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**RESEARCH PAPER**

# Studies on developing mixed consortia of $N_2$-fixing and Phosphate solubilizing liquid biofertilizers and population dynamics of biofertilizer strains in the consortia

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**Abstract:** An attempt was made to develop consortia of $N_2$-fixing and Phosphate solubilizing liquid biofertilizers. A total of four strains of biofertilizers, three recommended rhizobial strains namely, GR-2 for chickpea, NC-92 for groundnut, SB-120 for soybean and *Pseudomonas striata* a phosphate solubilizing bacterium (PSB) were used in developing mixed consortia. Intrinsic antibiotic resistance (IAR) of each strain for six antibiotics was studied and used in population enumeration of strains. In the first phase three rhizobial strains with seven different ratios were formulated and the effectiveness of each ratio was studied. Only two among the seven ratios namely; 1GR-2:1SB-120:1NC-92 which supported higher population and 2GR-2:1SB-120:2NC-92 which significantly influenced root biomass of chickpea (0.08 g plant$^{-1}$), soybean (0.05 g plant$^{-1}$) and groundnut (0.14 g plant$^{-1}$) were used to develop mixed consortia. In the second phase mixed consortia containing three strains of *Rhizobium* and a PSB were developed using the two efficient ratios of rhizobial consortia and liquid formulation of PSB. Six different ratios of mixed consortia were formulated and the population dynamics of each of the four strains was studied at ambient conditions over 100 days. The survival of individual strain of rhizobia and PSB in the mixed consortia was used to identify an efficient mixed consortium containing a PSB and three strains of *Rhizobium* suitable for three cross inoculation groups.

**Key words:** Liquid formulation, Mixed consortia, Phosphate solubilizing bacteria, *Rhizobial* consortia

## Introduction

Biofertilizers are complex products of live microbial inoculants which are able to ûx atmospheric nitrogen, solubilize soil phosphorus, decompose organic material or oxidize sulphur in the soil. Among the soil bacteria; *Rhizobium* form an unique group showing beneficial effect on the growth of legumes. *Rhizobium* exhibit host specificity and inoculation of any *Rhizobium* to different legumes across cross inoculation groups is not useful and this is a serious bottleneck in adoption of this technology by farmers. Hence, consortium of *rhizobial* strains belonging to different cross inoculation groups would help to resolve this problem. A formulation containing more than two compatable microorganisms is called as microbial consortium. Combinations of more than two beneficial microorganisms as inoculants have been found to perform better than single inoculations. Consortium containing *Rhizobium* strains belonging to different cross inoculation groups is not available for farmers use in this region. This kind of efforts however have been made in case of rhizobial consortia (Nitu and Haider, 2009, Bhuiyan *et al.,* 2014) and a consortium of *Rhizobium* strains suited to many groups of legumes can help farmer to use one inoculant to diverse legume hosts.

It is well known that Phosphate solubilizing bacteria (PSB) and *Rhizobium* have synergistic effect on crops. Development of consortia containing one strain of *Rhizobium,* PSB and PGPR have been attempted (Rather *et al.,* 2010; Anandaraj and Delapierre 2010; Bansal, 2015). The literature on combining two or more strains of rhizobia belonging to different cross inoculation groups with PSB to develop mixed consortium appears to be scarce. Formulation having combination of *rhizobial* strains and PSB help to increase the availability of at least two different nutrients using one single formulation in a broad group of legume crops. Hence, an attempt was made to develop consortia of liquid biofertilizers containing three strains of rhizobia covering groundnut, soybean and chickpea groups and a phosphate solubilizing bacterium with an objective to identify an efficient mixed consortium.

## Material and methods

The rhizobia used to develop *rhizobial* consortia included three recommended strains of *rhizobia* namely SB-120 for soybean, GR-2 for chickpea and NC-92 for groundnut. The phosphate solubilizing bacterium (PSB) used to develop mixed consortia was *Pseudomonas striata*. This study was conducted at the Department of Agricultural Microbiology, College of Agriculture and the Institute of Organic Forming, University of Agriculture Sciences, Dharwad during 2016-17.

Liquid formulation technology of *Rhizobium* strains developed at the Institute of organic farming and of *Pseudomonas striata* (Parvathi and Patil, 2018) were borrowed and used in this study.The consortia were prepared in two phases. In the first phase rhizobial consortia in different ratios of GR-2, SB-120 and NC-92 were prepared (Table 1). In the second phase two promising rhizobial consortia from first phase were selected and used to prepare mixed consortia containing different ratios of PSB (Table 2). Different ratios based on population of individual biofertilizers strain in each liquid formulation were prepared and their effectiveness was studied.

Table 1. Composition of different *rhizobial* consortia and their influence on root dry matter production in three legumes at 30 days after inoculation

| Sl. No. | Composition of *Rhizobial* consortia GR-2:SB-120:NC-92 | Volume of each of the three strains of *Rhizobium* in the final 200 ml of *rhizobial* consortium | | | Root dry weight (g plant$^{-1}$) of three legumes in response to inoculation | | |
|---|---|---|---|---|---|---|---|
| | | SB-120 | NC-92 | GR-2 | Groundnut | Chickpea | Soybean |
| 1 | 1:1:1 | 72.02 | 68.50 | 59.48 | 0.10 | 0.03 | 0.03 |
| 2 | 1:1:2 | 53.64 | 102.03 | 44.31 | 0.08 | 0.04 | 0.02 |
| 3 | 1:2:2 | 84.60 | 80.45 | 34.94 | 0.12 | 0.06 | 0.02 |
| 4 | 1:2:1 | 105.90 | 50.35 | 43.73 | 0.12 | 0.04 | 0.03 |
| 5 | 2:2:1 | 86.89 | 41.32 | 71.77 | 0.09 | 0.04 | 0.04 |
| 6 | 2:1:2 | 43.91 | 83.53 | 72.55 | 0.14 | 0.08 | 0.05 |
| 7 | 2:1:1 | 55.50 | 52.78 | 91.90 | 0.11 | 0.03 | 0.02 |
| | | S.Em± | | | 0.003 | 0.001 | 0.002 |
| | | C.D. (1 %) | | | 0.01 | 0.001 | 0.010 |

Before development of consortium, all strains were examined *in vitro* for their compatibility on selective medium by cross streak method (Ganesan and Gnanamanickam,1987). The intrinsic antibiotic resistance of all three *rhizobial* strain *viz.,* GR-2, SB-120 and NC-92 and *Pseudomonas striata* (PSB) to six different antibiotics was studied in the laboratory (Table 3). All strains were individually grown in yeast extract mannitol broth containing gum arabica, poly vinyl pyrolidone, poly ethylene glycol and tween 20 as required by each strain as followed in the mass production unit at the Institute of Organic Farming, University of Agricultural Sciences, Dharwad. To know the effectiveness of individual strain in consortia they were mixed in seven different ratios based on population and the population of each strain based on its ISR was enumerated on selective medium. The seven different ratios of *rhizobial* consortia formulated (Table 1) were studied for their effectiveness.

Each consortium with desired ratio was prepared by mixing individual strain in formulation to obtain a final volume of 200 ml. Volume of individual liquid formulation required for consortia preparation was calculated as detailed in Table 1. The formulated consortia were packed separately in a 250 ml capacity High Density Poly Ethylene (HDPE) narrow mouth bottles (Tarsons) and stored at ambient temperature for five months to assess shelf life. The population of individual strain of biofertilizers was enumerated following dilution plate count technique on respective selective medium (yeast extract mannitol agar with congo red for *Rhizobium* and Pikovoskya's agar for PSB) containing required quantity of antibiotics based on the results of IAR studies done earlier (Table 3).

Table 2. Composition of mixed microbial consortia containing *rhizobial* consortia and phosphate solubilizing bacteria in defined ratios

| Sl. No. | Mixed consortia with ratios | Volume of *Rhizobial* consortium in 100 ml of mixed consortium | Volume of PSB liquid formulation in 100 ml of mixed consortium |
|---|---|---|---|
| 1. | 1 part of *rhizobial* consortium (1:1:1):1 part of PSB | 42.20 | 57.80 |
| 2. | 1 part of *rhizobial* consortium (1:1:1):2 part of PSB | 26.80 | 73.20 |
| 3. | 2 part of *rhizobial* consortium (1:1:1):1 part of PSB | 59.35 | 40.65 |
| 4. | 1 part of *rhizobial* consortium (2:1:2):1 part of PSB | 34.65 | 65.35 |
| 5. | 1 part of *rhizobial* consortium (2:1:2):2 part of PSB | 20.95 | 79.05 |
| 6. | 2 part of *rhizobial* consortium (2:1:2):1 part of PSB | 51.45 | 48.55 |

Two efficient *rhizobial* consortia were selected for developing mixed microbial consortia. One was based on the population stability of individual *Rhizobium* strain in each consortium as estimated using direct plate count technique (DPCT). Selection of the other one was based on its inoculation effect on root biomass of three legumes namely; groundnut, chickpea and soybean (Table 1). Fresh seeds of these legumes were surface sterilized by dipping in 70 per cent alcohol for five minutes followed by rinsing with sterile distilled water six times. These seeds were transferred to small plastic pots (9.5 cm height × 10 cm width) containing sterile sand sieved through 2 mm mesh and amended with N-free nutrient solution (Wan *et al.,* 1986). These three legume crops were grown for 30 days under green house conditions. The root system was recovered after 30 days by carefully dipping each pot in fresh water held in plastic containers and gentle washing with running water. The roots were examined for nodulation and as nodule development was in very early stages the root system was dried to constant weight at $60^0$ C in hot air oven. The dry biomass was recorded using an electronic balance 0.01 mg sensitivity and this parameter was used to identify a ratio of *rhizobial* consortium that was very effective in promoting root biomass.

These two ratios of *rhizobial* consortia were further used to develop mixed microbial consortia. *Rhizobial* consortia and phosphate solubilizing bacteria were mixed in three different ratios as indicated in Table 2. Consequently, mixed consortia with different ratios based on population of *Rhizobia* in *rhizobial* consortia and population of *Pseudomonas striata* in liquid formulations were prepared and evaluated.

Into each of the two selected ratios of *rhizobial* consortia the required volume of PSB in liquid formulation was added

and mixed to obtain a final volume of 100 ml of mixed microbial consortia containing the three strains of *Rhizobium* and the PSB in desired ratio (Table 2). Volume of liquid *rhizobial* consortia and liquid formulation of *Pseudomonas striata* to achieve required ratio in a final 100 mL volume is presented in Table 2. This was packed in 250 ml capacity High Density Poly Ethylene (HDPE) narrow mouth bottles (Tarsons) and stored at ambient temperature for 100 days for assessing the population dynamics of the prepared formulations following direct plate count technique (DPCT) using selective medium with antibiotics.

**Statistical analysis**

The data obtained from the experiments were subjected to statistical analysis using Completely Randomized Design (CRD). Interpretation of the data was carried out in accordance with Panse and Sukhatme (1985). The levels of significance used in the 'F' and't' test was P=0.01. The critical difference values were calculated wherever the 'f' test values were significant.The treatment means were compared by applying Duncan's Multiple Range Test (DMRT).

**Results and discussion**

The effects of six different antibiotics at four varied concentrations on growth of rhizobia and PSB (*Pseudomonas striata*) are indicated in Table 3.

The population (CFU/ml) of strain GR-2, SB-120 and NC-92 grown on respective liquid formulation for 60 h were $7.66 \times 10^8$, $6.33 \times 10^8$ and $6.66 \times 10^8$ respectively. Among them the population of SB-120 was the least. One ml of liquid formulation of SB-120 containing $6.33 \times 10^8$ CFU/ml was equivalent to 0.826 ml of GR-2 containing $7.66 \times 10^8$ and 0.951 ml of NC-92 containing $6.66 \times 10^8$. This approach was used to develop *rhizobial* consortia with different ratios. These three strains were mixed in required proportion to achieve seven defined consortial ratios of 200 ml each (Table 1).

Population of individual *Rhizobium* strain was in consortia calculated using direct plate count technique based on their intrinsic antibiotic resistance (Table 3). On Congo red yeast extract mannitol(CRYEMA) without any antibiotics the total population of three *rhizobial* strains was enumerated (A). On CRYEMA amended with Streptomycin sulphate at 25 ppm only colonies of SB-120 appeared (B). While on CRYEMA amended with Chloramphenicol at 25 ppm the combined population of Rhizobium strains GR-2 and SB-120 could be enumerated (C). To record the population of only GR-2, from the value of C which contained population of GR-2 and SB-120 the value of B which contained population of the SB-120 only were substracted. Similarly, to enumerate the population of strain NC-92, value of A (three strains) was subtracted with C (contained population of GR-2 and SB-120).

Intrinsic antibiotic resistance (IAR) of *Rhizobium strains* has been used by earlier workers for enumerating population of specific strains (Junior *et al.,* 2012; Cigdem and Merih, 2008) and differentiating contaminants. To study population

Table 3. Intrinsic antibiotic resistance (IAR) displayed by three *Rhizobium strains* and *P. striata*

| Antibiotics | *Rhizobium strains* | | | *P. striata* |
|---|---|---|---|---|
| | SB-120 | GR-2 | NC-92 | |
| Novobiocin | | | | |
| | 25 ppm | + | + | - - |
| | 50 ppm | + | + | - - |
| | 75 ppm | + | + | - - |
| | 100 ppm | + | + | - - |
| Tetracylin | 25 ppm | - | - | - - |
| | 50 ppm | - | - | - - |
| | 75 ppm | - | - | - - |
| | 100 ppm | - | - | - - |
| Chloramphenicol | 25 ppm | + | + | - + |
| | 50 ppm | + | + | - - |
| | 75 ppm | + | + | - - |
| | 100 ppm | + | + | - - |
| Amoxyllin | 25 ppm | + | - | - + |
| | 50 ppm | + | - | - + |
| | 75 ppm | + | - | - + |
| | 100 ppm | + | - | - + |
| Streptomycin | 25 ppm | + | - | - - |
| | 50 ppm | + | - | - - |
| | 75 ppm | + | - | - - |
| | 100 ppm | + | - | - - |
| Penicillin | 25 ppm | + | + | - + |
| | 50 ppm | + | + | - + |
| | 75 ppm | + | + | - + |
| | 100 ppm | + | + | - + |

Note: + indicates growth on antibiotic amended medium displaying resistance to the antibiotic.
- Indicates no growth on antibiotics amended medium displaying resistance to the antibiotic.

dynamics of individual strains of *Rhizobium* in consortia with different ratios and to understand effective ratios of rhizobial strains could be a new application of IAR trait. The results of this study clearly demonstrated this new application of their IAR trait as evident from the findings of this study. The population of each individual strain of *Rhizobium* was higher in the consortium with equal populations (1:1:1). Although the populations of both GR-2 and SB-120 were higher in consortium with 1:1:1 ratio as compared to any other ratio from 30 days after incubation until 135 days after incubation, the population of NC-2 which was lower initially was significantly higher only from 45 days after incubation until 150 days after incubation (Table 4a, 4b and 4c). This clearly indicated differences in the adoption of the three *Rhizobium* strains to varying ratios in formulations. Although, significant reduction in population of all three strains of *rhizobia* were observed in consortia with different ratio only consortia with 1:1:1 ratio consistently recorded higher population of all three strains as compared to other ratios. This clearly brought out the need to formulate consortia with different ratios of *Rhizobium* strains varying in their growth rate and host specificity for identifying the right proportion of each to be used in the final formulation. Besides, such an approach helps to understand the behavior, population stability of each strain over a period of incubation consortia. This eventually will be an important consideration in identifying

Table 4a. Population of *Rhizobium strain*, SB-120 in the *rhizobial* consortia at different interval

| Ratios of *rhizobial* strain in consortium GR-2:SB120:NC-92 | Population of SB-120 (CFU x $10^4$) at regular intervals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 15 DAI | 30 DAI | 45 DAI | 60 DAI | 75 DAI | 90 DAI | 105 DAI | 120 DAI | 150 DAI |
| 1:2:2 | 6.81 (650) | $6.48^{b}$ (300) | $6.00^{b}$ (100) | $4.00^{c}$ (0) | $4.00^{c}$ (0) | $4.00^{c}$ (0) | $4.00^{d}$ (0) | $4.10^{d}$ (0.7) | $4.00^{c}$ (0.5) |
| 1:2:1 | 6.90 (1000) | $6.45^{b}$ (300) | $6.39^{b}$ (250) | $6.39^{b}$ (250) | $6.30^{b}$ (200) | $6.15^{b}$ (200) | $6.30^{b}$ (150) | $4.69^{b}$ (5.3) | $4.30^{bc}$ (2.0) |
| 1:1:1 | 7.15 (1400) | $6.72^{a}$ (950) | $6.81^{a}$ (850) | $6.89^{a}$ (800) | $6.89^{a}$ (800) | $6.93^{a}$ (650) | $6.98^{a}$ (600) | $5.29^{a}$ (19.7) | $4.80^{a}$ (7) |
| 2:1:2 | 6.95 (950) | $6.39^{bc}$ (250) | $6.39^{b}$ (250) | $6.00^{b}$ (150) | $6.00^{b}$ (100) | $6.15^{b}$ (100) | $6.00^{b}$ (100) | $4.26^{cd}$ (2.0) | $4.15^{c}$ (1.5) |
| 1:1:2 | 7.03 (1100) | $6.30^{b}$ (200) | $6.30^{b}$ (200) | $6.30^{b}$ (200) | $6.15^{b}$ (150) | $6.15^{b}$ (150) | $6.00^{b}$ (100) | $4.49^{bc}$ (3.3) | $4.15^{c}$ (1.5) |
| 2:1:1 | 6.72 (600) | $6.48^{b}$ (300) | $6.15^{b}$ (150) | $6.15^{b}$ (150) | $6.15^{b}$ (150) | $6.15^{b}$ (150) | $4.00^{d}$ (0) | $4.20^{d}$ (1.7) | $4.15^{c}$ (1.5) |
| 2:2:1 | 6.95 (900) | $6.30^{b}$ (200) | $6.15^{b}$ (150) | $6.15^{b}$ (150) | $6.00^{b}$ (100) | $6.00^{b}$ (100) | $5.24^{c}$ (150) | $4.65^{b}$ (6.7) | $4.45^{b}$ (3) |
| S.Em | 0.16 | 0.07 | 0.09 | 0.11 | 0.09 | 0.08 | 0.09 | 0.07 | 0.08 |
| L. S. D. | NS | 0.23 | 0.33 | 0.40 | 0.40 | 0.23 | 0.33 | 0.24 | 0.23 |

Table 4b. Population of *Rhizobium strain*, GR-2 in the *rhizobial* consortia at different interval

| Ratios of *rhizobial* strain in consortium GR-2:SB120:NC-92 | Population of GR-2 (CFU x $10^4$) at regular intervals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 15 DAI | 30 DAI | 45 DAI | 60 DAI | 75 DAI | 90 DAI | 105 DAI | 120 DAI | 150 DAI |
| 1:2:2 | 6.65 (450) | $6.48^{b}$ (300) | $4.00^{e}$ (0) | $4.00^{c}$ (0) | $4.00^{e}$ (0) | $4.00^{d}$ (0) | $4.00^{d}$ (0) | $4.20^{c}$ (1.3) | $4.00^{c}$ (1) |
| 1:2:1 | 6.81 (650) | $6.54^{b}$ (350) | $6.57^{ab}$ (450) | $6.54^{ab}$ (400) | $6.39^{b}$ (250) | $6.39^{ab}$ (250) | $6.24^{b}$ (200) | $5.08^{ab}$ (12.3) | $4.45^{ab}$ (3) |
| 1:1:1 | 7.15 (1400) | $6.99^{a}$ (1000) | $7.00^{a}$ (1000) | $6.85^{a}$ (750) | $6.78^{a}$ (650) | $6.74^{a}$ (550) | $6.69^{a}$ (500) | $5.32^{a}$ (21) | $4.65^{a}$ (4.5) |
| 2:1:2 | 7.13 (2100) | $6.30^{b}$ (200) | $6.24^{b}$ (200) | $6.24^{b}$ (200) | $6.15^{c}$ (150) | $6.00^{b}$ (100) | $6.00^{b}$ (100) | $4.68^{bc}$ (5.6) | $4.24^{b}$ (2) |
| 1:1:2 | 6.83 (700) | $6.30^{b}$ (200) | $6.24^{b}$ (200) | $6.15^{b}$ (150) | $5.30^{d}$ (200) | $5.15^{c}$ (100) | $5.00^{bc}$ (50) | $4.53^{c}$ (4.6) | $4.54^{a}$ (3.5) |
| 2:1:1 | 6.52 (600) | $6.54^{b}$ (350) | $6.24^{b}$ (200) | $6.15^{b}$ (150) | $5.00^{e}$ (50) | $4.00^{d}$ (0) | $4.00^{c}$ (0) | $4.42^{c}$ (2.6) | $4.00^{c}$ (1) |
| 2:2:1 | 6.48 (300) | $6.39^{b}$ (250) | $6.15^{b}$ (150) | $6.00^{b}$ (100) | $6.00^{c}$ (100) | $5.24^{c}$ (150) | $5.15^{c}$ (100) | $4.28^{c}$ (3) | $4.00^{c}$ (1) |
| S.Em | 0.17 | 0.06 | 0.20 | 0.16 | 0.09 | 0.04 | 0.04 | 0.17 | 0.07 |
| L. S. D. | NS | 0.23 | 0.66 | 0.52 | 0.17 | 0.22 | 0.20 | 0.52 | 0.23 |

Table 4c. Population of *Rhizobium strain*, NC-92 in the *rhizobial* consortia at different time interval

| Ratios of *rhizobial* strain in consortium GR-2:SB120:NC-92 | Population of NC-92 ( CFU x $10^4$) at regular intervals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 15 DAI | 30 DAI | 45 DAI | 60 DAI | 75 DAI | 90 DAI | 105 DAI | 120 DAI | 150 DAI |
| 1:2:2 | 7.11 (1300) | 6.60 (400) | $6.39^{c}$ (250) | $6.15^{b}$ (150) | $4.00^{b}$ (0) | $4.00^{c}$ (0) | $4.00^{c}$ (0) | $4.52^{bc}$ (3.3) | $4.00^{c}$ (0.5) |
| 1:2:1 | 7.38 (3050) | 6.69 (500) | $6.66^{b}$ (500) | $6.59^{b}$ (400) | $6.45^{a}$ (300) | $6.35^{ab}$ (300) | $6.15^{b}$ (150) | $4.49^{bc}$ (6.0) | $4.15^{c}$ (1.5) |
| 1:1:1 | 7.30 (2150) | 7.10 (1300) | $7.01^{a}$ (1050) | $7.00^{a}$ (1050) | $7.00^{a}$ (1000) | $6.95^{a}$ (900) | $6.92^{a}$ (850) | $5.35^{a}$ (22.3) | $5.25^{a}$ (18) |
| 2:1:2 | 7.44 (3200) | 6.74 (550) | $6.60^{b}$ (500) | $6.59^{b}$ (400) | $6.54^{a}$ (400) | $6.39^{ab}$ (250) | $6.15^{b}$ (150) | $4.56^{bc}$ (5.0) | $4.30^{c}$ (2) |
| 1:1:2 | 7.41 (2800) | 6.72 (550) | $6.72^{b}$ (600) | $6.60^{b}$ (500) | $6.54^{a}$ (400) | $6.39^{ab}$ (250) | $6.35^{ab}$ (300) | $5.09^{a}$ (13.7) | $4.45^{bc}$ (3) |
| 2:1:1 | 7.57 (3900) | 6.78 (600) | $6.50^{b}$ (350) | $6.35^{b}$ (300) | $6.30^{a}$ (350) | $6.00^{b}$ (100) | $6.15^{b}$ (150) | $4.39^{c}$ (3.0) | $4.24^{c}$ (1.5) |
| 2:2:1 | 6.66 (500) | 6.63 (450) | $6.50^{b}$ (350) | $6.45^{b}$ (300) | $6.39^{a}$ (350) | $6.30^{ab}$ (250) | $6.15^{b}$ (150) | $4.94^{ab}$ (9.3) | $4.70^{b}$ (5) |
| S.Em | 0.21 | NS | 0.07 | 0.09 | 0.22 | 0.18 | 0.18 | 0.14 | 0.09 |
| L. S. D. | NS | NS | 0.23 | 0.33 | 0.7 | 0.62 | 0.57 | 0.42 | 0.33 |

Note: The values in parenthesis are real values next to the log transformed values. CFU – Colony Forming Unit, DAI- Days After Incubation

efficient ratio for developing a consortium based on populations.

Apart from the population the real effectiveness of a consortium is of practical significance. Identification of an effective formulation could be done with a rapid plant response test. Hence, response of three legumes to inoculation with different ratios of *rhizobial* consortia was studied. Although the extent of nodulation in each legume influenced by different ratios would have been very useful, this data could not be generated as the nodulation in each of the legume roots was in very early stages and very tiny outgrowths resembling nodules and not mature nodules were observed. Hence, root biomass produced was estimated to make comparisons and identify effectiveness of each ratio. It was interesting to know that in all these three legumes inoculation with consortia containing 2 parts of GR-2, 1 part of SB-120 and 2 parts of NC-92 produced the highest root biomass in chickpea (0.08 g plant$^{-1}$), soybean (0.05 g plant$^{-1}$) and groundnut (0.14 g plant$^{-1}$).

Hence, only two of the seven rhizobial consortia namely one with 1:1:1 (1 part of GR-2, 1 part of SB-120 and 1 part of NC-92) and the other with 2:1:2 (2 parts of GR-2, 1 part of SB-120 and 2 parts of NC-92) ratios were found to be efficient in terms of population (Table 5a, 5b and 5c) it supported and root biomass of seedling (Table 1) produced respectively. Formulation containing strains effective in different cross inoculation groups of legumes help the farmers to use one biofertilizer for a broad group of legumes which could have greater adoptability in agriculture. Hence, the findings have relevance for developing one *Rhizobial* inoculum for a broad groups of legumes.

Subsequently, mixed consortia of *rhizobial* strain and PSB with three different ratios based on their populations were prepared. The population of *rhizobia* at the time of preparation of mixed consortia was 15× $10^8$ CFU ml$^{-1}$ in consortium with 1:1:1 ratio and 20.5 ×$10^8$ CFU ml$^{-1}$ with 2:1:2 ratio while the population of PSB in the liquid formulation used to develop mixed consortia

Table 5a. Population of *Rhizobium* strain, SB-120 in mixed microbial consortia at different interval

| Mixed microbial consortium | Population of SB-120 ( CFU x $10^6$) at regular intervals | | |
|---|---|---|---|
| | 15 DAI | 75 DAI | 100 DAI |
| $M_1C_1$ [1part (1:1:1): 1 part PSB] | 8.16$^a$ (147) | 7.28 (19) | 7.13 (14) |
| $M_1C_2$ [1part (1:1:1): 2 parts PSB] | 8.13$^a$ (137) | 7.35 (23) | 7.27 (19) |
| $M_1C_3$ [2 parts (1:1:1): 1 part PSB] | 8.38$^a$ (247) | 6.89 (8) | 6.74 (5.5) |
| $M_2C_1$ [1 part (2:1:2): 1 part PSB] | 8.17$^a$ (163) | 7.02 (11) | 6.92 (8.5) |
| $M_2C_2$ [1 part (2:1:2): 2 parts PSB] | 7.69$^b$ (50) | 6.65 (6) | 6.57 (4.5) |
| $M_2C_3$ [2 parts (2:1:2) : 1 part PSB] | 7.82$^b$ (67) | 6.69 (5) | 6.45 (3) |
| S.Em± | 0.07 | 0.16 | 0.15 |
| L.S.D. | 0.30 | NS | NS |

Table 5b. Population of *Rhizobium* strain, NC-92 in mixed microbial consortia at different interval

| Mixed microbial consortium | Population of NC-92 ( CFU x $10^4$) at regular intervals | | |
|---|---|---|---|
| | 15 DAI | 75 DAI | 100 DAI |
| $M_1C_1$ [1part (1:1:1): 1 part PSB] | 8.58$^a$ (406.6) | 7.36 (23.5) | 7.23 (17) |
| $M_1C_2$ [1part (1:1:1): 2 parts PSB] | 7.40$^a$ (36.67) | 6.69 (5) | 6.45 (3) |
| $M_1C_3$ [2 parts (1:1:1): 1 part PSB] | 8.09$^a$ (123.3) | 7.51 (33) | 7.29 (20) |
| $M_2C_1$ [1 part (2:1:2): 1 part PSB] | 8.19$^a$ (156.6) | 7.25 (18) | 7.10 (13) |
| $M_2C_2$ [1 part (2:1:2): 2 parts PSB] | 7.99$^a$ (100) | 6.80 (7) | 6.75 (6) |
| $M_2C_3$ [2 parts (2:1:2) : 1 part PSB] | 8.02$^a$ (126.6) | 6.60 (5) | 6.45 (3) |
| S. Em± | 0.15 | 0.16 | 0.12 |
| L. S. D. | 0.84 | NS | NS |

Table 5c. Population of *Rhizobium* strain, GR-2 in mixed microbial consortia at different interval

| Mixed microbial consortium | Population of GR-2 (CFU x $10^4$) at regular intervals | | |
|---|---|---|---|
| | 15 DAI | 75 DAI | 100 DAI |
| $M_1C_1$ [1part (1:1:1): 1 part PSB] | 8.72$^a$ (543.3) | 7.41 (26) | 7.18 (16.5) |
| $M_1C_2$ [1part (1:1:1): 2 parts PSB] | 7.69$^a$ (50) | 7.04 (11) | 6.94 (9) |
| $M_1C_3$ [2 parts (1:1:1): 1 part PSB] | 7.63$^a$ (63.3) | 7.15 (15) | 7.09 (12.5) |
| $M_2C_1$ [1 part (2:1:2): 1 part PSB] | 8.03$^a$ (106.7) | 7.36 (23) | 7.21 (16.5) |
| $M_2C_2$ [1 part (2:1:2): 2 parts PSB] | 7.91$^a$ (83.3) | 6.95 (9) | 6.83 (7) |
| $M_2C_3$ [2 parts(2:1:2): 1 part PSB] | 7.99$^a$ (126.7) | 6.54 (4) | 6.39 (2.5) |
| S. Em± | 0.17 | 0.12 | 0.12 |
| L. S. D. | 0.65 | NS | NS |

Table 5d. Population of *Pseudomonas* striata in mixed microbial consortia at different interval

| Mixed microbial consortium | Population of PSB (CFU x $10^4$ ) at regular intervals | | |
|---|---|---|---|
| | 15 DAI | 75 DAI | 100 DAI |
| $M_1C_1$ [1part (1:1:1): 1 part PSB] | 8.44$^a$ (276.7) | 7.57 (37.5) | 7.39 (25) |
| $M_1C_2$ [1part (1:1:1): 2 part PSB] | 7.74$^c$ (60) | 7.40 (25) | 7.24 (17.5) |
| $M_1C_3$ [2 part (1:1:1): 1 part PSB] | 8.17$^{ab}$ (150) | 7.20 (16) | 7.12 (13.5) |
| $M_2C_1$ [1 part (2:1:2): 1 part PSB] | 8.36$^a$ (230) | 7.35 (22.5) | 7.24 (17.5) |
| $M_2C_2$ [1 part (2:1:2): 2 part PSB] | 8.03$^b$ (106.7) | 7.30 (20) | 7.15 (15) |
| $M_2C_3$ [2 part (2:1:2): 1 part PSB] | 8.00$^b$ (100) | 7.45 (30) | 7.39 (25) |
| S. Em± | 0.06 | 0.07 | 0.10 |
| L. S. D. | 0.21 | NS | NS |

Note: The values in parenthesis are real values next to the log transformed values.

CFU – Colony Forming Unit, DAI- Days After Incubation

was $11\times10^8$ CFU $ml^{-1}$. To obtain the various ratios in mixed consortia of the three *Rhizobium* strains and one PSB the following approach based on their populations was applied.

One ml liquid formulation of PSB containing $11\times10^8$ (CFU/ml) was equivalent to 0.73ml of *rhizobial* consortium with 1:1:1 ratio (1GR-2: 1SB-120: 1NC-92) and 0.53ml of 2:1:2 rhizobial consortium with (2GR-2: 1SB-120: 2NC-92). This proportion was used to develop mixed consortia with six ratios (Table 2).

The volume of *rhizobial* consortia and liquid formulation of PSB in 100 ml each of mixed microbial consortia (Table 2) were evaluated for their ability to support population of each strain in mixed consortia over 100 days (Table 2).

Mixed microbial consortia having three ratios of nitrogen fixers (*Rhizobial* consortium) and phosphate solubilizing bacterial population was enumerated using direct plate count technique using IAR marker of respective strain (Table 5a,5b,5c and 5d). Among the six different mixed consortia the one which supported higher population of each biofertilizers strain up to 15 days after formulation was the consortium with 1 part of 1:1:1 *rhizobial* consortia and 1 part of PSB. This ratio consistently supported higher population of each biofertilizers strain although there were no significant differences with other ratios from 75 days after incubation. It was also observed that this ratio of mixed consortium (1: 1: 1) accounted for lesser decline in the population of individual biofertilizers strains (both rhizobia and PSB) and hence was better than the rest of the ratios of mixed microbial consortia. Bansal *et al.* (2015) have reported that mixed microbial consortium containing one strain each of *Rhizobium,* PSB and PGPR was effective in their attempt. The approach of this study was to know an effective formulation of mixed microbial consortia which included three strains of *Rhizobium* each belonging to a different cross inoculation group and a PSB based on different ratios of *Rhizobium* and PSB. Considering that currently the recommended *Rhizobium* for each group of legumes is different, additionally if PSB is to be inoculated to the same legume to mobilize phosphorus availability another biofertilizers formulation is required. Therefore one mixed formulation of these biofertilizers strains suitable to a group of legumes will boost adoption and spread of biofertilizers technology.

## Conclusion

In this study *rhzobial* consortia with 1:1:1 and 2:1:2 proved effective with respect to population stability of individual strain and effectiveness of consortia on seedling root biomass. Further, when these two ratios were used to formulate mixed consortia of three strains of *Rhizobium* and a PSB only the formulation containing one part each of *rhizobial* consortium and PSB was found to support higher populations of all four strains as compared to other ratios (Table 5a, b,c,d). This mixed consortium can be used to inoculate chickpea, soybean and groundnut. The findings show avenues for developing mixed consortia for other legumes and mixed consortia including more strains of *Rhizobium* and PSB as one biofertilizers formulation for legume crops.

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