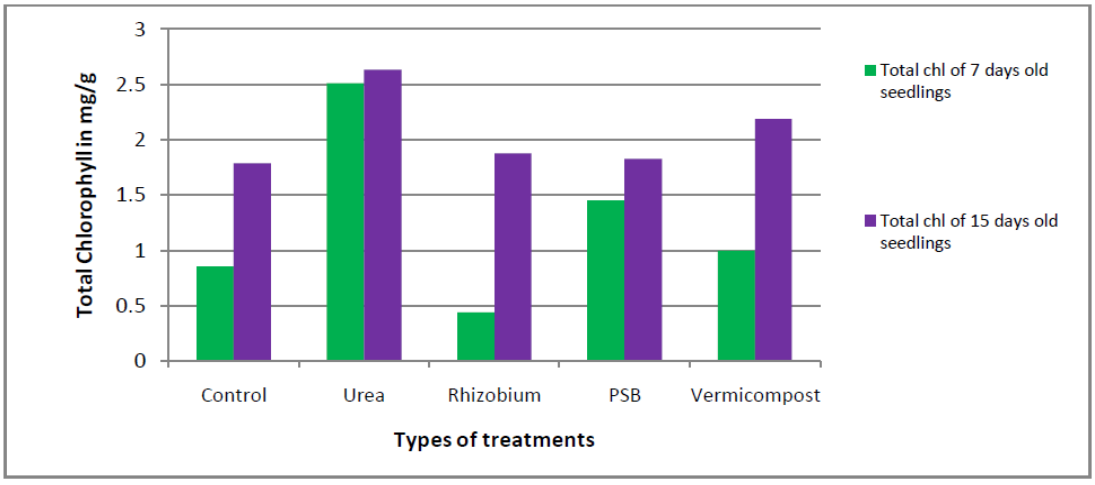

Figure 9: Effect on Total Chlorophyll content of soybean

The total Chlorophyll content in seven day old untreated seedlings of soybean was 0.8529±0.631 mg/g while the total Chlorophyll of seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 2.5099±0.026 mg/g, 0.60887±0.13 mg/g, 1.4481±0.009 mg/g and 0.99303±0.033 mg/g respectively. Increase in total Chlorophyll was observed in Urea and PSB treated seedlings as

compare to control. Change was insignificant in PSB as compare to control. Highly significant increase was observed in Urea as compare to control. While insignificant decrease was observed in Rhizobium treated seedlings while insignificant increase was observed in PSB treated seedlings (Figure 9).

Calculated value (20.23) of F is greater than the table value (3.48), thus the difference is significant in total Chlorophyll content of soybean under the influence of four fertilizers.

The total Chlorophyll content of fifteen days old untreated seedlings of soybean was 1.7861±0.341 mg/g while the total Chlorophyll of seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 2.6292±0.027 mg/g, 1.8729±0.032 mg/g, 1.8245±0.0417 mg/g and 2.1830±0.022 mg/g respectively. Increase in total Chlorophyll was observed in all treatments. Significant increase was observed in Vermicompost as compare to control. Highly significant increase was observed in Urea while non-significant increase was observed in Rhizobium and PSB as compare to control (Figure 9.).

Calculated value (15.69) of F is greater than the table value (3.48), thus the difference is significant in total Chlorophyll content of soybean under the influence of four fertilizers.

In seven days and fifteen days old seedlings of soybean total Chlorophyll content was found higher in Urea treated seedlings as compared to control.

As shown in table 3 the Protein content in seven days old untreated seedlings of soybean was 23.67±3.55 mg/g while in seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 27.06±0.42 mg/g, 30±1.67 mg/g, 35.89±5.39 mg/g and 25.28±3.94 mg/g respectively. Increase in Protein content was observed in all treatments as compare to control. These increase were significant ($p<0.05$) in Rhizobium and PSB as compare to control. Increase was non-significant in Urea and Vermicompost as compare to all other treatments (Figure 10.). Calculated value (5.78) of F is greater than the table value (3.48), thus the difference is significant in Protein content of soybean.

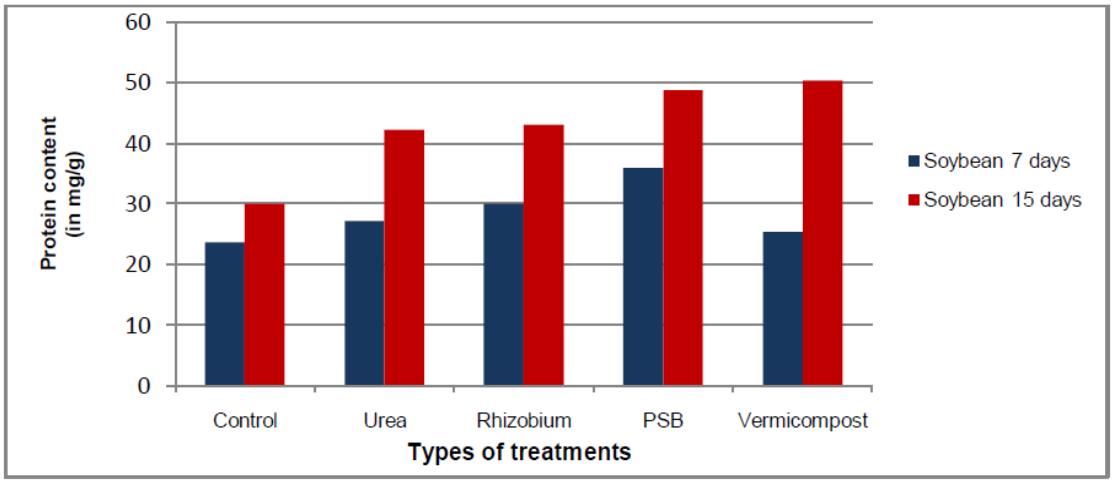

Figure 10: Effect on Protein content of soybean

The Protein content in fifteen days old untreated seedlings of soybean was 29.94±1.59 mg/g while in seedlings treated with Urea, Rhizobium, PSB and Vermicompost were 42.17±4.65 mg/g, 43±3.28 mg/g, 48.76±4.09 mg/g and 50.28±2.55 mg/g respectively. Increase in Protein content was observed in all treatments as compare to control. These increase were extremely significant ($p<0.001$) in PSB and Vermicompost treatments as compare to control. Highly significant increase was observed in Urea and Rhizobium as compared to control (Figure 10.).

Calculated value (16.62) of F is greater than the table value (3.48), thus there is significant difference in the average Protein content under the four varieties of fertilizers.

In seven days old seedlings highest increase in Protein content was observed in PSB treated seedlings as compare to control. The result is in accordance with the work of L. Naresh [15] who found that Protein content in *Westiellopsis* sp. treated flax plants were enhanced as compare to control. Results of present study is also supported by Dhaliwal [16] also reported that Protein % was increased under the influence of biofertilizers application. In fifteen days old seedlings of soybean highest increase in Protein content was observed in Vermicompost treated seedlings as compare to control.

Conclusion

It was concluded that biofertilizers are more efficient in promoting growth of soybean seedlings as compared to chemical fertilizer and do not cause any harm to soil. The present study suggests that the Vermicompost is best suited for getting soybean rich in protein.

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