



## **Influence Of Integrated Weed Management Practices On Biofertilizers and Soil Enzyme Activity in Soybean [*Glycine max (L.) Merill*] In Southern Telangana Agro- Climatic Zone**

**BHARAT BHUSHAN RAO, C.H.**

Department of Agronomy, College of Agriculture  
Professor Jayashankar Telangana State Agricultural University.  
Rajendranagar, Hyderabad – 500030.  
Corresponding author email: [chirubharat@gmail.com](mailto:chirubharat@gmail.com)

### **ABSTRACT**

*A field experiment entitled "Influence of integrated weed management practices on bio fertilizers and soil enzyme activity in soybean [*Glycine max (L.) Merill*] in southern Telangana agro- climatic zone" was conducted at the Agricultural College farm, Rajendranagar, Hyderabad, Telangana State during 2014 and 2015. Maximum of 10.62 and 12.73 x log<sub>10</sub> CFU g<sup>-1</sup> soil colony forming units of *Rhizobium* developed at 30 days during 2014 and 2015 in the hand weeding treatment. The CFU of 9.71 and 11.61 x log<sub>10</sub> CFU g<sup>-1</sup> soil due to the integrated weed management treatment were also on par. They were significantly low due to pre and post emergence herbicide applications. Maximum colony forming units of 2.68 x log<sub>10</sub> CFU g<sup>-1</sup> soil at 30 days and 2.97 x log<sub>10</sub> CFU g<sup>-1</sup> soil phosphate solubilising bacteria per gram soil were recorded at 30 days during 2015. The improvement in the colony forming units of this bacteria increased over fertilizer application when *Rhizobium*, phosphorus and potassium solubilising bacteria were also added. This effect was significant at 30 days during 2014 and 60 days during 2015. The interactions were not significant. The dehydrogenase and urease activity, the acid and alkaline phosphatases were not significantly influenced by the weed management treatments at 30 and 60 days during 2014 or 2015. The application of nutrient mobilizing cultures also did not influence any of these parameters in the two years.*

**Keywords:** *Glycine max*, IWM

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### **INTRODUCTION**

Soybean [*Glycine max (L.) Merill*] is a miracle golden bean of the 20<sup>th</sup> century. It occupies third place among oilseed crops of Telangana State. It is a rich source of protein (40-42 %) and quality oil (20-22%). Protein is rich in valuable amino acid with 5% lysine. It also contains good amount of minerals, salts and vitamins. Its sprouting grain contains considerable amount of vitamin A and Vitamin C. Commercial cultivation of seeded soybean is comparatively of recent origin. Earlier, low yielding black seeded shattering type of varieties were grown under different names in hills and scattered pockets in plains. Soybean suffers from heavy infestation of complex weed flora belonging to grasses, broad leaf weeds, sedges and perennial types. The herbicides are apprehended to have direct or indirect consequences on non-targeted organisms including soil micro flora in the field. Diversified effect of both toxic as well as beneficial effect of herbicides on soil micro-organisms and soil characteristics were studied in different ways in the recent past. Scientific information indicates that there is no universal pattern of herbicidal effect on soil micro-organisms [1]. Since soybean is a legume, it meets nitrogen requirements both through soil in the form of NO<sub>3</sub><sup>-</sup> and the atmosphere through symbiotic fixation in the root nodules [2]. Being a legume the phosphorus requirement of soybean is high. It helps in better root and nodule formation. N<sub>2</sub> fixing and P-solubilising bacteria increase the N and P uptake. They play a crucial role as plant growth-promoting *Rhizobacteria* in the bio-fertilization [3]. obtained maximum biological nitrogen fixation by the inoculation of seeds with efficient *Bradyrhizobium* inoculants in low N soils without N fertilizer application. Similarly, The micro organisms play a crucial role to enhance the enzyme activities, phosphorus and the process of its solubilization.

## MATERIAL AND METHODS

A field experiment entitled "Influence of integrated weed management practices on bio fertilizers and soil enzyme activity in soybean [*Glycine max (L.) Merrill*] in southern Telangana agro-climatic zone" was conducted at the Agricultural College Farm, Rajendranagar, Hyderabad during 2014 and 2015. The soil was sandy loam in texture having 7.8 pH and EC 0.21 d S m<sup>-1</sup>. It was very poor in nutrient status with 0.35% OC and 226 kg ha<sup>-1</sup> available N, available P was 18 kg ha<sup>-1</sup> and available K was 236 kg ha<sup>-1</sup>. The layout was a split plot design. The main treatments were: (W1) Pre-emergence application of pendimethalin @ 1.0 kg a.i ha<sup>-1</sup> followed by hand weeding 25 DAS, (W2) Pre emergence application of pendimethalin @ 1.0 kg a.i ha<sup>-1</sup> followed by post-emergence application of imazethapyr @ 100 g a.i ha<sup>-1</sup> + quizalofop-p-ethyl @ 50 g a.i ha<sup>-1</sup> 25DAS, (W3) Pre-emergence application of pendimethalin @ 1.0 kg a.i ha<sup>-1</sup> followed by post-emergence application of odyssey i.e. imazethapyr + imazamox @ 70 g a.i ha<sup>-1</sup> at 25 DAS, (W4) Hand weeding at 25 and 45 DAS and (W5) un-weeded check. The sub plot treatments comprising of (F1) Recommended dose of fertilizers @ 30:60:40 kg ha<sup>-1</sup> NPK, (F2) RDF+ seed treatment with rhizobium @ 250g10kg<sup>-1</sup> seed, (F3) RDF+seed treatment with rhizobium @ 250g10kg<sup>-1</sup> seed + phosphate solubilising bacteria @ 5 kg ha<sup>-1</sup>, (F4) RDF + seed treatment with rhizobium @ 250 g10 kg<sup>-1</sup> seed + phosphate solubilising bacteria @ 5 kg ha<sup>-1</sup> + potassium solubilising bacteria @ 5kg ha<sup>-1</sup>. Recommended fertilizer dose of 30:60:40 kg ha<sup>-1</sup> NPK was calculated for the dimensions of each sub plot and applied at the time of sowing in the form of urea. Single super phosphate and muriate of potash. Seed rate was @ 63 kg ha<sup>-1</sup>. The bio fertilizers- *brady rhizobium japonica* and phosphate solubilising bacteria were mixed as per the treatment in jaggery solution prepared @ 250 g for 10 kg seed. The seed was thoroughly mixed with the solution and shade dried. The potassium solubilising bacteria were applied @ 5 kg ha<sup>-1</sup> after mixing with FYM. The seeds were dibbled at the rate of two per hill 10 cm apart in 30cm interval. The crop was sown on 10<sup>th</sup> July in 2014 and 18<sup>th</sup> June in 2015. For isolation of rhizobacteria, the method proposed by Vlassak *et al.* [4] was followed. In this procedure 10 g of soil from each soil sample was taken in a conical flask to which 90 ml of normal saline was added. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. Serial dilutions prepared for different bacteria. 0.1ml of respective dilutions were spread on sterilized petri plates containing specific media. Specified media were used for enumeration of different bacterial count, yeast extract mannitol agar (YEMA) for *Rhizobium* spp [5]; Pikovskaya's agar for enumeration of phosphate solubilising bacteria (Pikovskaya, 1948) and Aleksandrov agar medium for enumeration of potash mobilizing bacterial isolates [5].

### Dehydrogenase

Method was described by Casida *et al.*, [6]. The enzyme activity in 1g soil was determined by adding 0.05g of CaCO<sub>3</sub>, 2.5 ml distilled water and by using 1 ml of 3% TTC where it is reduced to light pink TPF on the incubation for 24 h. Later it was dissolved in 10 ml of methanol and finally made up to 25 ml. The intensity of the red color was measured on a spectrophotometer at 485 nm.

### Urease activity

Urease activity in soil was assayed by quantifying the ratio of release of NH<sub>4</sub><sup>+</sup> from the hydrolysis of urea [7]. Soil (5g) was taken in a 50 ml volumetric flask, after adding 0.2 ml of Toluene and 9 ml tris-hydroxymethyl amino methane (THAM) buffer, the flask was swirled for a few seconds to mix the contents and 1 ml of 0.2 M urea solution was added and swirled the flask again for a few seconds.

Then the flask was stoppered and placed in an incubator at 37°C. After 2 hours, the stopper was removed, and approximately 35 ml of KCl-Ag<sub>2</sub>SO<sub>4</sub> solution was added, swirled the flask for a few seconds and allowed flask to stand until the contents cooled to room temperature (about 5 minutes). The contents were made up to 50 ml by addition of KCl-Ag<sub>2</sub>SO<sub>4</sub> solution. The flask was stoppered and inverted several times to mix the contents. NH<sub>4</sub><sup>+</sup>- N was determined in the resulting soil suspension by pipetting out 20 ml aliquot of the suspension distilling with 0.2 g of MgO for 4 minutes. Controls were maintained by following the same procedure described for assay of urease activity, except the addition of 1 ml of 0.2 M urea solution after the addition of KCl Ag<sub>2</sub>SO<sub>4</sub> solution.

### Phosphatase enzyme

The procedure followed was of Tabatabai and Bremner [7] (Acid phosphatases) and Eivazi and Tabatabai [8] (Alkaline phosphatases). One gram of soil sample was taken in glass tube. Then 0.2 ml of Toluene was added followed by 4 ml of modified universal buffer (MUB) pH 6.5 (for acid phosphatase), 4ml MUB buffer pH 11.0 (for alkaline phosphatase) and 1 ml of p- nitro phenyl phosphate (only for samples) was added glass tubes swirled for few seconds, stoppered and incubated for one hour at 37°C. After incubation, one ml of 0.5 M CaCl<sub>2</sub> 2H<sub>2</sub>O and 4 ml 0.5 M Na OH was added, swirled and filtered. The intensity of yellow colour was measured with spectrophotometer at 420 nm.

## RESULTS AND DISCUSSIONS

The results obtained in the field experiment entitled "Influence of integrated weed management practices on bio fertilizers and soil enzyme activity in soybean [*Glycine max (L.) Merrill*] in southern Telangana agro-climatic zone". The data on response of colony forming units of *Rhizobium* and phosphate solubilizing bacteria in the soil is presented in table 1. The initial soil sample had 7.29 colony forming units of *Rhizobium*. They increased enormous at 30 days age of the crop. Maximum CFU of 10.62 and 12.73  $\times 10^8$  CFU  $g^{-1}$  soil were formed at 30 days during 2014 and 2015, respectively due to hand weeding treatment. The colony forming units due to integrated weed management was significantly superior over unweeded check. They reduced significantly due to the pre and post emergence application of herbicides. The consortium of bio-fertilizers *Rhizobium*, phosphate and potassium solubilising bacteria significantly increased the CFU to 9.60 compared to 7.84  $\times 10^8$  CFU  $g^{-1}$  soil applied with recommended dose of 30:60:40 kg  $ha^{-1}$  N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O during 2014. The respective treatments had 11.58 and 9.81  $\times 10^8$  CFU  $g^{-1}$  soil during 2015. The interactions were not significant. Initially the colony forming units were 3.0  $\times 10^8$  CFU  $g^{-1}$  soil for phosphate solubilising bacteria. Maximum colony forming units of 3.56  $\times 10^8$  CFU  $g^{-1}$  soil and 5.05  $\times 10^8$  CFU  $g^{-1}$  soil was recorded at 30 and 60 days during 2015 in the hand weeding treatment. They reduced due to the herbicide applications. The trends were not clear at 30 days and not significant at 60 days in the previous year. The trends due to bio-fertilizers were not consistent. The interactions due to weed management treatments and bio-fertilizers were not significant. The data on colony forming units of potassium solubilizing bacteria per gram of soil is presented in table 4.24. The weed management treatments did not influence the colony forming units of potassium solubilising bacteria at 30 or 60 days during 2014 and 2015. The supplementation of *Rhizobium*, phosphate and potassium solubilising bacteria significantly increased the colony forming units over the recommended dose of fertilizers at 30 days during 2014. Maximum colony forming units of 20.26 and 21.29  $\times 10^8$  CFU  $g^{-1}$  soil were recorded at 30 and 60 days compared to 15.51 and 18.06  $\times 10^8$  CFU  $g^{-1}$  soil due to recommended dose of fertilizers applied during 2015. The interaction due to different weed management and nutrient improvement techniques had no significant influence.

### Enzyme activity

The data on dehydrogenase and urease activity is presented in table 4.25. Initially the dehydrogenase activity was 3.6  $\mu g$  TPF  $g^{-1}$  day<sup>-1</sup> and urease activity was 52  $\mu g$  NH<sub>4</sub><sup>+</sup>  $g^{-1}$  2h<sup>-1</sup>. The weed management treatments and bio-fertilizers had no significant influence on these activities in either of the two years. The interactions were also not significant. The acid phosphatase activity was 82  $\mu g$  TPF  $g^{-1}$  day<sup>-1</sup> while alkaline phosphatase activity was 51  $\mu g$  TPF  $g^{-1}$  day<sup>-1</sup> (Table ). They were not influenced due to the weed management treatments, bio-fertilizers and their interactions.

Table 1. *Rhizobium* (**log<sub>10</sub> CFU g<sup>-1</sup> soil**) and Phosphate solubilising bacteria log<sub>10</sub> CFU g<sup>-1</sup> soil at 30 and 60 DAS as influenced by weed management treatments and bio-fertilizers during 2014 and 2015

Treatment	<i>Rhizobium</i> (log <sub>10</sub> CFU g <sup>-1</sup> soil)				PSB (log <sub>10</sub> CFU g <sup>-1</sup> soil)			
	2014		2015		2014		2015	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
<b>Weed management</b>								
W1:PE Pendimethalin @ 1kg <i>a.i ha<sup>-1</sup>fb</i> Hand weeding at 25DAS	9.71	12.40	11.61	13.70	2.68	3.25	2.73	4.29
W2:PE Pendimethalin @ 1kg <i>a.i ha<sup>-1</sup>fb</i> PoE Imazethapyr @100g <i>a.i ha<sup>-1</sup></i> + Quizalofop- P-ethyl @ 50 g <i>a.i ha<sup>-1</sup></i> 25 DAS	7.29	11.11	9.22	12.41	2.35	3.35	2.39	4.27
W3:PE Pendimethalin @ 1kg <i>a.i ha<sup>-1</sup>fb</i> PoE Imazethapyr + Imazamox @ 70 g <i>a.i ha<sup>-1</sup></i> 25 DAS	7.70	11.14	9.64	12.44	2.65	3.16	1.99	3.86
W4:Hand weeding at 25 and 45 DAS	10.62	12.70	12.73	14.00	2.39	3.18	3.56	5.05
W5:Unweeded check	8.00	12.34	10.00	13.90	2.25	3.17	2.36	3.17
SE±	0.54	0.73	0.53	0.70	0.10	0.34	0.37	0.24
CD(P=0.05)	1.26	NS	1.25	NS	0.25	NS	0.80	NS
<b>Bio fertilizers</b>								
F1:Fertilizers @ 30:60:40 kg $ha^{-1}$ N:P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O	7.84	10.81	9.81	12.13	2.29	3.19	2.80	3.83
F2:F1 + <i>Rhizobium</i> @ 250g10 kg <sup>-1</sup> seed	8.26	11.65	10.23	13.10	2.41	3.27	2.40	4.09
F3:F2 + Phosphate solubilising bacteria@ 5 kg $ha^{-1}$	8.98	12.48	10.95	13.80	2.57	3.45	2.93	4.43
F4:F3+ Potassium solubilising bacteria @ 5 kg $ha^{-1}$	9.60	12.81	11.58	14.13	2.68	3.58	2.97	4.46

SE±	0.40	0.82	0.40	0.83	0.11	0.12	0.30	0.18
CD(P=0.05)	0.82	NS	0.82	NS	0.23	NS	NS	0.30
<b>Weed Management x Bio fertilizers</b>								
SE±	0.89	1.83	0.89	1.85	0.25	0.29	0.62	0.42
CD(P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Initial *Rhizobium* 7.29 (log<sub>10</sub> CFU g<sup>-1</sup> soil)

Phosphate solubilising bacteria 3.0 (log<sub>10</sub> CFU g<sup>-1</sup> soil)

Table 2 . Potassium solubilising bacteria (log<sub>10</sub> CFU g<sup>-1</sup> soil) at 30 and 60 DAS as influenced by weed management treatments and bio-fertilizers during 2014 and 2015

Treatment	KSB (log <sub>10</sub> CFU g <sup>-1</sup> soil)			
	2014		2015	
	30 DAS	60 DAS	30 DAS	60 DAS
<b>Weed management</b>				
W1:PE Pendimethalin @ 1kg <i>a.i</i> ha <sup>-1</sup> fb Hand weeding at 25DAS	15.76	18.70	16.83	19.94
W2:PE Pendimethalin @ 1kg <i>a.i</i> ha <sup>-1</sup> fb PoE Imazethapyr @100g <i>a.i</i> ha <sup>-1</sup> +Quizalofop- P-ethyl @ 50g <i>a.i</i> ha <sup>-1</sup> 25 DAS	16.16	17.41	17.23	18.41
W3:PE Pendimethalin @ 1kg <i>a.i</i> ha <sup>-1</sup> fb PoE Imazethapyr + Imazamox @ 70 g <i>a.i</i> ha <sup>-1</sup> 25 DAS	16.93	17.44	18.00	18.44
W4: Hand weeding at 25 and 45 DAS	17.29	18.70	17.94	20.12
W5: Unweeded check	17.06	19.04	17.79	21.09
SE±	1.41	0.73	1.46	1.08
CD(P=0.05)	NS	NS	NS	NS
<b>Bio-fertilizers</b>				
F1:Fertilizers @ 30:60:40 kg ha <sup>-1</sup> N:P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O	14.64	17.13	15.51	18.06
F2:F1 + Rhizobium @ 250g10 kg <sup>-1</sup> seed	15.81	17.97	16.74	18.95
F3:F2 + Phosphate solubilising bacteria@ 5 kg ha <sup>-1</sup>	16.78	18.80	17.71	20.10
F4:F3+ Potassium solubilising bacteria @ 5 kg ha <sup>-1</sup>	19.34	19.13	20.26	21.29
SE±	0.91	0.82	0.91	0.80
CD(P=0.05)	1.80	NS	2.00	1.60
<b>Weed management x Bio-fertilizers</b>				
SE±	2.00	1.83	2.04	1.80
CD(P=0.05)	NS	NS	NS	NS

Initial 14.64 (log<sub>10</sub> CFU g<sup>-1</sup> soil)

Table 3 . Dehydrogenase (µgTPF g<sup>-1</sup> day<sup>-1</sup>) and urease activity (µgNH<sub>4</sub><sup>+</sup> g<sup>-1</sup> h<sup>-1</sup>) in soil at harvest as influenced by weed management treatments and bio-fertilizers during 2014 and 2015

Treatment	Dehydrogenase activity (µgTPF g <sup>-1</sup> day <sup>-1</sup> )		Urease activity (µgNH <sub>4</sub> <sup>+</sup> g <sup>-1</sup> h <sup>-1</sup> )	
	2014	2015	2014	2015
<b>Weed management</b>				
W1:PE Pendimethalin @ 1kg <i>a.i</i> ha <sup>-1</sup> fb Hand weeding at 25DAS	3.88	5.67	52.75	64.10
W2:PE Pendimethalin @ 1kg <i>a.i</i> ha <sup>-1</sup> fb PoE Imazethapyr @100g <i>a.i</i> ha <sup>-1</sup> +Quizalofop- P-ethyl @ 50 <i>a.i</i> ha <sup>-1</sup> 25DAS	3.81	5.65	56.33	68.40
W3:PE Pendimethalin @ 1kg <i>a.i</i> ha <sup>-1</sup> fb PoE Imazethapyr + Imazamox @ 70 g <i>a.i</i> ha <sup>-1</sup> 25 DAS	3.85	5.71	58.16	70.60
W4:Hand weeding at 25 and 45 DAS	3.93	6.06	53.83	65.40
W5:Unweeded check	3.83	5.65	52.33	63.60
SE±	0.13	0.32	4.06	4.87
CD(P=0.05)	NS	NS	NS	NS
<b>Bio-fertilisers</b>				
F1:Fertilizers @ 30:60:40 kg ha <sup>-1</sup> N:P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O	3.63	5.40	52.93	64.32
F2:F1 + Rhizobium @ 250g10 kg <sup>-1</sup> seed	3.82	5.71	53.80	65.36
F3:F2 + Phosphate solubilising bacteria@ 5 kg ha <sup>-1</sup>	3.99	5.86	56.06	68.08
F4:F3+ Potassium solubilising bacteria@ 5 kg ha <sup>-1</sup>	4.00	5.98	55.93	67.92
SE±	0.15	0.23	2.43	2.91
CD(P=0.05)	NS	NS	NS	NS
<b>Weed Management x Bio-fertilizers</b>				
SE±	0.35	0.52	5.43	6.52
CD(P=0.05)	NS	NS	NS	NS

Initial - Dehydrogenase activity 3.6  $\mu\text{g TPF g}^{-1} \text{ day}^{-1}$  ; Urease activity 52  $\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ 2 h}^{-1}$

Table 4. Acid ( $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$ ) and alkaline phosphatase ( $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$ ) in soil as influenced by weed management treatments and bio-fertilizers during 2014 and 2015

Treatment	Acid phosphatase ( $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$ )		Alkaline phosphatase ( $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$ )	
	2014	2015	2014	2015
<b>Weed management</b>				
W1:PE Pendimethalin @ 1kg a.i ha <sup>-1</sup> fb Hand weeding at 25 DAS	85.25	96.41	57.08	67.16
W2:PE Pendimethalin @ 1kg a.i ha <sup>-1</sup> fb PoE Imazethapyr @100g a.i ha <sup>-1</sup> +Quizalofop- P-ethyl @ 50 g a.i ha <sup>-1</sup> 25DAS	82.91	94.00	51.08	61.33
W3: PE Pendimethalin @ 1kg a.i ha <sup>-1</sup> fb PoE Imazethapyr + Imazamox @ 70 g a.i ha <sup>-1</sup> 25 DAS	85.25	96.41	54.41	64.33
W4:Hand weeding at 25 and 45 DAS	88.91	100.08	54.83	64.75
W5:Unweeded check	84.00	95.16	53.75	63.33
SE±	3.05	3.04	2.33	1.93
CD(P=0.05)	NS	NS	NS	NS
<b>Bio-fertilizers</b>				
F1:Fertilizers @ 30:60:40 kg ha <sup>-1</sup> N:P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O	85.13	96.30	53.86	63.53
F2:F1 + Rhizobium @ 250g 10 kg <sup>-1</sup> seed	83.33	94.50	52.93	62.60
F3:F2 + Phosphate solubilising bacteria @ 5 kg ha <sup>-1</sup>	85.93	97.10	54.00	63.66
F4:F3+ Potassium solubilising bacteria @ 5 kg ha <sup>-1</sup>	86.66	97.76	56.13	66.93
SE±	1.71	1.70	1.90	1.91
CD(P=0.05)	NS	NS	NS	NS
<b>Weed Management x Bio-fertilizers</b>				
SE±	3.82	3.81	4.26	4.27
CD(P=0.05)	NS	NS	NS	NS

Initial- Acid phosphatase activity 82  $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$  ; Alkaline phosphatase 51  $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$

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