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SHORT COMMUNICATION

In Vitro Sensitivity of *Rhizobium* and Phosphate Solubilising Bacteria to Herbicides

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Abstract Nitrogen fixing bacteria, rhizobia and phosphate solubilizing bacteria (PSB) are the commonly applied microbial inoculants in grain legumes (Pulses). It is important to apply herbicides to control weeds in order to augment yield of the crop. The herbicides may however, be incompatible with the microbial inoculants. This study compared the effect of the recommended pre-plant incorporated herbicide, fluchloralin (20.25×10^4 ppm) and pre-emergence herbicide, pendimethalin in two doses (9×10^4 and 15×10^4 ppm) on the growth and survival of mungbean *Rhizobium* and PSB, under laboratory conditions. These herbicides were also used under field conditions in conjunction with biofertilizers (R, PSB) to improve grain yield of mungbean. It was found that fluchloralin (20.25×10^4 ppm) and the lower dose of pendimethalin (9×10^4 ppm) had no adverse effect on growth of *Rhizobium* and PSB. The higher dose of pendimethalin (15×10^4 ppm) was safe on PSB but it imposed a retarding effect on the growth of *Rhizobium.*

Keywords Herbicides · PSB · *Rhizobium*

In India, mungbean was sown on 2.92 million hectares and had a production potential of 1.42 million tons in the year 2005 [1]. In Punjab, mungbean was sown on 15.0 thousand hectares and has a production potential of 12.0 thousand tons during 2004–2005 [2]. Frequent rains during monsoon in *kharif* (rainy) season result in high infestation of weeds, which compete with the crop for nutrients, moisture, space and sunlight thus reducing crop yield up to 79% in mungbean [3]. Herbicides are preferred over hand weeding for controlling weeds as manual weeding is costly and frequent rains do not permit hand weeding at proper time. The rhizobial inoculant and phosphate solubilizing bacteria (PSB) are commonly applied to seeds of legume crops to ensure effective nitrogen fixation by *Rhizobium* and solubilisation of native phosphorus by PSB, thereby making the two essential nutrients available to the crop [4]. The use of herbicides has become an integral and economically essential part of agriculture. However, the microbial biofertilizers, viz. *Rhizobium* and PSB may become exposed to these herbicides either present in soil at planting time or later during the season. There are reports which suggest that herbicides when applied indiscriminately have variable effects on legume *Rhizobium* symbiosis [5]. Herbicides may have negative effects on growth of rhizobia [6, 7] although other reports revealed no such adverse effects [8, 9]. Discrepancies between reports may be due to differences in concentration of chemicals used and strains of bacterium employed. Periodic evaluation of new herbicides for toxicity to *Rhizobium*/PSB may assist mungbean growers in selection of compatible herbicides.

Therefore, the present investigation was conducted to study the relative compatibility of two herbicides, one of them applied as pre-plant (fluchloralin) and the other as pre-emergence (pendimethalin) on growth of *Rhizobium* and PSB in vitro. The experiment was conducted under controlled conditions to test the effect of recommended dose of fluchloralin (20.25×10^4 ppm) and pendimethalin (9×10^4 and 15×10^4 ppm) on the microbial biofertilizers (*Rhizobium* and PSB). Recommended *Rhizobium* strain MA 1 and PSB strain *Pseudomonas striata* were procured from Department of Microbiology, Punjab Agricultural University, Ludhiana and Indian Agricultural Research

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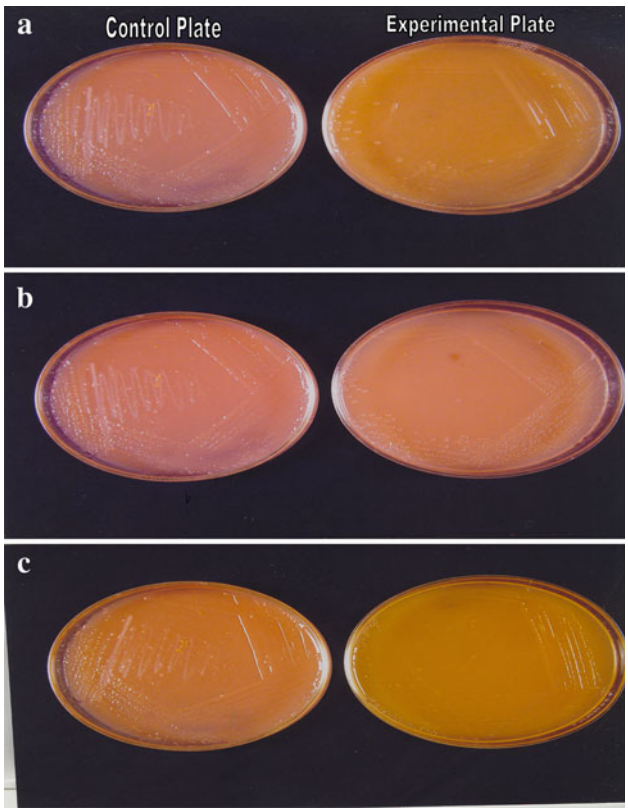
Institute, New Delhi, respectively. Cultures of *Rhizobium* and PSB were maintained on Yeast Extract Mannitol Agar (YEMA) [10] and Pikovskaya's agar media [11], respectively. The recommended field concentration of herbicides was converted to laboratory concentration on the basis of already reported calculations [12]. The sensitivity/resistance of herbicide on growth of *Rhizobium* and PSB was determined by two methods. In the first method viz. bacterial sensitivity—streak plate method, the calculated amount of herbicides was mixed with melted YEMA and Pikovskaya's agar media for *Rhizobium* and PSB, respectively and the medium was poured into sterile Petri plates. Plates without herbicides were prepared to serve as control. After solidification and storage for 2 days to ensure sterility, plates were streaked with 3–4 days old broth suspension [10^8 cells ml^{-1} of test organism (*Rhizobium* and PSB)]. The plates for *Rhizobium* were incubated at 28°C for 4–5 days and for PSB at 32°C up to 7 days, after which growth was examined. Growth was visually grouped into 4 categories: +++ (good), ++ (moderate), + (poor) and − (no growth). The second method was bacterial sensitivity—broth culture method in which culture bottles (250 ml) were prepared with 75 ml yeast extract mannitol broth and nutrient broth for *Rhizobium* and *P. striata*, respectively. Calculated amount of herbicide solutions (made in sterilized water) was added to the broth. One millilitre of the desired inoculum (10^8 cells ml^{-1}) was added to specific broths. Flasks were incubated at 28 and 32°C on rotary shaker for 5 and 7 days for *Rhizobium* and PSB, respectively. Growth of the desired organism was measured by recording optical density (OD) at 540 nm after regular intervals till the control (untreated culture) reached maximum OD of 1.2 [13].

The experiment was conducted in completely randomized design (CRD) with four replications. The data was subjected to statistical programme CPCS 1.

Rhizobium showed varied growth in vitro under different herbicide treatments. Fluchloralin @ 20.25×10^4 ppm and pendimethalin @ 9×10^4 ppm were not detrimental to the growth of rhizobia. The growth was categorized as good (+++) with these concentrations of the herbicides. However, moderate growth (++) of *Rhizobium* was observed with pendimethalin @ 15×10^4 ppm (Plate 1). The PSB was not sensitive to any of the herbicides used. Growth of *P. striata* under the influence of fluchloralin (20.25×10^4 ppm) and pendimethalin (9×10^4 and 15×10^4 ppm) was comparable with that of control plate and was found to be good (+++) (Plate 2).

In broth culture method, untreated rhizobia (control) had the highest OD (1.42) (Table 1). With fluchloralin, a reduction of 1.4 fold in rhizobial growth was observed, over the untreated rhizobia. The OD (1.47) recorded with lower concentration of pendimethalin (9×10^4 ppm) was

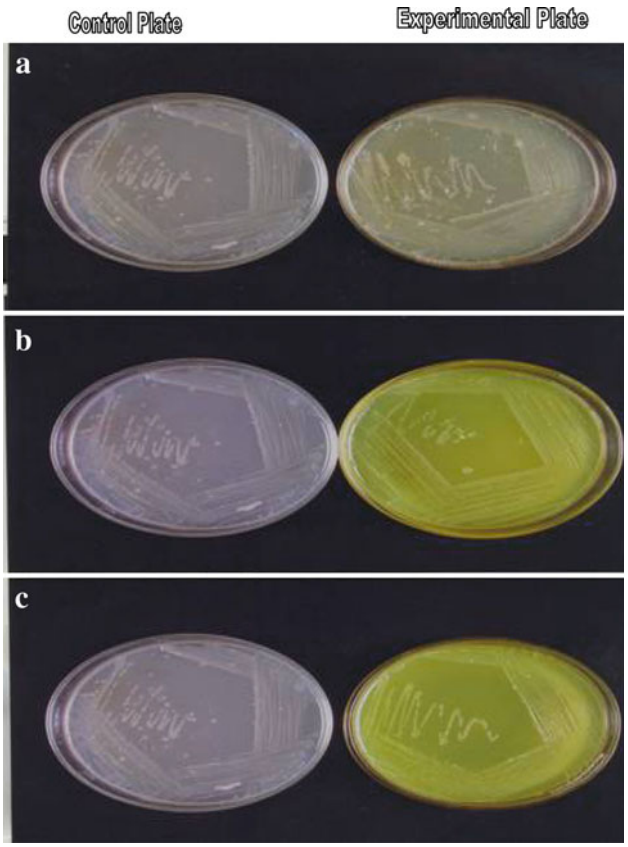


	TREATMENT (Experimental Plate)	GROWTH
a)	*Rhizobium* + Fluchloralin (20.25×10^4 ppm)	+++
b)	*Rhizobium* + Pendimethalin (9×10^4 ppm)	+++
c)	*Rhizobium* + Pendimethalin (15×10^4 ppm)	++

Plate 1 Effect of herbicides on growth of *Rhizobium* (R)

statistically on par with OD of untreated rhizobia (1.42), showing no inhibitory effect on growth. However, pendimethalin at higher dose (15×10^4 ppm) resulted in significant decrease in rhizobial growth (OD = 0.69) over the control. Comparable OD was recorded for *P. striata* in nutrient broth with herbicides fluchloralin (20.25×10^4 ppm) and pendimethalin (9×10^4 and 15×10^4 ppm) and nutrient broth without herbicides, thus showing no significant adverse impact of the used herbicides on its growth (Table 1).

It appears that fluchloralin (20.25×10^4 ppm) and lower dose of pendimethalin (9×10^4 ppm) is safe on *Rhizobium*, whereas the higher dose of pendimethalin (15×10^4 ppm) is damaging. Earlier reports with different herbicides (cinosulfuron and trifluralin) have also shown an inhibitory effect on growth of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* [14, 15] which probably happened due to impairment of metabolic activities resulting in reduced growth of microflora [16]. Reduced growth curves of crown vetch rhizobia in broth medium are reported only at highest concentration of tested herbicides (atrazine and



	TREATMENT (Experimental Plate)	GROWTH
a)	PSB + Fluchloralin (20.25 x 10^4 ppm)	+++
b)	PSB + Pendimethalin (9 x 10^4 ppm)	+++
c)	PSB + Pendimethalin (15 x 10^4 ppm)	+++

Plate 2 Effect of herbicides on growth of phosphate solubilizing bacteria (PSB)

Table 1 Effect of herbicides on growth (optical density) of *Rhizobium* and PSB

Treatment	Concentration of herbicides (ppm)	Optical density (540 mm)
Control	–	1.42
Rhizobium + fluchloralin	20.25 × 10^4	1.03
Rhizobium + pendimethalin	9 × 10^4	1.47
Rhizobium + pendimethalin	15 × 10^4	0.69
CD ($P = 0.05$)		0.20
Control	–	1.16
PSB + fluchloralin	20.25 × 10^4	1.13
PSB + pendimethalin	9 × 10^4	1.14
PSB + pendimethalin	15 × 10^4	1.12
CD ($P = 0.05$)		NS

Data based on four replicates in experiment

bifenox) [17]. However, the various rhizobial strains differ in their sensitivity to different rates of herbicides tested [18, 19]. The unaltered growth of *P. striata* suggests that its growth was unaffected by any of the tested herbicides. Earlier findings revealed that acetachlor (0.01–200.00 mg/dm^3) did not show any inhibitory effect on growth of *Bacillus* sp. (PSB), only a slight growth inhibition of PSB (*Bacillus* sp.) was observed in liquid broth with acetachlor at 7.0 and 10 mg/l [20]. It was also reported that fluchloralin (1,000 g/ha) was not toxic to *Pseudomonas* sp [21].

The adverse effect of herbicides, if any, may not be significant in soil. Reports suggest that the persistence of herbicide in soil is influenced by its adsorption by soil particles, its volatility, photodecomposition, leaching and degradation by soil microorganisms [22], thus reducing availability and effect of these chemicals on soil microbial flora.

In conclusion fluchloralin (20.25 × 10^4 ppm) and the lower recommended dose of pendimethalin (9 × 10^4 ppm) did not have any adverse effect on the growth of *Rhizobium* whereas, higher dose of pendimethalin (15 × 10^4 ppm) reduced the growth of *Rhizobium*. Different herbicides [fluchloralin (20.25 × 10^4 ppm) and pendimethalin (9 × 10^4 and 15 × 10^4 ppm)] did not affect the growth of PSB. Thus, it can be concluded that the use of *Rhizobium* and PSB with the recommended dose of fluchloralin (20.25 × 10^4 ppm) and the lower dose of pendimethalin (9 × 10^4 ppm) is innocuous for their growth.

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