

Original Research Article

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Effect of Co-inoculation of different Bacterial Cultures with *Rhizobium phaseoli* on Soil Biological Properties

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ABSTRACT

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The present experiment was conducted to study the changes occurred in biological property under the influence of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* in blackgram grown Vertisols at Research farm, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The pre-evaluated bacterial cultures i.e. *Rhizobium phaseoli* and seven other (*Bacillus megaterium*, *Bacillus subtilis*, *Bacillus polymyxa*, *Pseudomonas striata*, *Pseudomonas fluorescens*, *Azotobacter chroococcum* and *Azospirillum lipoferum*) in laboratory were used with RDF in randomized block design. Seed treatment of black gram was done with 50 ml of *Rhizobium phaseoli* and 50 ml of other bacterial cultures along with application RDF at the time of sowing. Results emerged out indicated that the soil microbial population i.e. fungi, bacteria and actinomycetes and enzymatic activity i.e. dehydrogenase, acid phosphatase and alkaline phosphatase were highly influenced by co-inoculation over mono-inoculation and control one. The treatments received co-inoculation of *Rhizobium phaseoli* with *Bacillus megaterium* (T₄), *Pseudomonas striata* (T₇) and *Pseudomonas fluorescense* (T₈) found strongly at par with each other and having more potential than the other combinations.

Introduction

Indiscriminate use of synthetic chemical fertilizers has led to the pollution and contamination of the soil, has polluted water basins, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility (Syed Ismail 2015). At this alarming stage to sustain

crop production and maintain soil fertility integrated nutrient management is prime and only option. The biofertilizers are one of the major components of integrated nutrient management having potential to work efficiently to reduce such hazards. Microbial inoculants are cost effective, eco-friendly and renewable sources of plant nutrients. Plant beneficial living microbial cultures (bio-

fertilizers) are supposed to be a safe supplement to chemical fertilizers in order to minimize the ecological disturbance.

The biochemical properties of soil have often been proposed as early and sensitive indicators of soil ecosystem health. Soil enzymes play an essential role in energy transfer environmental quality, organic matter decomposition, nutrient cycling and crop productivity (Mina *et al.*, 2011). A number of microorganisms are considered important for agriculture to promote better enzyme activity and biological health of soil.

Materials and Methods

Composite soil samples of 0-15 cm depth were collected from individual plots after harvesting the crop and stored at low temperature in deep freeze for determination of microbial population and enzymatic activity in soil after harvesting.

For the growth of bacteria, fungi and actinomycetes three different media i.e. Nutrient Agar, Rose Bengal and Ken knight medium respectively were used.

The serial dilution technique described by Dhingra and Sinclair (1993) was used for particular group of microbes. Enzymes i.e. dehydrogenase, acid phosphatase and alkaline phosphates which play important role in soil microbial respiration and phosphorous mobilization respectively were analyzed by using standard procedures described by Tabatabai and Bremner (1969).

The results obtained were statistically analyzed and appropriately interpreted as per the methods described by Panse and Sukhatme (1985). Appropriate standard error (S.E.) and critical differences (C.D.) at 5 per cent levels were worked out for interpretation of result.

Results and Discussion

Effect on Enzymatic activity

The data noted in Table 1 indicates the enzymatic activity of soil significantly influenced by co-inoculation of bacterial cultures with *Rhizobium phaseoli*.

Dehydrogenase activity

The activity of enzyme dehydrogenase ($39.47 \mu\text{g g}^{-1}$ soil) was significantly higher in treatment T₇ (RDF + *Rhizobium phaseoli* + *Pseudomonas striata*) over other treatments and found at par with treatment T₈ ($37.83 \mu\text{g g}^{-1}$ soil) having co-inoculation of *Pseudomonas fluorescens* with *Rhizobium phaseoli*. The minimum dehydrogenase activity ($29.57 \mu\text{g g}^{-1}$ soil) observed in treatment T₁ (absolute control). The dehydrogenase participates in electron transport system of oxygen metabolism so it reflects the extent of oxidative activity of soil microorganisms and is good indicator of microbial activity (Nannipieri *et al.*, 2002) and due to high substrate availability the dehydrogenase activity is also high.

Acid and alkaline phosphatase

The acid and alkaline phosphatase activities in soil recorded high in treatment T₈ (51.63 and $67.72 \mu\text{g g}^{-1}$ soil, respectively) receiving co-inoculation of *Pseudomonas fluorescens* with *Rhizobium phaseoli* which significantly differed from other treatments.

The second and third higher value of acid (50.84 and $50.44 \mu\text{g g}^{-1}$ soil, respectively) and alkaline phosphatase (66.07 and $65.76 \mu\text{g g}^{-1}$ soil, respectively) was recorded in treatment T₄ (RDF + *Rhizobium phaseoli* + *Bacillus megaterium*) and T₇ (RDF + *Rhizobium phaseoli* + *Pseudomonas striata*) which found at par with treatment T₈. The minimum acid

(30.25 $\mu\text{g g}^{-1}\text{soil}$) and alkaline phosphatase (54.42 $\mu\text{g g}^{-1}\text{soil}$) activities were recorded in treatment T₁ (absolute control). Soil phosphatase play a major role in the mineralization processes of organic P substrates and their activity can be influenced by soil microbial population (Sarapatka, 2003).

However, activities of these enzymes were not persistent, and sometimes found contrasting. The acid phosphatase activity was much lower than alkaline phosphatase activity, irrespective of the treatments, which may be due to the alkaline reaction of soil (Dick, 1994).

Similarly, Kaleeswari (2007) reported that activities of both acid and alkaline phosphatases were significantly improved over control levels in the rhizosphere up on inoculation. This might be due to increased microbial and root activities.

Further, Nihorimbere *et al.*, (2011) reported more microbial activities increased the dehydrogenase activity in rhizosphere due to more availability of food material for its growth. Badawi *et al.*, (2011) also studied and results revealed that, there was maximum value of nitrogenase activity in *Bradyrhizobium* + *Serratia marcescens* among different inoculation treatments.

Similarly, Badda *et al.*, (2013) concluded that triple inoculation of *A. laevis* + *T. viridae* + *P. Fluorescence* showed maximum increment in both acid and alkaline phosphatase activity.

Moreover, Bodkhe *et al.*, (2014) concluded that an application of 75 % RDF and dual inoculation significantly increased soil enzymes activity. Our findings were matched with results of Sable and Ismail (2017) that activity of alkaline phosphatase and acid phosphatase was noted significantly highest in treatment RDF + *Rhizobium* + *Bacillus megaterium*. Similarly, Vidhyashree *et al.*,

(2017) reported that dehydrogenase and alkaline phosphatase activity in co-inoculated treatment (PSB + *Aspergillus awamori*) showed significant increase.

Effect on Microbial population

The data depicted in Table 2 indicates that the soil microbial population shows distinct differences under the influence of co-inoculation of bacterial cultures with *Rhizobium phaseoli*.

Bacterial population

With respect to culturable microbial communities of the black gram soil the bacterial load was highest in the treatment T₇ (39 CFU X 10⁷) receiving co-inoculation of *Pseudomonas striata* and *Rhizobium phaseoli* which found at par with treatment T₄ (35.33 CFU X 10⁷) having co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli*.

The lowest bacterial population recorded in treatment T₁ (20.67 CFU X 10⁷) which is absolute control.

Due to co-inoculation of different bacterial cultures with *Rhizobium phaseoli* population of native and applied bacteria were increased by multiplication (Bodkhe *et al.*, 2014).

Actinomycetes population

Among treatments the treatment T₇ having co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* found significantly superior in case of actinomycetes population (32.67 CFU X 10⁵) over rest of treatments and found at par with treatment T₄ (32.33 CFU X 10⁵) having co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli* and treatment T₈ (30.67 CFU X 10⁵) receiving co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli*.

Table.1 Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on enzymatic activities in soil after harvest of black gram

Sr. No.	Treatments	Dehydrogenase ($\mu\text{g g}^{-1}$ soil)	Acid phosphatase ($\mu\text{g g}^{-1}$ soil)	Alkaline phosphatase ($\mu\text{g g}^{-1}$ soil)
T ₁	Absolute control	29.57	30.25	54.42
T ₂	Only RDF	30.83	39.03	55.64
T ₃	RDF + <i>Rhizobium phaseoli</i>	34.07	43.56	60.22
T ₄	T ₃ + <i>Bacillus megaterium</i>	37.43	50.84	66.07
T ₅	T ₃ + <i>Bacillus subtilis</i>	34.73	47.02	62.04
T ₆	T ₃ + <i>Bacillus polymyxa</i>	32.17	40.24	57.09
T ₇	T ₃ + <i>Pseudomonas striata</i>	39.47	50.44	65.76
T ₈	T ₃ + <i>Pseudomonas fluorescens</i>	37.83	51.63	67.72
T ₉	T ₃ + <i>Azotobacter chroococcum</i>	36.30	44.26	59.99
T ₁₀	T ₃ + <i>Azospirillum lipoferum</i>	31.43	47.25	59.76
	S.Em.±	0.59	0.69	1.50
	C.D. at 5 %	1.76	2.05	4.46
	Initial Soil Sample	28.60	29.96	52.20

Table.2 Effect of co-inoculation of different bacterial cultures with rhizobium on microbial population in soil after harvest of black gram

Sr. No.	Treatments	Bacteria (CFUX10 ⁷)	Actinomycetes (CFUX10 ⁵)	Fungi (CFUX10 ⁴)
T ₁	Absolute control	25.67	21.00	2.17
T ₂	Only RDF	29.67	22.33	2.33
T ₃	RDF + <i>Rhizobium phaseoli</i>	31.33	26.33	3.33
T ₄	T ₃ + <i>Bacillus megaterium</i>	35.33	32.33	4.33
T ₅	T ₃ + <i>Bacillus subtilis</i>	31.33	26.67	3.67
T ₆	T ₃ + <i>Bacillus polymyxa</i>	29.67	22.00	3.00
T ₇	T ₃ + <i>Pseudomonas striata</i>	39.00	32.67	5.00
T ₈	T ₃ + <i>Pseudomonas fluorescens</i>	34.67	30.67	4.67
T ₉	T ₃ + <i>Azotobacter chroococcum</i>	30.00	27.33	3.67
T ₁₀	T ₃ + <i>Azospirillum lipoferum</i>	27.00	23.33	4.00
	S.Em.±	1.25	1.93	0.30
	C.D. at 5 %	3.70	5.73	0.87
	Initial Soil Sample	23.00	19.00	2.00

Fungal population

In case of fungal population the treatment receiving RDF + *Rhizobium phaseoli* + *Pseudomonas striata* (T₇) recorded highest fungal load (5.00 CFU X 10⁴) which significantly differed from other treatments at 5 per cent significance level and treatment T₄ (4.33 CFU X 10⁴) and T₈ (4.67 CFU X 10⁴) having co-inoculation of *Rhizobium phaseoli* with *Bacillus megaterium* and *Pseudomonas fluorescens* respectively, found at par with treatment T₇. The lowest actinomycetes (21.00 CFU X 10⁵) and fungal (1.67 CFU X 10⁴) population were recorded in treatment T₁ (absolute control).

Increase in microbial population may be due to growth promoting substances secreted by crop during growth period. Similar results were obtained by Saini *et al.*, (2015) showing co-inoculation of endophytic bacteria with *Rhizobium* noted maximum microbial population.

In line with our work, Goutami *et al.*, (2015) found that the maximum bacterial and fungal population was noticed in the FYM inoculated with biofertilizers while the minimum population was recorded in treatment no biofertilizer and FYM. Similarly, Sable and Ismail (2017) conducted results indicated that highest values of actinomycetes and bacterial population were noted in treatment RDF + *Rhizobium* and *Bacillus megaterium* whereas, fungal population was highest in the RDF+ *Rhizobium*+ *Trichoderma sp.* treated soil. Further, Trabelsi and Mhamdi (2013) reported that soil or seed inoculation may lead to changes in the structure and population of indigenous microbial communities. The variation in efficacy of different treatment combinations indicates the specificity of the inoculation response. These results provide a basis for the selection of an appropriate combination of specific *Pseudomonas sp.* and

Rhizobium sp. which could further be utilized for verifying the symbiotic effectiveness and competitive ability of bio-inoculants under field conditions (Mishra *et al.*, 2011).

Significantly highest values of acid phosphatase and alkaline phosphatase were noted in treatment of *Pseudomonas fluorescens* along with *Rhizobium phaseoli* and RDF after harvest of black gram while, co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* helped in enhancement of dehydrogenase activity. The significant increase in bacteria, actinomycetes and fungi in soil after harvest of black gram were recorded with co-inoculation of *Pseudomonas fluorescens* along with *Rhizobium phaseoli* and RDF.

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