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RESEARCH NOTE

Assessing the compatibility of pre- and post-emergence herbicides with plant growth promoting rhizobacteria on performance of soybean

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ABSTRACT

The study was conducted to know the compatibility of different herbicides recommended for soybean with plant growth promoting rhizobacteria (PGPR) including the native strain of *Rhizobium* so that the tolerant microbes could be used as a potential herbicide tolerant microbial culture to support the soybean crop nutritionally and on the growth performance of the soybean (*Glycine max* L. Merill). In this study, the soybean crop was inoculated by cultures of *Bradyrhizobium daqingense, Paenibacillus polymyxa* and *Bradyrhizobium japonicum*. As recommended herbicides for soybean, pre-emergence herbicides diclosulam and pendimethalin were sprayed to the soybean plant 52 ppm/ha and 6 ppm/ha, respectively at 5 days after sowing. Post-emergence herbicides propaquizafop and imazethapyr were sprayed to the soybean plant 1.2 ppm/ha and 2 ppm/ha, respectively and their cocktail mix 4 ppm/ha at 18 days after sowing. The experiment was laid out in a completely randomized design with eighteen treatments repeated three times on a soybean crop (cv: *JS-9560*). The results of the investigation revealed that pendimethalin was comparatively more compatible with PGPRs than diclosulam under pre-emergence herbicide category. In case of post-emergence herbicides, propaquizafop was comparatively more compatible with PGPRs, than imazethapyr and cocktail mix of propaquizafop + imazethapyr. The propaquizafop was found safe herbicide to produce maximum biomass yield of soybean at 50 days after herbicide application (DAHA). The nodulation behavior was found significantly less affected by all the herbicidal application in presence of *Bradyrhizobium daqingense*.

Keywords: *Bradyrhizobium*, Diclosulam, Imazethapyr, *Paenibacillus polymyxa*, Pendimethalin, Propaquizafop, Soybean

Soybean (*Glycine max* L. Merill) belongs to family "Legumenaceae or Papilionaceae" has been called "Goldan bean" or "Miracle crop" of twentieth century consisting 40-42% protein and 18-22% oil (Masciarelli *et al*. 2014). Soybean produces 2-3 times more high-quality protein yield per hectare than other pulses and cholesterol free oil (Kumari *et al*. 2002). It is cultivated as the world's sixteenth most significant crop. (Foley *et al*. 2011). Soybean is mostly grown in *Kharif* (rainy) season and suffers from severe weed crop competition due to continuous rain, which do not permit hand weeding operation timely resulting in yield loss to the tune of 30–80% (Yaduraju 2002). Weeds are the major biotic factor responsible for poor soybean yield. Malik *et al.* (2006). have reported 55% soybean yield reduction with broad-leaved weeds (80%), grasses and sedges (20%) infestation throughout the crop season. Major broad-leaved weeds of soybean are *Celosia argentia, Digera arvensis, Commelina benghalensis, and Amaranthus viridis* (Singh Pratap and Rajkumar 2008). Pre-emergence herbicides are recommended in soybean production systems for management of weed species with extended emergence window (Norsworthy *et al.* 2012). Due to the widespread prevalence of glyphosate-resistant weeds and limited effective post-emergence herbicide options in soybean, the use of pre-emergence herbicides has become a standard recommendation for weed management. Benefits of incorporating pre-emergence into weed management programms include reduced early season weed competition and delayed critical time for weed removal, thus optimizing weed control strategies and minimizing potential crop yield loss (Oliveira *et al.* 2017, Knezevic *et al*. 2019). The availability of pre-emergence herbicides such as chlorimuron-ethyl, cloransulam-methyl, metribuzin, sulfentrazone, flumioxazin, saflufenacil, acetochlor, S-metolachlor, dimethenamid-P, pyroxasulfone, diclosulam and pendimethalin *etc*. and post-emergence herbicides such as imazethapyr, propaquizafop, acifluorfen, fomesafen, sethoxydim and fluazifop-p-butyl *etc*. for soybean crop has been noticed in recent years in Indian herbicide market. The ideal bio-fertilizers for soybean are *Rhizobium* and phosphate solubilizing bacteria. These crop beneficial microorganisms

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supplement substantial amount of nitrogen and phosphorus to crop which increase the productivity and simultaneously reduce the input cost of cultivation. Generally, the large farmers have initiated use of the above bio-fertilizers under the new techniques of cultivation but the small and marginal farmers are far behind. When we apply different herbicides to the soil for controlling the weeds, it may cause some effect on the applied bio-inoculant. Hence, an experiment was framed to assess the compatibility of different soybean herbicides with plant growth promoting rhizobacteria (PGPR) including the native strain of *Rhizobium* so that the tolerant microbes could be used as a potential herbicide tolerant microbial culture to support the soybean crop nutritionally.

The experiment was conducted under the open microcosm conditions in the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during 2021-22 with Soybean (*Glycine max*), Variety: *JS-9560*. The study area receives average annual rainfall of 1200-1400 mm, with temperature ranged from 12 °C in December to 45 °C in May. Polybags were used for conducting the experiment. A random collection of surface soil was done from 15 cm (6-inch) depth from the fields of real agricultural land near the College of Agriculture, Raipur and was thoroughly blended with compost samples. This soil was sieved and processed from a 2 mm sieve. Well-mixed sample of 8 kg soil, sand and compost in the ratio 3:1:1 was filled for facilitating proper drainage of water. The surface of the seeds was sterilized with 95% ethanol and 0.1 per cent mercuric chloride. The seeds were then rinsed 7 times with sterilized water and then placed on the Petri-plates to undergo seed treatments. A five per cent sugar solution was applied to each Petri-plate to help in adhesion.

Hundred micro-litre of each bacterial cultures, such as *Bradyrhizobium daqingense*, *Paenibacillus polymyxa* and *Bradyrhizobium japonicum* for thirty seeds were introduced by using a micropipette in aseptic surrounding. The treated seeds were sown in the holes made aseptically by the glass rod. Ten seeds were sown in each polybag. At 7 days after sowing (DAS), the seedlings were thinned and three seedlings were maintained in each pot. The nutrients such as nitrogen, phosphorus and potassium were added after seven days of sowing with the recommended dose of 20:80:40 NPK, kg/ha. The experiment was laid out in a completely randomized design with eighteen treatments. The details of the treatments and their scheduling are given in **Table 1**. The soybean crop was inoculated by cultures *of Bradyrhizobium daqingense, Paenibacillus polymyxa* and *Bradyrhizobium japonicum*. The required amount of pre-emergence herbicides diclosulam and pendimethalin were sprayed to the soybean plant 52 ppm/ha and 6 ppm/ha, respectively at 5 DAS. Post-emergence herbicides propaquizafop and imazethapyr were sprayed to the soybean plant 1.2 ppm/ha and 2 ppm/ha, respectively and also the cocktail mix of propaquizafop and imazethapyr was sprayed 4 ppm/ha, each at 18 DAS. The plants height (cm) was measured at 15, 30 and 50 days after pre- and post-emergence herbicide application, the number of weed population (area of 12.36 square inch) were measured at 10 days after pre- and post-emergence herbicide application, the number of nodules (nodules/plant), fresh and dry weight of nodules (g/plant), fresh and dry weight (g/plant) of shoot and root were recorded at 50 DAHA (days after herbicide application). All observations from this experimental study have been systematically tabulated. For their respective number of replications used, values were given as a means. For complete randomized design, the data were statistically analysed using ANOVA. The significant difference was tested at signifying level by F-test at 5 per cent. If F-test found significant in comparing treatment means then standard error of mean (SEm ±) and CD was calculated, (Panse and Shukhatme 1978).

The plants height: The Data on plant height of soybean was recorded at three different growth stages of the crop which was tabulated in **Table 1** and **Plate 1**. The data recorded at 15, 30 and 50 DAHA revealed that pre-emergence herbicides diclosulam and pendimethalin significantly reduced the plant height at all the stages in comparison to untreated check. Data recorded at different growth stages of crop growth indicated that among different PGPRs, highest plant growth was recorded due to *Bradyrhizobium daqingense* (33.9 cm) followed by *Bradyrhizobium japonicum* (31.4 cm) and *Paenibacillus polymyxa* (30.1 cm) at 30 DAHA. *Paenibacillus polymyxa* was least affected by pre-emergence herbicide diclosulam among the three PGPRs whereas local isolate *Bradyrhizobium japonicum* was severely affected by diclosulam application among the three PGPRs. In case of pendimethalin, the trend of plant growth inhibition was similar to that of diclosulam. Post- emergence herbicide propaquizafop did not inhibit the plant growth at all the growth stages of crop in presence of *Bradyrhizobium daqingense* and *Paenibacillus polymyxa*. However, in case of *Bradyrhizobium japonicum,* the propaquizafop found detrimental to reduce the plant height at 30 and 50 DAHA.

Growth performance of the crop PE (diclosulam) at 50 DAHA

Growth performance of the crop PE (pendimethalin) at 50 DAHA

Growth performance of the crop PoE (propaquizafop) at 50 DAHA

Growth performance of the crop PoE (imazethapyr) at 50 DAHA

Growth performance of the crop (cocktail mix of propaquizafop + imazethapyr) at 50 DAHA

Plate 1. Growth performance of the crop at 50 DAHA (days after herbicide application)

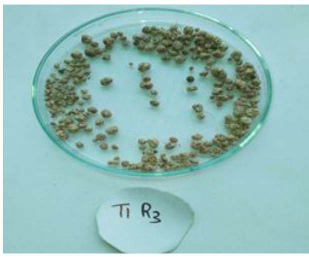
Bradyrhizobium daqingense + pendimethalin PE

Bradyrhizobium daqingense + diclosulam PE

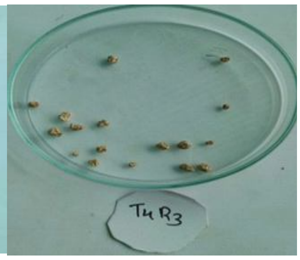
Bradyrhizobium daqingense + propaquizafop PoE

Bradyrhizobium daqingense + imazethapyr PoE

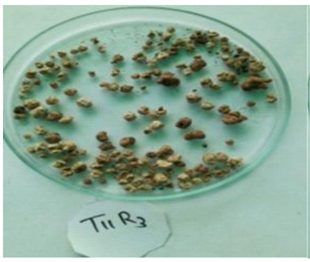
Plate 2. Biomass of soybean crop at 50 days as influenced by pre- and post -emergence herbicides



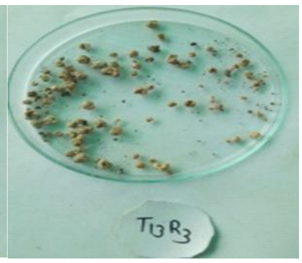
Bradyrhizobium daqingense + pendimethalin PE



Bradyrhizobium daqingense + diclosulam PE



Bradyrhizobium daqingense + propaquizafop PoE



Bradyrhizobium daqingense + imazethapyr PoE

Plate 3. Soybean nodulation behavior at 50 days after pre- and post- emergence herbicide application

Application of post- emergence herbicide imazethapyr alone and its cocktail mixture with propaquizafop significantly affected the plant growth at all the three stages over individual application of PGPRs, without treatment of herbicides. Thus, it was concluded that in case of pre-emergence herbicides, pendimethalin was comparatively more compatible with PGPRs, than diclosulam. Similar results were obtained by ESFA (2014) and it was revealed that the residues of pendimethalin were not detected at harvest in soil, soybean oil, defatted cake and straw from treated fields. Pendimethalin residues were below MRL in soybean. In our case, we found that post-emergence herbicides propaquizafop was comparatively more compatible with PGPRs, than imazethapyr and cocktail mix of propaquizafop + imazethapyr. Above observations were in close agreement with Renjith and Sharma (2014) as they reported that among herbicidal treatments, plants treated with propaquizafop 50 g/ha at 3 weeks after sowing (WAS) gave better performance.

Weed population: The data of weed population as affected by herbicide application are presented in **Figure 1**. It is apparent from the data that the weed population was significantly reduced in the treatments where herbicides were applied. Among pre-emergence herbicides, application of diclosulam did not allow the growth of any weed in the poly-bag having an area of 12.36 square inch. Similarly, application of pendimethalin also reduced the weed population to the extent of zero level in the treatment containing *Paenibacillus polymyxa*. Among post-emergence herbicides, propaquizafop was found better than imazethapyr for reduction of weed population. Minimum weed population (0.7/bag) was recorded in treatment received propaquizafop in presence of *Paenibacillus polymyxa*. In case of imazethapyr, the minimum weed population was 1.3/ bag recorded in treatment where *Paenibacillus polymyxa* was applied. Cocktail mix of post-emergence herbicides although significantly reduced the weed population but did not find more effective to reduce the population as comparison to the single application of post-emergence herbicides. Higher population of weeds was observed in pots treated with PGPRs compared to untreated check. Highest population of weeds was recorded in *Bradyrhizobium daqingense* (4.3/bag) treatment and minimum in *Paenibacillus polymyxa* (3.7/bag). Thus, it was concluded that in case of pre-emergence herbicides, diclosulam had comparatively more weed control efficiency as compared to other herbicidal treatments. Similar results were obtained by Singh *et al.* (2009), who revealed that diclosulam applied at 22 and 26 g/ha showed higher weed control efficiency as compared to other herbicidal treatments at all the stages of crop growth. It also provided higher value for all the characters of yield attributes and grain yield. In case of post-emergence herbicides, propaquizafop had comparatively more weed control efficiency as compared to imazethapyr & cocktail mix of propaquizafop + imazethapyr.

Plant biomass: The data on the effect of inoculation with different PGPRs, with and without application of herbicides on plant fresh and dry matter at 50 DAHA is presented in **Figure 1** and **Plate 2**. Data

Table 1. Effect of pre- and post-emergence herbicides on plant height (cm) of soybean

Treatment	Plant height (cm)		
	15 DAHA	30 DAHA	50 DAHA
Bradyrhizobium daqingense (control- I)	11.53	33.90	36.90
Paenibacillus polymyxa (control- II)	10.60	30.10	35.23
Bradyrhizobium japonicum (local strain – control -III)	10.80	31.40	35.80
Bradyrhizobium daqingense + diclosulam	8.83	19.53	24.43
Paenibacillus polymyxa + diclosulam	8.70	17.93	24.96
Bradyrhizobium japonicum + diclosulam	8.00	13.30	18.30
Bradyrhizobium daqingense + pendimethalin	9.03	21.20	26.40
Paenibacillus polymyxa + pendimethalin	9.10	21.40	28.30
Bradyrhizobium japonicum + pendimethalin	8.200	13.60	23.20
Bradyrhizobium daqingense + propaquizafop	10.43	32.90	34.80
Paenibacillus polymyxa + propaquizafop	11.00	32.73	36.30
Bradyrhizobium japonicum + propaquizafop	10.10	24.30	31.20
Bradyrhizobium daqingense + imazethapyr	8.50	17.43	26.56
Paenibacillus polymyxa + imazethapyr	8.20	13.93	24.23
Bradyrhizobium japonicum + imazethapyr	8.50	17.66	26.93
Bradyrhizobium daqingense + (propaquizafop + imazethapyr)	8.40	23.66	30.80
Paenibacillus polymyxa + (propaquizafop + imazethapyr)	8.30	23.33	30.60
Bradyrhizobium japonicum + (propaquizafop + imazethapyr)	8.00	20.76	28.43
LSD (p=0.05)	1.19	2.82	3.80

showed that application of pre-emergence herbicides significantly reduced the fresh and dry biomass of shoot but the root biomass was not affected except in the treatment received pendimethalin and treated with *Bradyrhizobium daqingense.* Among pre-emergence herbicides, diclosulam was found more effective to reduce the fresh and dry biomass of soybean than pendimethalin. Among post-emergence herbicides, the biomass yield of soybean was not affected by propaquizafop but significantly reduced due to application of imazethapyr. Among tested PGPRs, *Bradyrhizobium daqingense* was least affected due to application of imazethapyr whereas in case of propaquizafop along with *Paenibacillus polymyxa* performed best. Cocktail mix of post-emergence herbicides, imazethapyr significantly reduced the biomass yield of soybean at 50 DAHA. Data clearly indicated that combined effect of both the post-emergence herbicides reduced the shoot biomass in comparison to their individual application but the shoot dry biomass reduction due to cocktail application of post-emergence herbicides was significant than individual application. The results clearly elucidated that *Bradyrhizobium japonicum* was least affected by cocktail mix of propaquizafop and imazethapyr followed by *Bradyrhizobium daqingense*. Fresh and dry biomass of soybean root was unaffected by application of cocktail combination of post-emergence herbicides except the case in which *Bradyrhizobium daqingense* was applied**.** Similar results were obtained by Shaner and Singh (1992), who revealed that the reductions in fresh and dry matter content of maize and soybean seedlings in response to treatment with the different herbicides appeared as a result of concomitant alterations in certain metabolic processes. Protein and carbohydrate metabolism in several plant species can be affected by many herbicides with a reduced production of plant materials and, consequently, growth cessation.

Nodulation behavior: The data on the effect of different herbicides on number of nodules and their biomass are presented in **Table 2** and **Plate 3**. The number of nodules recorded at 50 days after herbicide application (DAHA) revealed that both pre- and post-emergence herbicides including cocktail mix of post-emergence herbicides significantly reduced the nodule number and their biomass. In case of pre-emergence herbicides, diclosulam affected the nodulation more than pendimethalin. Among pre-emergence herbicides, comparatively higher nodules were recorded in treatment of pendimethalin over diclosulam. Higher number of nodules was recorded 41 per plant) in the treatments of *B. daqingense*; in case of pendimethalin. It was reduced to (8.33 per plant) in treatment of *B. daqingense*, due to the treatment of diclosulam. Similar results were obtained by Praharaj and Dhingra (1995) who revealed that application of pendimethalin 0.5 kg/ha neither had any adverse effect on the nodulation and nitrogenase activity nor it influenced the efficiency of rhizobial inoculants in terms of BNF (biological nitrogen fixation) in soybean. Among PGPRs, *Bradyrhizobium*

Table 2. Effect of pre- and post-emergence herbicides on nodulation behavior of soybean at 50 DAHA

Treatment	Nodulation		
	No. of nodules/ plant	Fresh weight of nodules (g/plant)	Dry weight of nodules (g/plant)
Bradyrhizobium daqingense (control-I)	221.66	3.26	1.22
Paenibacillus polymyxa (control-II)	164.32	2.59	0.92
Bradyrhizobium japonicum (local strain – control-III)	101.43	2.04	0.70
Bradyrhizobium daqingense + diclosulam	8.33	0.14	0.04
Paenibacillus polymyxa + diclosulam	7.66	0.11	0.03
Bradyrhizobium japonicum + diclosulam	4.66	0.06	0.01
Bradyrhizobium daqingense + pendimethalin	41.00	0.52	0.21
Paenibacillus polymyxa + pendimethalin	39.33	0.82	0.26
Bradyrhizobium japonicum + pendimethalin	28.00	0.34	0.11
Bradyrhizobium daqingense + propaquizafop	85.00	1.63	0.53
Paenibacillus polymyxa + propaquizafop	106.00	1.48	0.49
Bradyrhizobium japonicum + propaquizafop	88.7	1.42	0.47
Bradyrhizobium daqingense + imazethapyr	75.67	0.76	0.24
Paenibacillus polymyxa + imazethapyr	33.00	0.48	0.20
Bradyrhizobium japonicum + imazethapyr	58.00	0.96	0.32
Bradyrhizobium daqingense + (propaquizafop + imazethapyr)	61.00	0.76	0.26
Paenibacillus polymyxa + (propaquizafop + imazethapyr)	33.67	0.77	0.28
Bradyrhizobium japonicum + (propaquizafop + imazethapyr)	41.33	0.52	0.16
LSD (p=0.05)	11.73	0.16	0.06

japonicum was severely affected by pre-emergence herbicides followed by *Paenibacillus polymyxa* to produce nodules. In case of nodule biomass, diclosulam significantly affected the fresh and dry biomass of nodules in comparison to pendimethalin. Among PGPRs, *Paenibacillus polymyxa* was least affected by pendimethalin followed by *Bradyrhizobium daqingense*. However, *Bradyrhizobium daqingense* was least affected by diclosulam followed by *Paenibacillus polymyxa* in both the cases of fresh and dry biomass of nodules. The study on the effect of post-emergence herbicides alone and combination revealed that number of nodules and their biomass was less affected by propaquizafop in comparison to imazethapyr. Among different PGPRs, *Paenibacillus polymyxa* found resistant over others and produced maximum number of nodules (106 per plant) and their biomass yield (1.48g/plant). However, in case of imazethapyr, *Bradyrhizobium daqingense* produced the maximum number of nodules (75 per plant) followed by Bradyrhizobium japonicum (58/plant). In case of biomass yield of nodules, the maximum yield was attributed with *Bradyrhizobium japonicum* (0.96 g/plant) followed by *Bradyrhizobium daqingense* (0.76 g/plant). Combined application of post-emergence herbicides

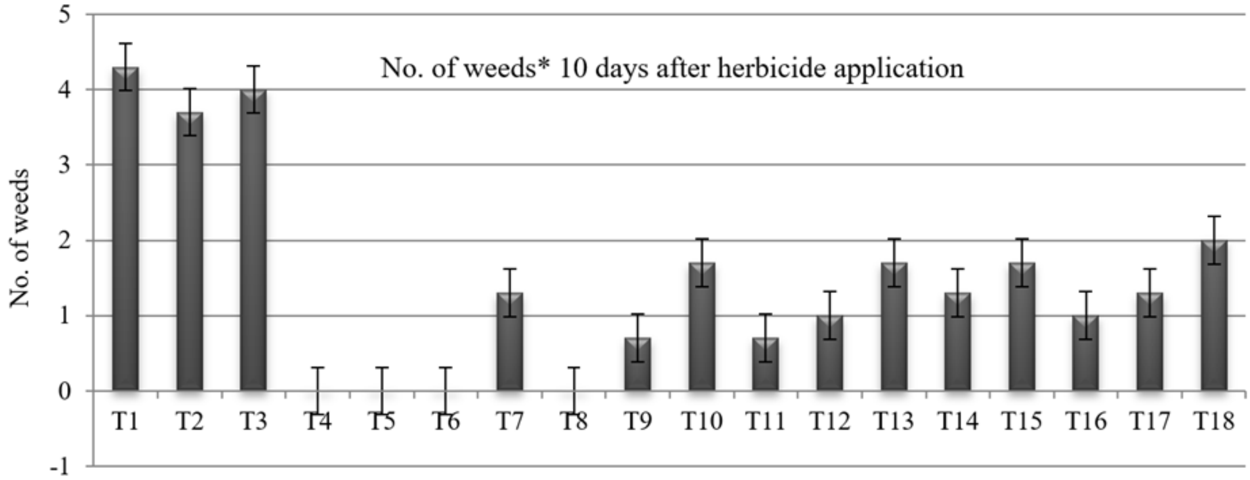


Figure 1. Effect of pre- and post-emergence herbicides on weed population

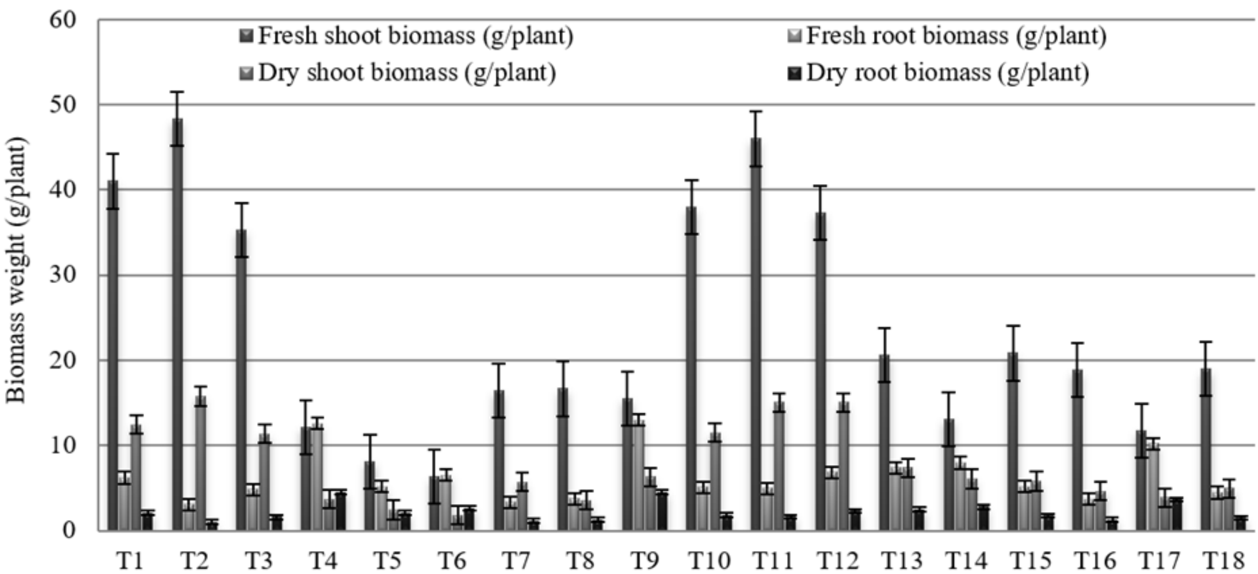


Figure 2. Effect of pre- and post-emergence herbicides on plant biomass of soybean at (50 DAHA)

T_1: *Bradyrhizobium daqingense* (control- I); **T_2:** *Paenibacillus polymyxa* (control- II); **T_3:** *Bradyrhizobium japonicum* (local strain – control -ÉÉÉ); **T_4:** *Bradyrhizobium daqingense* + diclosulam; **T_5:** *Paenibacillus polymyxa* + diclosulam**;** **T_6:** *Bradyrhizobium japonicum* + diclosulam; **T_7:** *Bradyrhizobium daqingense* + pendimethalin; **T_8:** *Paenibacillus polymyxa* + pendimethalin; **T_9:** *Bradyrhizobium japonicum* + pendimethalin; **T_{10}:** *Bradyrhizobium daqingense* + propaquizafop**;** **T_{11}:** *Paenibacillus polymyxa* + propaquizafop; **T_{12}:** *Bradyrhizobium japonicum* + propaquizafop; **T_{13}:** *Bradyrhizobium daqingense* + imazethapyr; **T_{14}:** *Paenibacillus polymyxa* + imazethapyr; **T_{15}:** *Bradyrhizobium japonicum* + imazethapyr; **T_{16}:** *Bradyrhizobium daqingense* + (propaquizafop + imazethapyr); **T_{17}:** *Paenibacillus polymyxa* + (propaquizafop + imazethapyr); **T_{18}:** *Bradyrhizobium japonicum* + (propaquizafop + imazethapyr) and *Bradyrhizobium daqingense* (61/lant) produced the maximum nodulation followed by *Bradyrhizobium japonicum* (41/per plant) However, the biomass yield of soybean nodules was found maximum with *Paenibacillus polymyxa* (0.77 g/ plant) followed by *Bradyrhizobium daqingense* (0.76 g/plant). In this study, it was proved that in case of pre-emergence herbicides, pendimethalin was comparatively more compatible with PGPRs than diclosulam to produce nodules. In case of post-emergence herbicides, propaquizafop was comparatively more compatible with PGPRs than imazethapyr and cocktail mix of propaquizafop + imazethapyr to produce nodules. Above observations were in close agreement with Sawicka and Selwet (1998), who claimed that both imazethapyr and linuron can cause decrease of root– nodule and bacterial nitrogenase activity. They also can stimulate development of bacteria and inhibitory growth of fungi.

From the above findings, it may be concluded that pendimethalin was comparatively more compatible with PGPRs than diclosulam under pre-emergence herbicides category. The same chemical was found suitable to produce maximum biomass yield of soybean at 50 DAHA. In case of post-emergence herbicides, propaquizafop was comparatively more compatible with PGPRs than imazethapyr and cocktail mix of propaquizafop + imazethapyr. Propaquizafop was found safe herbicide to produce maximum biomass yield of soybean at 50 DAHA. Among pre- and post-emergence herbicides, application of propaquizafop was found comparatively better molecule to produce maximum soybean biomass yield.

The salient findings of this investigation are (i) the nodulation behavior of soybean was least affected by all the herbicidal application in presence of *Bradyrhizobium daqingense*. Hence, it is proved that *Bradyrhizobium daqingense* is highly tolerant to different pre-and post-emergence herbicides. However the plant biomass was found to be less affected by pre-emergence herbicides in presence of *Bradyrhizobium daqingense* and post-emergence herbicides in presence of *Paenibacillus polymyxa* (ii) the rhizobial population in soil was comparatively less affected by application of pre-emergence and post-emergence herbicides up to 20 DAHA due to inoculation of *Bradyrhizobium daqingense*, but after 20 DAHA, the performance of local strain *Bradyrhizobium japonicum* was the best to accelerate the population.

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