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Identification of effective PGPR(s) for management of herbicidal stress towards successful cultivation of Soybean (*Glycine max* L. Merrill)

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Abstract
To assess 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 on the growth performance of the soybean (*Glycine Max* L. Merrill) crop. The study was carried out in the Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur, Chhattisarh, India during 2021-2022. In this study the soybean crop was inoculted 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 @ 52ppm/ha and @ 6ppm/ha, respectively at 5 days after sowing. Post emergence herbicides propaquizafop and imazethapyre were sprayed to the soybean plant @ 1.2ppm/ha and @ 2ppm/ha, respectively and their cocktail mix of propaquizafop and imazethapyre were sprayed to the soybean plant @ 4ppm/ha, at 18 days after sowing. Results from the investigation revealed that pendimethalin was comparatively more compitable with PGPRs than diclosulam under pre emergence herbicide category. In case of post emergence herbicides propaquizafop was comparatively more compitable with PGPRs, than imzethapyre & cocktail mix of propaquizafop + imazethapyre.

Keywords: Herbicides, basal soil respiration, dehydrogenase activity, *Bradyrhizobium*, *Paenibacillus polymyx*a, Soybean

Introduction

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) [6]. It is cultivated as the world’s sixteenth most significant crop. (Foley *et al.*, 2011) [4]. Soybean is an important oilseed crop playing a vital role in sustaining the oilseed production in India. Soybean production supports the livelihood of a large number of people associated with cultivation, trading, processing, industrial usages, value addition, and export of soybean and its products, in India and overseas (Dass *et al*., 2016) [3]. In India Soybean is a rainfed crop In India, with an area, production, and productivity of 11 million hectares, 13 million tons, and 11.92 quintal per hectare, respectively. (FAO, 2019).

Weeds are the major biotic factor responsible for poor soybean yield. Malik *et al*. (2006) [5]. have reported 55% soybean yield reduction with broad-leaved weeds (80%), grasses and sedges (20%) infestation throughout the crop season. Major weeds of soybean are monocot weeds, *viz*. *Echinochloa colona*, *Dinebra retroflexa* and *Cyperus iria*; and dicot weeds, *viz*. *Eclipta alba* and *Alternanthera philoxeroides*. Pre-emergence herbicides are recommended in soybean (*Glycine max* (L.) Merr.) production systems for management of weed species with extended emergence window (Norsworthy *et al*. 2012) [7]. Due to the widespread prevalence of GR 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 programs include reduced early season weed competition and delayed critical time for weed removal, thus optimizing weed control strategies and minimizing potential crop yield loss.

Basal Soil Respiration (BSR) has been extensively used as an indicator of this soil microbial activity (Thoumazeau *et al*.,2017) [13]. Microbial biomass, and soil dehydrogenase activity was significantly correlated with soil respiration (Cao *et al*.,2016) [2]. Chemical herbicides have been used on fields across the world, creating a buildup of adverse pollution in our environment, which continues to grow with every application. When herbicides are applied onto a surface, they travel outside their intended area of use by air, soil or water. Chemical herbicides not only deplete the nutritional value of our food, but they also contaminate it. This can lead to reduce weed, contaminate surface water and groundwater and injury of non-target species, including humans. Going organic allows us to start from scratch with the soil. Decreasing soil chemical contamination creates an overall "return to nature", bringing back nutrients and helpful organisms, and yielding clean, unaltered produce. The ideal biofertilizers for soybean are *Rhizobium* & phosphate solubilizing bacteria. These crop beneficial microorganisms supplement substantial amount of nitrogen & phosphorus to crop which increase the productivity and simultaneously reduce the input cost of cultivation. Although most of the large farmers applied the above biofertilizers under the new techniques of cultivation but the small & 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.

The present study is coming to know 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.

In the remaining of the paper, section 2 explains materials and methods, Section 3 describes result and discussion while section 4 describes the conclusion.

Materials and Methods

The experiment was conducted under the open microcosm condition in the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during 2021-2022 with Soybean (*Glycine max*), Variety: JS-9560. The experiment was carried out in a completely randomized design with three replication. The details of the treatments and their scheduling are given in Table 1.

Filling of poly bags for experimentation

A random collection of surface soil was done from a 15cm (6-inch) depth from the fields of real agricultural land near the College of Agriculture, Raipur and was thoroughly blended with compost samples. A 8Kg well-mixed sample in a ratio 3:1:1 for Soil: Sand: Compost was filled for facilitating proper drainage of water.

Preparation of the inoculums

In a 500 ml volumetric flask, new broths were prepared, and the cultures of *Bradyrhizobium daqingense*, *Paenibacillus polymyxa* and *Bradyrhizobium japonicum* were inoculated and incubated. The broths would be added to the soybean seeds according to the treatments through seed treatment.

Seed sterilization and seed inoculation

The seeds were chosen and the surfaces of the seeds were sterilised with 95 percent ethanol and 0.1 percent mercuric chloride. The seeds were then rinsed 7 times with sterilised water and then placed on the petri-plates to undergo seed treatments. A five percent 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, according to the treatments and the petri-plates were then gently shaken in a circular motion for 15 to 10 minutes before being set aside.

Sowing and thinning

One day before sowing, the soil-filled poly bags were moistened. With the aid of forceps, the treated seeds were sown in the holes made aseptically by the glass rod. Ten seeds were sown in each poly bag. At 7 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.

Herbicide application

As a recommended herbicide for soybean pre emergence herbicides diclosulam and pendimethalin were sprayed to the soybean plant @ 52ppm/ha and @ 6ppm/ha, respectively at 5 days after sowing. Post emergence herbicides propaquizafop and imazethapyre were sprayed to the soybean plant @ 1.2ppm/ha and @ 2ppm/ha, respectively at 18 days after sowing and their cocktail mix of propaquizafop and imazethapyre were sprayed to the soybean plant @ 4ppm/ha, at 18 days after sowing.

Observations recorded during experiment

Soil sampling was done at 10, 20, 30 and 50 DAHA (days after herbicide application) and the samples were analyzed for basal soil respiration rate, dehydrogenase activity and population of soil rhizobial, phospho-bacterial (PSB).

Dehydrogenase activity

Dehydrogenase enzymatic activity in soil was estimated by UV/VIS Spectrophotometer with the wavelength of 485 nm which based on the principles of reduction of 2,3,5-triphenylotetrazolium chloride (TTC) to red-coloured triphenylformazon (TPF), which were quantified spectrophotometrically.

Basal Soil Respiration study

This study was conducted to know the respiration rate of microflora present in the crop rhizosphere soil. Basal soil respiration was calculated by measuring the CO2 evolution rates.

Microbial enumeration

The soil microbial populations were enumerated by using selective media by standard spread plate dilution technique. YEMA medium (Subba Rao, 1988) [11] for *Rhizobium* and Phosphobacterial population was enumerated by using Pikovskaya medium (Pikovskaya, 1948) [8].

Spot analysis

For testing the compatibility of PGPRs with herbicides, microbial estimation with respect B*radyrhizobium daqingense*, *Paenibacillus polymyxa* (PSB) and *Bradyrhizobium japonicum* (Local strain) was done.

Results and Discussion

Basal soil respiration

Pre emergence herbicide

The data on the effect of pre emergence herbicides on basal soil respiration rate recorded at different intervals after their application are presented in Table 1. The basal soil respiration (BSR) rate was reduced significantly at 10 days after herbicide application (DAHA). In all the herbicide applied treatments, except the treatments received *Bradyrhizobium japonicum* as a PGPRs, diclosulam was found more effective to reduce BSR in comparison to pendimethalin. Comparatively higher BSR was noticed in 20 DAHA in all the treatments over 10 DAHA. Significantly better BSR rate recorded in pendimethalin over diclosulam herbicide received the same PGPR. i.e. *Bradyrhizobium daqingense* (0.98 mg CO_2/h/100g) at 20 DAHA. At 30 and 50 DAHA the BSR rate was observed gradually increased over 10 and 20 DAHA in all the treatments. At both the interval the diclosulam treated treatments have showed significantly lower BSR in comparison to control. In case of pendimethalin only *Bradyrhizobium daqingense* (1.22 mg CO_2/h/100g) containing treatments had showen significantly lower BSR over control (1.49 mg CO_2/h/100g) at 45 DAHA. In this study it is proved that pendimethalin was comparatively more compitable with PGPRs, than diclosulam. Among PGPRs, *Paenibacillus polymyxa* had shown more tolerance towards pendimethalin over others.

Post emergence herbicide

The Data on the effect of post emergence herbicides and their cocktail mix on basal soil respiration rate (BSR) was presented in Table 2. The Data showed that *Paenibacillus polymyxa (*1.14, 1.19, 1.26 and 1.33 mg CO_2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), was significantly affected by both the post emergence herbicides in propaquizafop treated treatment and (1.13, 1.17, 1.26 and 1.31 mg CO_2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), in imazethapyre treated treatment at all intervals after their application. The two other PGPRs, *Bradyrhizobium daqingense* (1.21, 1.33, 1.38 and 1.69 mg CO2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), in propaquizafop treated treatment, and *Bradyrhizobium japonicum* (1.11, 1.14, 1.18 and 1.44 mg CO2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), in propaquizafop treated treatment and *Bradyrhizobium daqingense* (1.18, 1.30, 1.34 and 1.67 mg CO_2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), in imazethapyre treated treatment, *Bradyrhizobium japonicum* (1.04, 1.11, 1.16 and 1.38 mg CO_2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), in imazethapyre treated treatment were not significantly affected by application of propaquizafop and imazethapyre. The cocktail mix of above herbicides significantly lowered the BSR at different DAHA. The cocktail mix was reduced the rate of BSR at maximum level incase of *Bradyrhizobium japonicum* (0.98, 1.06, 1.10 and 1.22 mg CO_2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), and minimum in case of *Bradyrhizobium daqingense* (1.12, 1.18, 1.27 and 1.34 mg CO_2/h/100g BSR rate at 10, 20, 30 and 50 DAHA, respectively), It is revealed from the study that the BSR was more affected by imzethapyre compared to propaquizafop. The above observation are in close agreement with Ahmad *et al*. (2018) [1], who stated that under rice-chickpea cropping sequence application of imazethapyr at different doses significantly reduced the BSR rate soon after their application. The toxic effect of these chemicals was traced upto 10 DAS and there after a gradual increment BSR rate was noticed. Among PGPRs, local isolate *Bradyrhizobium japonicum* was found more susceptible to post emergence herbicides than others. However, in all stages *Bradyrhizobium daqingense* had showen the maximum tolerance towards individual and cocktail combination of propaquizafop and imazethapyre.

Dehydrogenase activity

Pre emergence herbicide

The data on the effect of pre emergence herbicides on dehydrogenase activity (DHA) of soil recorded at different intervals after their application are presented in Table 1. The study revealed that DHA activity was reduced significantly at 10 days after herbicide application (DAHA). In all the herbicide applied treatments diclosulam was found more effective to reduce DHA in comparison to pendimethalin. diclosulam was reduced DHA significantly (20.50, 22.79 and 25.94 g TPF/h/g DHA at 10, 20 and 30 DAHA, respectively); in *Bradyrhizobium daqingense* treated treatment; (20.63, 22.99 and 26.04 µg TPF /h/g DHA at 10, 20 and 30 DAHA, respectively), in *Paenibacillus polymyxa* treated treatment and (20.45, 22.54 and 25.77 µg TPF /h/g DHA at 10, 20, and 30 DAHA, respectively); in *Bradyrhizobium japonicum* treated treatment over pendimethalin (23.55, 25.92 and 28.54 µg TPF /h/g DHA at 10, 20 and 30 DAHA, respectively); in *Bradyrhizobium daqingense* treated treatment; (23.67, 25.94 and 28.98 µg TPF /h/g DHA at 10, 20 and 30 DAHA, respectively); in *Paenibacillus polymyxa* treated treatment; (23.53, 25.72 and 28.74 µg TPF /h/g DHA at 10, 20, and 30 DAHA, respectively); in *Bradyrhizobium japonicum* treated treatment at 10 to 30 DAHA. At 30 and 50 DAHA the DHA was observed gradually increased over 10 and 20 DAHA in all the treatments. At both the interval the diclosulam treated treatments have showed significantly lower DHA in comparison to control. At 50 DAHA the effect of both diclosulam and pendimethalin was found equal for all the three studied organisms. Local strain of *Bradyrhizobium japonicum* was non-significantly affected by the pre emergence herbicide as the DHA value of the concerned treatment was found at par with control. *Paenibacillus polymyxa* was found effective to tolerate both the herbicides after 30 days of their application. In this study it is proved that pendimethalin was comparatively more compitable with PGPRs, than diclosulam. Among PGPRs, local strain of *Rhizobium japonicum* had shown more tolerance towards pendimethalin over others. The result is in agreement with the finding of Sebiomo *et al*., (2011) [10] who observed that the application of herbicides to the soils led to a significant drop in dehydrogenase activity with respect to control soil samples. Suresh and Qureshi (2010) [12] reported that application of herbicide reduced the activity of dehydrogenase enzyme. The decreases in enzymatic activity of dehydrogenase with increase in herbicidal concentration.

Post emergence herbicide

The Data on the effect of post emergence herbicides and their cocktail mix on dehydrogenase activity (DHA) of soil are presented in Table 2. The data recorded from the study revealed that *Paenibacillus polymyxa* (27.13, 29.20, 33.27 and 36.34 µg TPF /h/g DHA at 10, 20, 30 and 50 DAHA, respectively), was significantly affected by post emergence herbicides in propaquizafop treated treatment and (27.12, 29.18, 33.27 and 36.32 µg TPF /h/g DHA at 10, 20, 30 and 50 DAHA, respectively) in imazethapyre treated treatment at all intervals after their application. The two other PGPRs, *Bradyrhizobium daqingense* and *Bradyrhizobium japonicum* were not significantly affected by application of propaquizafop and imazethapyre. The cocktail mix of above herbicides significantly lowered the DHA at different DAHA. The cocktail mix was reduced the rate of DHA at maximum level incase of *Bradyrhizobium japonicum* and minimum incase of *Bradyrhizobium daqingense*. Although the DHA gradually increased from 10 DAHA to 45 DAHA in all the treatments.

It is revealed from the study that the DHA was more affected by imzethapyre compared to propaquizafop. The cocktail mix of above herbicides was found more effective to reduce to the DHA at different DAHA over their individual application in all the treatments. Among PGPRs, local isolate *Bradyrhizobium japonicum* was found more susceptible to post emergence herbicides than others. However, in all stages *Bradyrhizobium daqingense* had shown the maximum tolerance towards individual and cocktail combination of propaquizafop and imazethapyre.

Population study

(a) Effect of pre emergence herbicides on rhizobial population: The data on the effect of pre emergence herbicides on soil rhizobial population (Bradyrhizobium daqingense and Bradyrhizobium japonicum) recorded at different intervals after their application are presented in figure 1 and Plate 1. The population of Rhizobium recorded at different growth stages of crop revealed that rhizobial population in soil seriously affected by application of diclosulam & pendimethalin soon after its application.

At 10 days after herbicide application (DAHA) all the treatment exhibited their effect on *Rhizobium* as the population was significantly reduced over control. Among herbicides comparatively higher rhizobial population was recorded in treatment of pendimethalin over diclosulam. Population of *B. daqingense* and *B. japonicum* was recorded as (44.17 and 39.08 x 10^4 g^{-1} soil in case of pendimethalin, respectively) which was reduced to (40.11 and 37.18 x 10^4 g^{-1} soil due to the treatment of diclosulam, respectively). This proved that in all the herbicide applied treatments diclosulam was found more effective to reduce Rhizobium population in comparison to pendimethalin. Diclosulam was reduced rhizobial population significantly in all the treatments from 10 to 30 DAHA. However, pendimethalin reduced the population of *Rhizobium* in the treatments of *B.daqingense* and *P. polymyxa*. The rhizobial population was gradually increased from 30 to 50 DAHA in all the treatments. This is in agreement with of Sah *et al*. (2018) [9] where they mentioned that The bacterial population was ranged between 6.85 to 8.37 x10^6 CFU / g soil. There was least effect on bacterial population when pendimethalin was sprayed.

At 50 DAHA the residual effect of Diclosulam was remained in soil, as the population of *Rhizobium* was observed significantly lower in all the treatments comparison to control. However, in case of pendimethalin the population of *Rhizobium* was affected only in treatment of *B. daqingense*. In rest of the treatments the effect was found non-significant. Highest population (118.55 x 10^5 g^{-1} soil) was observed in case of local strain of *B. japonicum* followed by *B. daqingense* (98.28 x 10^5 g^{-1} soil). In this study it was proved that pendimethalin was comparatively more compitable with PGPRs, than diclosulam. Among PGPRs, local strain of *B. japonicum* had shown more tolerance towards pendimethalin over others.

(b) Effect of pre emergence herbicides on Phosphobacterial population: The data on the effect of pre emergence herbicides on soil phosphobacterial (PSB) population (Paenibacillus polymyxa) recorded at different intervals after their application are presented in figure 1 and Plate 1. The population of PSB recorded at different growth stages of crop revealed that PSB population in soil seriously affected by application of diclosulam & pendimethalin soon after their application. At 10 days after herbicide application (DAHA) all the treatment exhibited their effect on P. polymyxa as the population was significantly reduced over control. Among herbicides comparatively higher PSB population was recorded in treatment of pendimethalin over diclosulam. Population of P. polymyxa was recorded as 46.28 x 10^3 g^{-1} soil, in case of pendimethalin, which was reduced to 35.28 x 10^3 g^{-1} soil, due to the treatment of diclosulam. This proved that in all the herbicide applied treatments diclosulam was found more effective to reduce PSB population in comparison to pendimethalin. Diclosulam was reduced PSB population significantly in all the treatments from 10 to 50 DAHA. The PSB population was gradually increased from 30 to 50 DAHA in all the treatments.

At 50 DAHA the residual effect of diclosulam was observed in soil, as the population of PSB was observed significantly lower in all the treatments comparison to control. However, in case of pendimethalin the population of PSB was affected only in treatment of *B. daqingense*. In rest of the treatments the effect was found non-significant. Highest population 134.39 x 10^4 g^{-1} soil was observed in case of *P. polymyxa*. In this study it was proved that pendimethalin was comparatively more compitable with *Paenibacillus polymyxa*, than diclosulam. This is in agreement with of Uma *et al*. (2017) [14] where they mentioned that the phosphobacterial population in soil soon after application of the herbicide and the decrease in the population was visualized up to 20 DAS. At this stage, the reduction in PSB population was reached to maximum At 50 DAS., the PSB population in soil reached to a maximum in the whole crop growth period under study.

(c) Effect of post emergence herbicides on rhizobial population: The data on the effect of post emergence herbicides on soil rhizobial population (*Bradyrhizobium daqingense* and *Bradyrhizobium japonicum*) recorded at different intervals after their application are presented in figure 2 and Plate 1. The population of *Rhizobium* recorded at different growth stages of crop at 10 days after herbicide application (DAHA) revealed that rhizobial population in soil seriously affected by application of propaquizafop, imazethapyre & cocktail mix of propaquizafop + imazethapyre soon after its application.

At 10 DAHA all the treatment exhibited their effect on *Rhizobium* as the population was significantly reduced over control. Among herbicides comparatively higher rhizobial population was recorded in treatment of propaquizafop over imazethapyre & cocktail mix of propaquizafop + imazethapyre. Population of *B. daqingense* and *B. japonicum* was recorded as 81.32 and 75.18 x 10^4 g^{-1} soil, respectively in case of propaquizafop, which was reduced to 78.28 and 71.48 x 10^4 g^{-1} soil, respectively due to the treatment of imazethapyre & 69.72 and 64.28 x 10^4 g^{-1} soil, respectively due to the treatment of cocktail mix of propaquizafop + imazethapyre. This proved that in all the herbicide applied treatments imazethapyre and cocktail mix of propaquizafop + imazethapyre was found more effective to reduce *Rhizobium* population in comparison to propaquizafop. At 45 DAHA the residual effect of cocktail mix of propaquizafop + imazethapyre was observed in soil, as the population of *Rhizobium* was observed significantly lower in all the treatments comparison to control. However, in case of propaquizafop & imazethapyre the population of *Rhizobium* was affected only in treatment of *P. polymyxa*. In rest of the treatments the effect was found non-significant. Highest population of *B. japonicum* and *B. daqingense* was recorded as 128.22 x 10^5 g^{-1} soil and 117.23 x 10^5 g^{-1} soil, respectively in case of propaquizafop, followed by 126.22 x 10^5 g^{-1} soil and 115.59 x 10^5 g^{-1} soil respectively due to the treatment of imazethapyre; and 123.41 and 109.48 x 10^4 g^{-1} soil, respectively due to the treatment of cocktail mix of propaquizafop + imazethapyre.

It is revealed from the study that the rhizobial population was more affected by imzethapyre compared to propaquizafop. The cocktail mix of above herbicides was found more effective to reduce to the rhizobial population in significant level at different DAHA over their individual application in all the treatments. Among PGPRs, local isolate *B. daqingense* was found more susceptible to post emergence herbicides than others at 30 & 50 DAHA. However, at 20 DAHA *Bradyrhizobium daqingense* had shown the maximum tolerance towards individual and cocktail combination of propaquizafop and imazethapyre. In this study it was proved that propaquizafop was comparatively more compitable with PGPRs, than imzethapyre & cocktail mix of propaquizafop + imazethapyre. This is in agreement with of Sah *et al*. (2018) [9] where they mentioned that among herbicides, significantly highest *Rhizobium* population was observed in pendimethalin and significantly lowest population of *Rhizobium* was noticed in imazethapyr applied treatment (1.51 x 10^4 CFU/g of soil).

(d) Effect of post emergence herbicides on Phosphobacterial population: The data on the effect of post emergence herbicides on soil soil phosphobacterial (PSB) population (*Paenibacillus polymyxa*) recorded at different intervals after their application are presented in in figure 2 and Plate 1. The population of PSB recorded at different growth stages of crop at 10 days after herbicide application (DAHA) revealed that PSB population in soil seriously affected by application of propaquizafop, imazethapyre & cocktail mix of propaquizafop + imazethapyre soon after its application.

At 10 DAHA all the treatment exhibited their effect on PSB, as the population was significantly reduced over control. Among herbicides comparatively higher PSB population was recorded in treatment of propaquizafop over imazethapyre & cocktail mix of propaquizafop + imazethapyre. Among the herbicide applied treatments Population of *P. polymyxa* was recorded 88.91 x 10^3 g-1 soil, in case of propaquizafop, which was reduced to 80.41 x 10^3 g^{-1} soil, due to the treatment of imazethapyre; and 74.32 x 10^3 g^{-1} soil, due to the treatment of cocktail mix of propaquizafop + imazethapyre. This proved that among all the herbicide applied treatments imazethapyre & cocktail mix of propaquizafop + imazethapyre was found more effective to reduce PSB population in comparison to propaquizafop. AT 20 & 30 DAHA reduced the population of PSB in all the treatments of *P. polymyxa*. was significantly affected by both the post emergence herbicides at all intervals after their application. Two other PGPR treatments of *B. daqingense* and *B. japonicum* were not significantly affected by application of Propaquizafop and Imazethapyre. At 45 DAHA the residual effect of Propaquizafop, Imazethapyre & their cocktail mix was noticed in *P. polymyxa* treated treatments, as the population of PSB was observed significantly lower in the above treatments comparison to control. In rest of the treatments of *B. daqingense* and *B. japonicum* the effect was found non-significant. Highest population of PSB was recorded as 138.76 x 10^4 g^{-1} soil, in case of propaquizafop followed by 135.41 x 10^4 g^{-1} soil due to the treatment of imazethapyre; and 128.11 x 10^4 g^{-1} soil due to the treatment of cocktail mix of propaquizafop + imazethapyre.

In this study it was proved that Propaquizafop was comparatively more compitable with PSB, than imzethapyre & cocktail mix of propaquizafop + imazethapyre. Significant decrease in phosphate solubilizing bacteria (Enterobacter asburiae) was reported by Ahmad and Khan (2010) [1] due to quizalafop-pethyl, clodinifop, metribuzin, glyphosate herbicides.

Spot analysis: *Compatibility of PGPRs with herbicides in vitro (Spot analysis):-*

Diclosulam (pre emergence)-Colony size study:- The mean value of colony diameter (in mm) of different concentration of diclosulam herbicide are presented in table in Table 3. The diameter size of microbial strains showed that diclosulam significantly reduced the colony size of all the PGPRs. the size of the colonies was reduced with increasing concentration of diclosulam in media. The local strain Bradyrhizobium japonicum (Diameter 11.98, 11.59 and 11.03mm due to 52,56 and 60 ppm concentration of diclosulam, respectively) affected less in comparison to two others by diclosulam followed by Bradyrhizobium daqingense (Diameter 9.40, 9.12 and 8.21mm due to 52,56 and 60 ppm concentration of diclosulam, respectively), and Penbacillus polymyxa (Diameter 5.48, 5.10 and 4.52mm due to 52,56 and 60 ppm concentration of diclosulam, respectively).

Pendimethalin (pre emergence)-Colony size study: The mean value of colony diameter (in mm) of PGPRs at different concentration of pendimethalin herbicide are presented Table 3. The pendimethalin at 8 and 10 ppm concentration significantly reduced the colony size of different PGPRs. The local strain Bradyrhizobium japonicum (Diameter 13.04 & 12.46mm due to 8 &10 ppm concentration of pendimethalin, respectively) affected less in comparison to two others by pendimethalin followed by Bradyrhizobium daqingense (Diameter 9.28 & 8.56mm due to 8 &10 ppm concentration of pendimethalin, respectively) and Penbacillus polymyxa (Diameter 5.21 & 4.82mm due to 8 &10 ppm concentration of pendimethalin, respectively). The 6 ppm concentration of pendimethalin was found ineffective to reduce the colony size of all the PGPRs.

Propaquizafop (post emergence)-Colony size study: The mean value of colony diameter (in mm) of PGPRs at different concentration of propaquizafop herbicide are presented in Table 4. The propaquizafop at 1.5 and 2.0 ppm concentration significantly reduced the colony size of different PGPRs. The local strain Bradyrhizobium japonicum (Diameter 13.58 & 13.28mm due to 1.5 & 2.0 ppm concentration of propaquizafop, respectively) affected less in comparison to two others by propaquizafop followed by Bradyrhizobium daqingense (Diameter 9.47 & 9.03mm due to 1.5 & 2.0 ppm concentration of propaquizafop, respectively) and Penbacillus polymyxa (Diameter 5.92 & 5.67mm due to 1.5 & 2.0 ppm concentration of propaquizafop, respectively). The 1.2 ppm concentration of propaquizafop was found ineffective to reduce the colony size of all the PGPRs.

Imazethapyre (post emergence)-Colony size study: The mean value of colony diameter (in mm) of PGPRs at different concentration of imazethapyre herbicide are presented in Table 4. imazethapyre significantly reduced the size of the colony of different PGPRs in different concentraction. The size of the colonies was reduced with increasing concentration of imazethapyre. The local strain Bradyrhizobium japonicum (Diameter 13.63, 13.39 and 13.08mm due to 2,2.5 and 3.0 ppm concentration of imazethapyre, respectively) affected less in comparison to two others by imazethapyre followed by Bradyrhizobium daqingense (Diameter 9.34, 9.19 and 8.89mm due to 2,2.5 and 3.0 ppm concentration of imazethapyre, respectively) and Penbacillus polymyxa (Diameter 5.88, 5.52 and 5.01mm due to 2, 2.5 and 3.0 ppm concentration of imazethapyre, respectively).

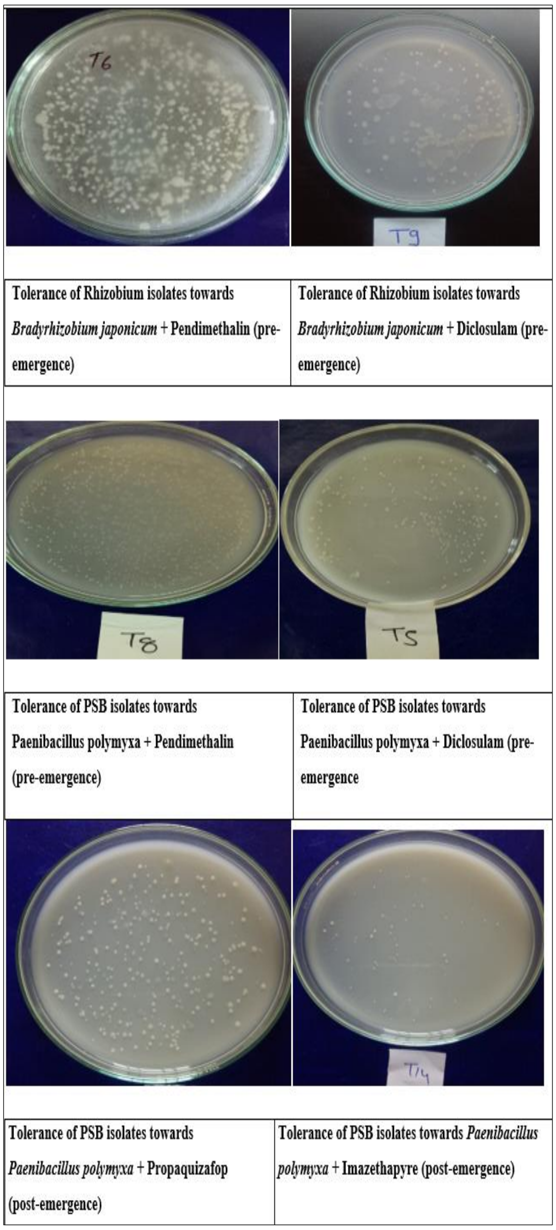


Plate 1: Microbial Population Study

Table 1: Effect of pre emergence herbicides on Basal Soil Respiration rate (mg CO_2/h/100g) and Dehydrogenase activity (µg TPF /h/g) at different growth stages of soybean

Treatment No.	Treatment	Basal Soil Respiration Days after herbicide application				Dehydrogenase activity Days after herbicide application			
		10 DAHA	20 DAHA	30 DAHA	50 DAHA	10 DAHA	20 DAHA	30 DAHA	50 DAHA
T_1	*Bradyrhizobium daqingense* (control- I)	0.74	1.13	1.27	1.49	25.75	28.14	31.28	36.50
T_2	*Paenibacillus polymyxa* (control- II)	0.79	1.13	1.28	1.51	25.80	28.14	31.29	36.52
T_3	*Bradyrhizobium japonicum* (Local strain – control -III)	0.53	0.78	1.13	1.28	25.54	27.79	31.14	36.29
T_4	*Bradyrhizobium daqingense* + Diclosulam	0.49	0.78	0.93	1.04	20.50	22.79	25.94	31.05
T_5	*Paenibacillus polymyxa* + Diclosulam	0.62	0.98	1.03	1.18	20.63	22.99	26.04	31.19
T_6	*Bradyrhizobium japonicum* + Diclosulam	0.44	0.53	0.76	1.12	20.45	22.54	25.77	31.13
T_7	*Bradyrhizobium daqingense* + Pendimethalin	0.54	0.98	1.03	1.22	23.55	25.92	28.54	33.23
T_8	*Paenibacillus polymyxa* + Pendimethalin	0.66	1.00	1.18	1.37	23.67	25.94	28.98	33.55
T_9	*Bradyrhizobium japonicum* + Pendimethalin	0.49	0.71	1.03	1.14	23.53	25.72	28.74	33.20
	SE(m)	0.02	0.03	0.04	0.05	0.68	0.73	0.81	1.05
	CD(0.05)	0.07	0.09	0.12	0.15	2.02	2.18	2.41	3.10

Table 2: Effect of Post emergence herbicides on Basal Soil Respiration rate (mg CO_2/h/100g) and Dehydrogenase activity (µg TPF /h/g) at different growth stages of soybean.

Treatment No.	Treatment	Basal Soil Respiration rate Days after herbicide application				Dehydrogenase activity Days after herbicide application			
		10 DAHA	20 DAHA	30 DAHA	50 DAHA	10 DAHA	20 DAHA	30 DAHA	50 DAHA
T_1	*Bradyrhizobium daqingense* (control- I)	1.27	1.44	1.49	1.85	31.28	34.45	39.50	43.86
T_2	*Paenibacillus polymyxa* (control- II)	1.28	1.49	1.51	1.88	31.29	34.50	39.52	43.89
T_3	*Bradyrhizobium japonicum* (Local strain – control -III)	1.13	1.25	1.28	1.56	31.14	34.26	39.29	43.57
T_{10}	*Bradyrhizobium daqingense* + Propaquizafop	1.21	1.33	1.38	1.69	27.22	29.34	33.39	36.70
T_{11}	*Paenibacillus polymyxa* + Propaquizafop	1.14	1.19	1.26	1.33	27.13	29.20	33.27	36.34
T_{12}	*Bradyrhizobium japonicum* + Propaquizafop	1.11	1.14	1.18	1.44	27.12	29.15	33.19	36.45
T_{13}	*Bradyrhizobium daqingense* + Imazethapyre	1.18	1.30	1.34	1.67	27.19	29.31	33.35	36.68
T_{14}	*Paenibacillus polymyxa* + Imazethapyre	1.13	1.17	1.26	1.31	27.12	29.18	33.27	36.32
T_{15}	*Bradyrhizobium japonicum* + Imazethapyre	1.04	1.11	1.16	1.38	27.05	29.12	33.18	36.39
T_{16}	*Bradyrhizobium daqingense* + (Propaquizafop + Imazethapyre)	1.12	1.18	1.27	1.34	27.13	29.19	33.28	36.55
T_{17}	*Paenibacillus polymyxa* + (Propaquizafop + Imazethapyre)	1.09	1.13	1.24	1.29	27.10	29.14	33.25	36.30
T_{18}	*Bradyrhizobium japonicum* + (Propaquizafop + Imazethapyre)	0.98	1.06	1.10	1.22	26.99	28.07	32.11	35.23
	SE(m)	0.04	0.05	0.05	0.06	1.35	1.69	2.04	2.34
	CD(0.05)	0.13	0.15	0.17	0.19	4.11	5.21	6.15	7.22

Table 3: Assessment of compatibility of different PGPRs with Diclosulam and Pendimethalin herbicides *in vitro* in terms of colony diameter (mm).

Treatment No.	Treatment	Concentration			Treatment No.	Treatment	Concentration		
		52 ppm	56 ppm	60 ppm			6 ppm	8 ppm	10 ppm
T_1	*Bradyrhizobium daqingense* (control- I)	10.00*	10.00*	10.00*	T_1	*Bradyrhizobium daqingense* (control- I)	10.00*	10.00*	10.00*
T_2	*Paenibacillus polymyxa* (control- II)	6.50*	6.50*	6.50*	T_2	*Paenibacillus polymyxa* (control- II)	6.50*	6.50*	6.50*
T_3	*Bradyrhizobium japonicum* (Local strain – control -III)	13.80*	13.80*	13.80*	T_3	*Bradyrhizobium japonicum* (Local strain – control -III)	13.80*	13.80*	13.80*
T_4	*Bradyrhizobium daqingense + Diclosulam*	9.40	9.12	8.21	T_4	*Bradyrhizobium daqingense* + Pendimethalin	9.59	9.28	8.56
T_5	*Paenibacillus polymyxa + Diclosulam*	5.48	5.10	4.52	T_5	*Paenibacillus polymyxa* + Pendimethalin	5.98	5.21	4.82
T_6	*Bradyrhizobium japonicum+ Diclosulam*	11.98	11.59	11.03	T_6	*Bradyrhizobium japonicum* + Pendimethalin	13.56	13.04	12.46
	SE(m)	0.32	0.16	0.16			0.29	0.19	0.14
	CD(0.05)	1.01	0.50	0.50			0.92	0.61	0.44

= without herbicide application

Table 4: Assessment of compatibility of different PGPRs with Propaquizafop herbicide *in vitro* in terms of colony diameter (mm).

Treatment No.	Treatment	Concentration			Treatment No.	Treatment	Concentration		
		1.2 ppm	1.5 ppm	2.0 ppm			2 ppm	2.5 ppm	3.0 ppm
T_1	*Bradyrhizobium daqingense* (control- I)	10.00*	10.00*	10.00*	T_1	*Bradyrhizobium daqingense* (control- I)	10.00*	10.00*	10.00*
T_2	*Paenibacillus polymyxa* (control-II)	6.50*	6.50*	6.50*	T_2	*Paenibacillus polymyxa* (control-II)	6.50*	6.50*	6.50*
T_3	*Bradyrhizobium japonicum* (Local strain – control -III)	13.80*	13.80*	13.80*	T_3	*Bradyrhizobium japonicum* (Local strain – control -III)	13.80*	13.80*	13.80*
T_4	*Bradyrhizobium daqingense* + propaquizafop	9.68	9.47	9.03	T_4	*Bradyrhizobium daqingense* + Imazethapyre	9.34	9.19	8.89
T_5	*Paenibacillus polymyxa* + propaquizafop	6.02	5.92	5.67	T_5	*Paenibacillus polymyxa* + Imazethapyre	5.88	5.52	5.01
T_6	*Bradyrhizobium japonicum* + propaquizafop	13.71	13.58	13.28	T_6	*Bradyrhizobium japonicum* + Imazethapyre	13.63	13.39	13.08
	SE(m)	0.32	0.03	0.08			0.01	0.06	0.15
	CD(0.05)	0.99	0.11	0.24			0.06	0.19	0.47

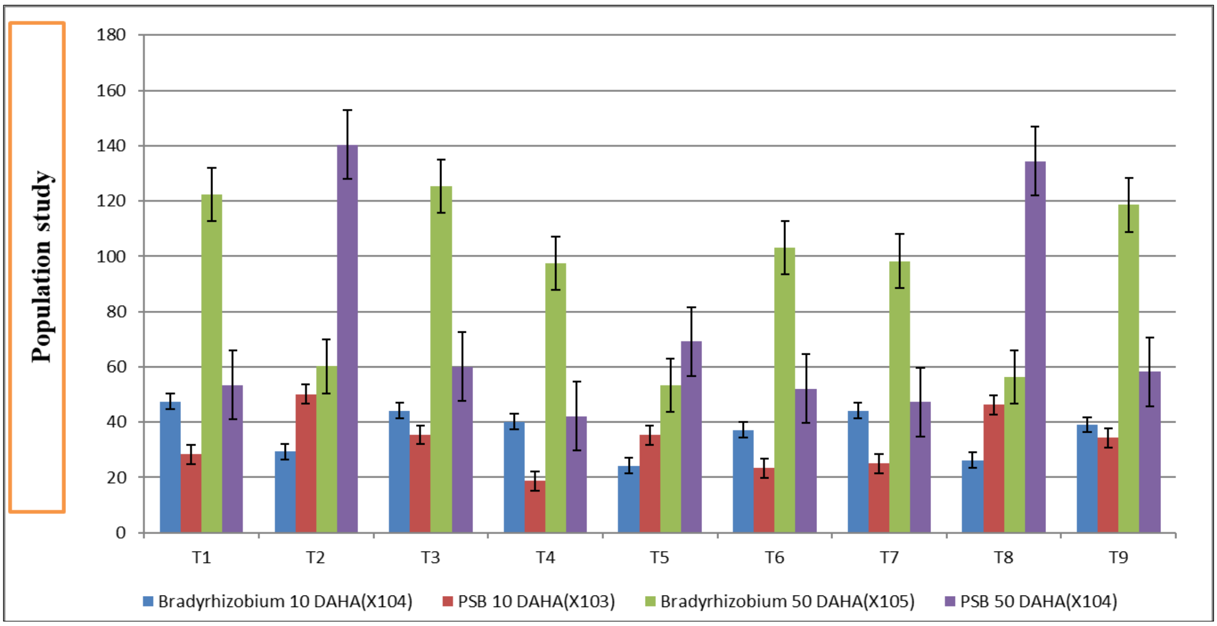
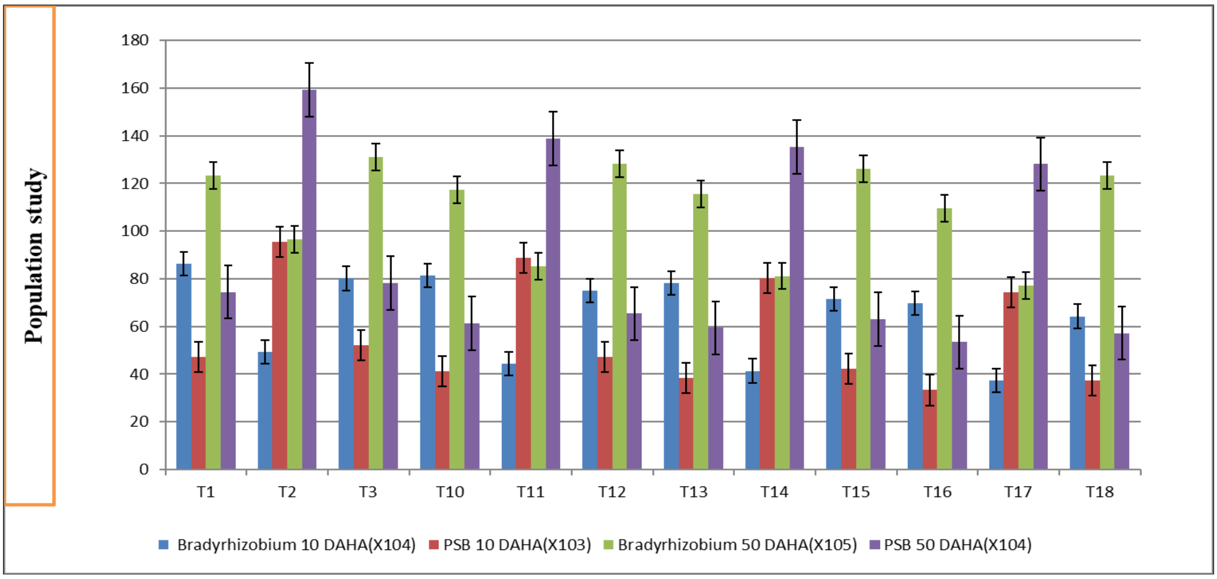


Fig 1: Effect of pre emergence herbicides on rhizobial ($x10^5$ g-1 soil) and phosphate solubilizing bacterial population ($x10^3$ g-1 soil) in soil at different growth stages of soybean.



= without herbicide application

Fig 2: Effect of post emergence herbicides on rhizobial ($x10^5$ g-1 soil) and phosphate solubilizing bacterial population ($x10^3$ g-1 soil) in soil at different growth stages of soybean.

Conclusion

It can be concluded from the study that (i) In all the herbicide applied treatments, diclosulam was found more effective to reduce BSR in comparison to pendimethalin. Significantly better BSR rate recorded in pendimethalin over diclosulam herbicide received the same PGPR. In case of post emergence herbicides BSR was more affected by imzethapyre compared to propaquizafop. all the herbicide applied treatments diclosulam was found more effective to reduce DHA in comparison to pendimethalin. *Bradyrhizobium daqingense* had shown the maximum tolerance towards individual and cocktail combination of propaquizafop and imazethapyre. (ii) in case of pre emergence herbicides pendimethalin was comparatively more compitable with PGPRs, than diclosulam to produced rhizobial population. In case of post emergence herbicides rhizobial population was more affected by imzethapyre compared to propaquizafop. The cocktail mix of above herbicides was found more effective to reduce to the rhizobial population in significant level at different DAHA. (iii) Compatibility of PGPRs with herbicides *in- vitro* was studied by Spot analysis method- The local strain Bradyrhizobium japonicum affected less in comparison to two others by pendimethalin followed by Bradyrhizobium daqingense, In case of post emergence herbicides propaquizafop at 1.5 and 2.0 ppm concentration significantly reduced the colony size of different PGPRs. The local strain *Bradyrhizobium japonicum* affected less in comparison to two others by propaquizafop followed by *Bradyrhizobium daqingense*.

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