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Research Article

Statistical Analysis of the Effect of Bacterial Consortia in Soybean Production

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Abstract

Soybean (*Glycine max*) is known as one of the most important legume plants, making a huge commercial contribution to vegetable oil production, meat production, even human nutrition. For marginalized farmers whose livelihood is dependent on soy, organic farming can be a great solution to improve its production. The use of organic farming essentially replaces the use of chemical fertilizers/pesticides and promotes the growth of Plant Growth Promoting Rhizobacteria (PGPR) which are eco-friendly and beneficial for plants in many ways. Different strains of bacteria M3, M7, M1 of *Bacillus sp.* can act as potential PGPRs. As part of the experiment, soil was collected and characterized by biochemical analysis in order to get an idea of how to improve soil quality. The CFU count of the soil was determined through serial dilutions and standard plate count technique followed by proper incubation. Two types of soil were considered: Garden soil (only) as normal control and garden soil plus vermicompost as the positive control. The treatments/inoculum included solid media, LB Broth, and water suspension which were applied separately. The pots were then prepared accordingly as positive and normal control setups and three different treatments had been applied. The growth was closely monitored for several weeks. The changes in the morphological and reproductive parameters for different treatments were quantified by Causal Impact Analysis for vegetative characteristics. For Reproductive Characteristics, ANOVA and LSD are performed. The results of this study indicated that LB treatments in normal, as well as, in the positive control, showed an overall better growth than the rest. Based on the above-mentioned tests, it is evident that the overall performance was best in the consortia when applied to LB broth. Additionally, the correlation coefficient shows that the vegetative and reproductive characteristics are highly correlated.

Keywords: Inoculum; Normal Control; Positive Control; Consortia; Strain; Vermicompost; Serial Dilution

Abbreviations

CFU: Colony Forming Units; PGPR: Plant Growth Promoting Rhizobacteria; LB Broth: Luria Bertani Broth; ANOVA Test: Analysis of Variance; LSD Test: Least Significant Difference

Introduction

Soybean is an important commercial crop that has a remarkable place in the world as the most important legume plant which contributes to about 25% of the global vegetable oil production

and to two thirds of the protein concentrate for feeding the livestock as well as human beings. Currently, the crop is grown in 103 million ha globally with an annual production of 261 million tons and average productivity of 2533 kg per ha (FAOSTAT, 2013, 8). India ranks 5th in terms of Soybean production over the world. Starting from an area of just 30000 ha in 1970 Soybean production has reached 10.69 million ha in 2012 (SOPA, 2013, 8). At present, the area under soybean is mainly spread in the states of Madhya Pradesh, Maharashtra, Rajasthan, Chhattisgarh, Andhra Pradesh, and Karnataka (Bhatia., *et al*. 2008) [8]. Despite the spectacular growth in area and production, the average productivity of the crop (1.2 t/ha) in India is less than half the world average (2.53 t/ha) and one-third of its climatic potential (3.5 t per ha) (Bhatia., *et al*. 2008) [8]. As the livelihood of millions of small and marginalized farmers depend on this crop there is an urgent need to improve the cultivation of the Soybean crop. It can be done with the help of organic farming in which the chemical pesticides will be replaced by the eco-friendly Plant Growth Promoting Rhizobacteria (PGPR) which can colonise the plant roots and help in the growth of the plants by fixation of Atmospheric Nitrogen, Solubilisation of Phosphate and Potassium.

There are a number of mechanisms by which PGPR promotes plant growth and development in diverse environmental conditions. The growth promotion occurs by the alteration of the entire microbial community in the rhizospheric niche through the production of various substances. Sometimes they supply the nutrients directly to the plants like Nitrogen, Phosphorus and Potassium and can also modulate the level of several plant hormones. They can decrease the inhibitory effect of various plant-borne pathogens. The PGPR plays a vital role in the production of Siderophore which are low molecular weight compounds used by the plants to trap iron in an iron-deficient environment. Flores-Felix showed that the growth of Strawberries is promoted by siderophore-producing Phylobacteria strain [7]. Some bacterial strains produce Volatile Organic Compounds which improve the plant growth and induce systemic resistance. Acetoin and 2,3 Butanediol produce by Bacillus sp. are responsible for improving plant growth.

PGPR helps the plants to cope with various types of abiotic stresses like hypersaline conditions, less availability of water, very high temperature, acidic soil etc. Like the growth of mung beans has been improved by *Pseudomonas aeruginosa* (Sarma and Saikia) [6]. Marulanda., *et al*. [5] reported that the strains of *Bacillus megatherium* inoculated into the maize roots increased its capability to absorb water in hypersaline conditions. Gonzalez., *et al*. showed that *Azospirillum brasilense* can be used to increase the salt tolerance of jojoba plant.

Various Strains of bacteria are getting discovered day by day which can act as potential PGPR and help in the growth of commercial crops. Soybean (*Glycine max*) can be grown well using the bacterial strains *Bacillus sp.* which have been isolated from one of the barren lands of Diamond Harbour in South 24 Parganas of West Bengal. Soybean has been selected because of its short life cycle and good yield of products in cool and moist climates which is prevalent in the months of February and March in West Bengal. The High Yielding Variety of the seeds JS335 has been chosen for our experiment and it was procured from the Department of Agriculture, Calcutta University.

Materials and Methods

Collection and Characterization of Soil

The Biochemical analysis of the soil gives us specific information so that we can improve their quality if required. The extent to which soil fertility can be improved depends on the inherent properties of the site- soil texture, climate, slope and mineralogy. (Dr. Christine Watson., *et al*.) [3].

Firstly, we had collected all the necessary materials from Jadavpur Railway Nursery. Materials Collected were Earthen Pots (7 inch) - 30, Garden Soil (3-3.5 kg per pot) Vermicompost (500g per pot), Soybean seeds (JS335), Tissue Paper, Small sticks (for giving support to the plants), Cocopeat and Neem Khol. A small quantity of the soil that we had collected from the Jadavpur Railway Nursery was given to the Bharat Foundations soil testing centre at GangulyPukur near Jadavpur Police Station in Kolkata for determination of the chemical properties of the soil like p^{H}, Organic Carbon, Nitrogen, Phosphorus and Potassium content (Meenakshi Mukhopadhyay., *et al.*) [15].

The CFU count of the soil was determined in our own Microbiology Lab. First, we had prepared the Nutrient Agar medium with the components Peptone (5g/L), Beef Extract (3g/L), NaCl (5g/L), Agar (15g/L) and Distilled Water. Since we had required

only two plates of 20 ml media each so we prepared 40 ml of the media. All the components were weighed in the digital weighing balance for preparing 40 ml of the media (0.2g of Peptone, 0.12g of Beef extract, 0.2g of NaCl, and 0.6g of agar powder). They were dissolved in 20 ml of distilled water. Next, the p^H of the media was checked with a p^H paper and it came to 7.3 which was in the range of 7.4 ± 0.2. So we didn't require to pour any acid or alkali to adjust the p^H. So the volume was made 40 ml by adding distilled water. For the purpose of serial dilution of the soil sample, 4 test tubes were prepared. One was filled with 10 ml of distilled water for preparing the stock and the rest three were filled with 9 ml of distilled water. The conical flask in which the agar was prepared and the test tubes were properly plugged with cotton. Now, these components were sent for autoclaving for the purpose of sterilization. Before autoclaving, the test tubes, conical flask, Glass spreader, and the micropipette were properly wrapped with newspaper (Lee., *et al*.) [2].

After autoclaving, the media was poured into the two petriplates and allowed to solidify. Using 1g of soil sample the stock solution of soil was made in one test tube containing 10 ml of distilled water. Now 1 ml of solution was transferred from the stock to the next test tube containing 9 ml of distilled water. So it was the 10^{-1} dilution tube. Correspondingly 10^{-2} and 10^{-3} dilutions were made. Now, 100 µL of the soil solution from 10^{-2} and 10^{-3} dilution tubes were taken and plated over the surface of the two Agar plates respectively. The plates were kept in the incubator for 24 hours at 37ºC. On the next day, the colonies that were obtained in the two plates were counted and the CFU was calculated.

Designing of the treatments

After isolating the bacteria from the soil sample three different treatments were made. For the solid treatment, the bacterial cultures were freshly grown in the LB medium and mixed in sterile water. For the Luria Broth treatment, a loopful of culture was taken and mixed in 20 ml of autoclaved sterile LB broth and kept overnight in the shaker. Then the broth was mixed with sterile water. For the water suspension treatment, freshly grown bacterial cultures were kept in shaker for 25 hours and centrifuged at 10,000 rpm for 20 min. The Pellet that was obtained was mixed with sterile water.

According to our need, we have procured the 7-inch pots from the nursery along with the garden soil and the vermicompost.

Treatment	Control	Bacterium
LB	Garden Soil	M1
		M3
		M7
		M1 + M3 + M7
	Garden Soil + Vermicompost	M1
		M3
		M7
		M1 + M3 + M7
Water Suspension	Garden Soil	M1
		M3
		M7
		M1 + M3 + M7
	Garden Soil + Vermicompost	M1
		M3
		M7
		M1 + M3 + M7
Solid	Garden Soil	M1
		M3
		M7
		M1 + M3 + M7
	Garden Soil + Vermicompost	M1
		M3
		M7
		M1 + M3 + M7

Table 1: Outline of the Treatments.

In some treatments, the pots have to be filled up with only garden soil. In those pots, 3.5 kg of the garden soil was stuffed properly. In the other treatments, there is the requirement for vermicompost. In those pots $3/4^{th}$ of them were filled with the garden soil and the rest $1/4^{th}$ was made with an equivalent mixture of garden soil and vermicompost in the surface layer. So in these set, we have required around 3 kg of garden soil and 500g of vermicompost per pot.

During the process of stuffing the soil in the pots, it was ensured that no big clumps of soil particles were there. If some clumps were present they were crushed.

The soil-filled pots were watered properly so that the soil remains moist.

After the seeds were procured they were kept over some layers of moist tissue paper. Above them, another layer of moist tissue papers was given. The process of germination was started on 2nd March 2021. After 3 days the seeds were ready to be placed in the pots. The seeds were sown in the pots on 5th March 2021.

Small holes of around 1.5-2 cm were made in the soil and the seeds were placed in these holes keeping the radical in the downward direction. Now the holes were filled up with the soil and some amount of water was sprinkled over the regions where the seeds have been sown. We have sown 4 properly germinated seeds in one pot.

Everyday the soil of the pots was watered in two phases- one in the early morning at around 6.30 am and the other during the evening after sunset at around 5.30-6 pm. During the course of the experiment, it was ensured every time that the soil does not get dry. Excess watering was also not done.

We had bought sealed mineral water bottles and opened them inside the Laminar Air Flow Chamber and the water is poured in some other containers. The medium was poured inside the bottles and given to us. In total each member had carried 4 bottles for M1, M3, M7 and the consortia (M1+M3+M7) respectively.

In the case of the liquid treatments, we have directly applied the inoculums in the respective labeled pots. About 10 ml of the inoculum was applied per plant surrounding the region of the roots and not directly over the roots. So for each pot 40 ml of inoculum was given.

In case of the Solid Treatments, we have mixed 10 ml of the inoculums in LB with 10 g of vermicompost and around 20g of such mixture was added to each of the five respective labeled pots in the circumference of all the plants by digging the soil a little. The inoculum was applied on 10th April 2021, at the end of the 5th Week.

Every week the various morphological parameters were noted for each of the treatments and they include the vegetative characters like the length of the plant, internode length, no of leaves, size of the leaves and photosynthetic surface area, and the reproductive characteristics like No of Flowers per node and no of pods per plant.

All the important dates during which some important changes had occurred were also noted. The notable changes before inoculation and after inoculation were also noted. The length of the plant is calculated with a centimeter scale. The leaf surface area is measured with the help of a mm graph. In the graph, the area occupied by 10×10 smallest squares is 1 cm^2. A leaf is placed on the graph paper and the no of 1 cm^2 boxes were calculated.

Statistical analysis

Two types of the characteristic of the plant, vegetative and reproductive, are tested to conclude which treatment is preferred over others. And for those characteristics, two different types of analysis are performed because the vegetative characteristic is the parameter that is measured throughout the growth period (time-dependent) and reproductive characteristic is a parameter that is measured to know the yield at a certain time [at the 10th week, here] (time-independent).

For vegetative characteristics

To determine the effectiveness of an experiment, we conducted a causal impact analysis after a certain period of time [here, 5 weeks] with respect to the subject and the control.

Among all the vegetative characteristics, photosynthetic area was selected as that is the major representative of all.

The data were separated into three categories according to their treatments, viz., Solid treatment, LB treatment and Water treatment. Then the data were further decomposed with respect to each bacterium used. Then a particular treatment is taken, say LB treatment. In that, a specific bacterium is taken, for example, M3 bacterium, and then Causal Impact Analysis is performed once with respect to the control Garden Soil and again with respect to the control Garden Soil with vermicompost.

Causal Impact Analysis is to infer the temporal evolution of impact by incorporating empirical priors on the parameters in a fully Bayesian treatment. For this time series data, structural time series models or state-space models are used. The model is represented by two equations. One is the observation equation another one is the state equation. The two equations are followed by,

$$y_t = Z_t^T \alpha_t + \varepsilon_t \quad \text{---------- (1)}$$

$$\alpha_{t+1} = T_t\alpha_t + R_t\eta_t \quad \text{---------- (2)}$$

Where, $\varepsilon_t N(0, \sigma_t^2)$ and η_t $N(0, Q_t)$ are independent of all other unknowns. The first equation is the observation equation which links the observed data y_t to a latent d-dimensional state vector α_t. The second equation is the state equation which governs the evolution of the state vector α_t through time (Kay H. Brodersen., *et al.*) [14].

For reproductive characteristics

Between 'number of flowers' and 'number of pods', 'number of pods' is selected to conclude which treatment gives the best yield. ANOVA is performed here to know if there exists any significant difference between the different treatments. Further to infer which treatment is the best LSD test is performed. To conclude the required ANOVA is carried out three times. Once to get which bacterium gives the best result with respect to both normal control and with positive control, then to get which control shows better performance, whether normal control or positive control and lastly which treatment LB, Solid or Water Suspension gives the best yield.

For ANOVA test, H_0 (null hypothesis): means of the different groups are the same against H_1 (alternative hypothesis): at least one sample mean is not equal to the others. The test statistic for ANOVA is

$$F\ statistic = \frac{S^2_{between}}{S^2_{within}}$$

Where, $S^2_{between}$ is variance between sample means and is the variance within samples or residual variance. Here, in the result part we reject the null hypothesis is the p-value is less than 0.05.

And for LSD test the calculative formula is

$$LSD_{A,B} = t_{0.025,DFW}\sqrt{MSW\left(\frac{1}{n_A}+\frac{1}{n_B}\right)}$$

Where, DFW = degrees of freedom of the errors within groups; = upper 0.025 point of t-distribution; MSW =Mean Square Within, obtained from the results of your ANOVA test; n_A and n_B = number of scores used to calculate the means.

Correlation between vegetative and reproductive characteristics

Further the correlation between the vegetative and reproductive characteristic is determined. As mentioned in above, Photosynthetic area represents the vegetative property and number of pods represents the reproductive property. The respective values of the properties at the 10th week are collected and correlation coefficient (spearman correlation) is calculated.

The formula to calculate spearman correlation coefficient is

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2-1)}$$

Where, n = number of observations and d_i = difference between the two ranks of each observation.

Results and Discussions

Soil characterization

After the chemical analysis of the soil it was seen that its p^H was 7.31 slightly alkaline which is suitable for growing the respective crop. The Nitrogen, Phosphorus, and Potassium content were 132.1, 56.1 and 29.3 mg/kg respectively. It contains 1.22% of organic carbon and has a water holding capacity of 20.2%.

In the 10^{-2}dilution plate we had obtained 32 colonies. So 1 ml of the soil sample solution contained 32×10^3 colonies. Since during serial dilution 1g of the soil was dissolved in 10 ml distilled water to prepare the stock solution so the CFU count per gram of soil sample is 32×10^4.

Statistical analysis results

Performing the statistical tests the results we get is: (All statistical tests were completed using the statistical program R (ver. 4.1.2)).

Graphical representation of Causal Impact analysis for vegetative characters

The interpretation of the graphs is mentioned as follows:

- **Original:** Plots the synthetic baseline created by the control groups in a dotted black line. The actual performance of the test group is plotted in solid black. The blue outline represents the bounds of a 95% confidence interval.
- **Pointwise:** Plots the difference between the observed outcome and the predicted outcome. In the example above, the difference between actual and predicted outcomes is 0 until the intervention period. In most real-world datasets there will be some variation from 0 in the pre-period. If variations in the pre-period are significant and the confidence interval very wide, either the pre-period dates or control groups should be adjusted to narrow the confidence interval.

- **Cumulative:** Plots the cumulative difference between the observed outcome and the predicted outcome.

LB treatment

Figure 1: M1 is imposed after 5 weeks in LB.

In the control (Figure 1) the black solid line is less above the dotted line, i.e., the value if nothing was imposed, than that of the positive control. The relative effectiveness on the photosynthetic area is around 1000 cm/sq. in normal control (from cumulative section of left graph), whereas it is around 2000 cm/sq. in positive control (from cumulative section of right graph). Clearly, positive control gives effective result.

Figure 2: M3 is imposed after 5 weeks in LB.

In figure 2 the black solid line is less above the dotted line in normal control, i.e., the value if nothing was imposed, than that of the positive control. The relative effectiveness on the photosynthetic area is around 1500 cm/sq. in control (from cumulative section of left graph), whereas it is around 3700 cm/sq. in the positive control (from the cumulative section of right graph). Clearly, positive control gives effective results again.

Figure 3: M7 is imposed after 5 weeks in LB.

Figure 3 explains the black solid line almost coincides the dotted line in normal control, i.e., the value if nothing was imposed. Whereas in the positive control, it is much above the dotted line. The relative effectiveness on the photosynthetic area is around 150 cm/sq. in normal control (from cumulative section of left graph), but it is around 4000 cm/sq. in the positive control (from the cumulative section of right graph).

Figure 4: M1+M3+M7 is imposed after 5 weeks in LB.

In the left plot in figure 4 the black solid line is less above the dotted line, i.e., the value if nothing was imposed, than that of the right plot. The relative effectiveness on the photosynthetic area is around 1000 cm/sq. in normal control (from cumulative section of left graph), whereas it is around 4100 cm/sq. in positive control (from cumulative section of right graph). Moreover, the steep of this graph is more than that of the graph in figure 2.

Therefore, looking on at all the 4 figures, it is clear that positive control gives better result than that of control. Now the ordering of the effectiveness of the bacteria (observed from above) is M1<M3<M7<Consortia (though in normal control M7 shows poor performance of all).So the consortia is statistically proved to be the best in this treatment.

Water suspension

Figure 5: M1 is imposed after 5 weeks in Water.

Figure 5 explains the black solid line almost coincides with the dotted line in normal control, i.e., the value if nothing was imposed. Whereas in the positive control, it is much above the dotted line. The relative effectiveness on the photosynthetic area is around 800 cm/sq. in normal control (from cumulative section of left graph), whereas it is around 2000 cm/sq. in the positive control (from cumulative section of right graph). Therefore, positive control gives effective result.

Figure 6: M3 is imposed after 5 weeks in Water.

In the left graph in figure 6 the black solid line is less above the dotted line, i.e., the value if nothing was imposed, than that of the right graph. The relative effectiveness on the photosynthetic area is around 3000 cm/sq. in the positive control (from cumulative section of right graph). In control the cumulative graph shows negative. That implies the intervention is very less effective.

Figure 7: M7 is imposed after 5 weeks in Water.

The black solid line is below the dotted line (Figure 7), i.e., the value if nothing was imposed. The relative effectiveness on the photosynthetic area is around 2000 cm/sq. in normal control (from cumulative section of right graph). But in control the effectiveness is very low.

Figure 8: M1+M3+M7 is imposed after 5 weeks in Water.

In the left one in figure 8, the black solid line is below the dotted line, i.e., the value if nothing was imposed. The relative effectiveness on the photosynthetic area is around 3000 cm/sq. in normal control (from cumulative section of right graph). Again normal control shows very poor performance.

Looking at the results we can say that normal control shows very poor performance in water suspension. So in the case of positive control the order can be concluded as M1 M7 < M3 Consortia. From the graphical representation it is clear that whenever the bacterial inoculums are applied in water suspension, the efficiency of the nutrient uptake by the plants actually decreases which is very much reflected in their growth parameters.

Solid treatment

Figure 9: M1 is imposed after 5 weeks in Solid.

Figure 9 explains the black solid line almost coincides with the dotted line in both cases, i.e., the value if nothing was imposed. The relative effectiveness on the photosynthetic area is around 20 cm/sq. in normal control (from cumulative section of left graph), whereas it is around 1000 cm/sq. in positive control (from cumulative section of right graph).

Figure 10: M3 is imposed after 5 weeks in Solid.

In both, the plots in figure 10 the black solid line coincides with the dotted line, i.e., the value if nothing was imposed, but in the right plot, it is much higher. The relative effectiveness on the photosynthetic area is around 10 cm/sq. in case of normal control. But again positive control gives 10 cm/sq. effectiveness after 10 weeks.

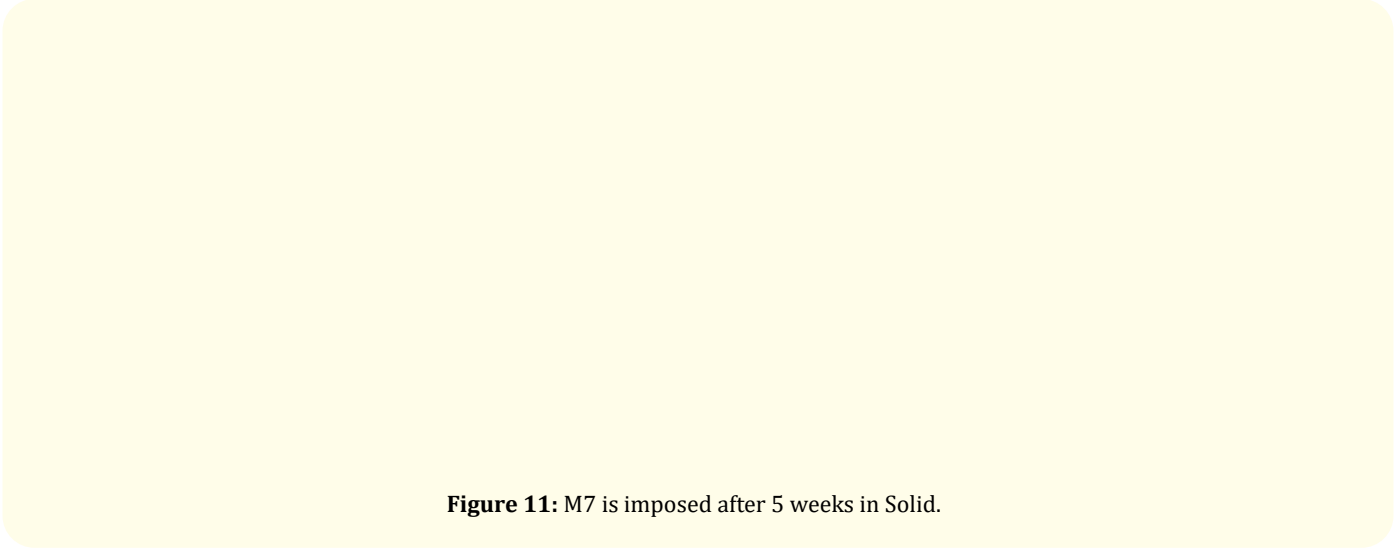

Figure 11: M7 is imposed after 5 weeks in Solid.

The black bold line almost coincides with the dotted line in figure 11. The relative effectiveness on the photosynthetic area is around 100 cm/sq. in normal control (from cumulative section of left plot). And in the right plot the effectiveness is around 500 cm/sq.

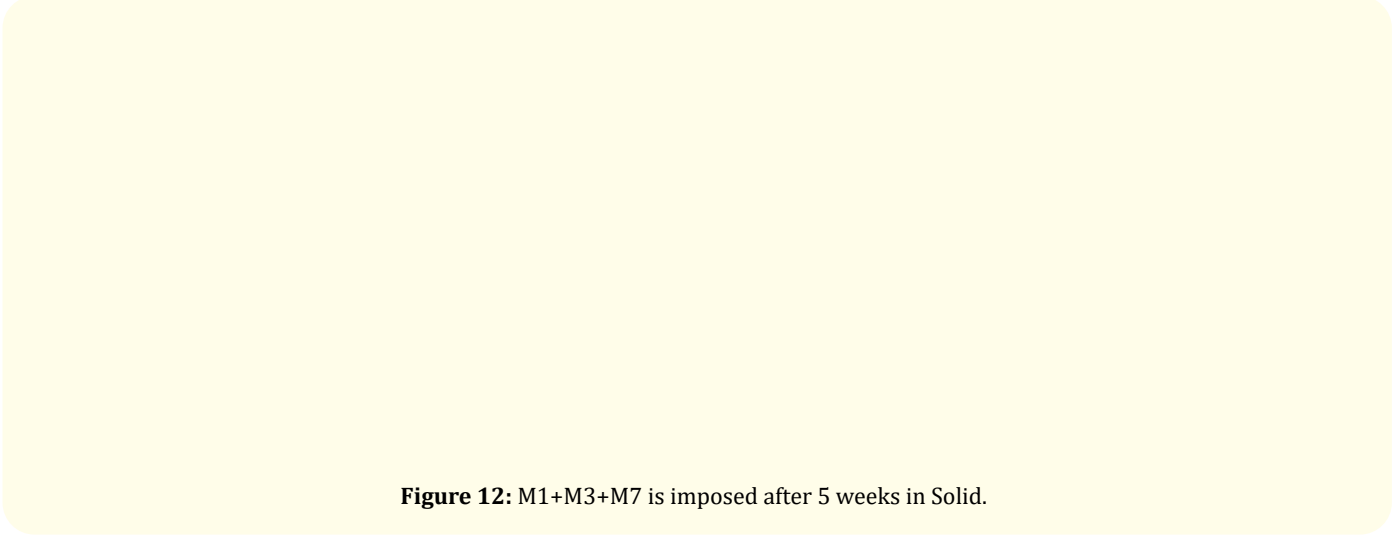

Figure 12: M1+M3+M7 is imposed after 5 weeks in Solid.

Figure 12 explains that normal control is very less effective as the dotted line is above the black bold line. But positive control gives very high effectiveness, which is around 1500 cm/sq. Therefore, positive control is better.

By observing the results we get, positive control is again very fruitful. Now, in the case of the application of the bacteria in solid treatment the order is as follows: M3 < M7 < M1 < Consortia.

Thus in each of the three treatments, it is observed that Consortia gives the best result. Now, if all the relative effectiveness mentioned above is compared LB in positive control is to be preferred. And among all it is clear that consortia in LB with positive control is the best treatment (which gives 4100 cm/sq. as relative effectiveness).

Correlation coefficient

Spearman correlation coefficient is calculated between the vegetative (photosynthetic area) and the reproductive (number of pods) characteristics. The result gives 0.8126.

Analysis of reproductive characters with ANOVA

Now, for the reproductive characteristic the results we have got are as follows: (All statistical tests were completed using the statistical program R (ver. 4.1.2)).

The result from ANOVA test, both in garden soil with normal control and with the positive control, shows that there is a significant difference among the bacteria, viz., p-value = 0.00118(<0.05) and p-value = 0.00165(<0.05) respectively in normal and positive control. Now, as there exists a significant difference we have done LSD test to know which bacterium is the best. On performing LSD test we get Consortium to be the best in both normal and positive control among all other bacteria used. The result or order we get in LSD test is as follows:

In normal control, Consortia (=15.22222) > M1 (=14.11111) > M3 (=13.66667) > M7 (=13.33333) and in positive control, Consortia (=22.66667) > M1 (=19.83333) > M3 (=18.66667) > M7 (=18.33333).

Now, the result of ANOVA test in between normal and positive control shows there exists a significant difference between them. The p-value is found to be 0.049 (<0.05). Again LSD test is performed and found that positive control gives the best result. Positive Control (=18.13333) > Normal Control (=17.11111).

Again, the result of the ANOVA test among the three treatments also shows there exists a significant difference between them. Here, p-value is found as <2e-16, which is <<<0.05. Further to conclude which treatment yields the better result, LSD test is performed. LB treatment is got to be the best and water suspension is the worst. LB (=20.200000) > Solid (=18.266667) > Water (=9.866667).

Discussion

In the Journal "Bacterial Consortium and Microbial Metabolites Increase Grain Quality and Soybean Yield" by Luis Gustavo Moretti., *et al*. [9] it has been shown that whenever several strains of the bacteria are applied together in consortia then the yield of the Soybean crop as well as its quality increases to a great extent. In our experiment, we had also proved that the consortia of M1, M3 and M7 gave the maximum yield by statistical analysis.

Similarly, if we look into "Effect of Microbial Consortia on Growth, Nodulation, Yield and Nutrient Uptake of Soybean in Vertisol of Central India" by Yaduwanshi., *et al*. [10] we can identify that using different microbes can increase different plant parameters like Length of the stem, biomass in terms of leaves as well as quality and quantity of seeds than the uninoculated plant. Likewise in our experiment, the plants in which we had applied different bacteria showed many good results from the ones in which no such bacterial inoculums were added.

From the viewpoint of "Plant Growth Stimulation by Microbial Consortia" by Gustavo Santoyo., *et al*. [11] the interaction of different microbial species in consortia facilitates the overall growth and development of the plants much better than the cases when they are applied alone. We had analysed the different results of the individual bacterial strains M3, M7 and R1 and pointed out that there are some positive effects in the inoculated plants and these effects further increase when the microbes are applied in consortia.

The correlation Coefficient helps to signify a linear relationship between any two variables and its value range from -1 to +1 (Correlation Coefficients: Appropriate Use and Interpretation, Schober., *et al*. [12]. We had obtained a value of 0.8 when we were comparing the relation between vegetative and reproductive character, which suggest clearly that they were following a linear trend.

From our experimental set up we had seen that the effect of consortia in LB is much more effective than that of water suspension. The probable reason may be that the bacterial cells can utilize the nutrients present in the LB broth much more effectively and they can replicate into huge numbers in a short time interval. So when applied in the soil they can solubilize the nutrients rapidly. On the other hand, water suspension is very poor in nutrients and hence the efficacy rate of the same bacterial species decreases when applied in water suspension. "The use of vermicompost in organic farming: overview, effects on soil and economics" by Su Lin Lim., *et al*. [13] states that vermicompost can enhance the characteristics of the soil which in turn accelerates the plant growth. In our experiment, we had encountered that the growth results in a positive control set that contains both soil and vermicompost are better than the normal control which contains only garden soil in most of the cases.

Conclusion

Ultimately from our experiments, it has been proved that M1, M3 and M7 strains of the *Bacillus sp.* are positively modulating the vegetative and the reproductive characteristics of the Soybean plant. We know the correlation coefficient lies between -1 to 1. And as the value is very near 1, we can say that there exists a high correlation between the chosen parameters. Therefore, we can state that vegetative characteristic and reproductive characteristic is highly correlated. From ANOVA Test, in case of reproductive characteristics as well, we get that Consortia in LB treatment with positive control gives the maximum yield.

The different results that we have got are statistically significant. The effect of different strains of bacteria together is much more significant than a single strain. The use of consortia in LB media applied in garden soil and vermicompost is the best of all treatments. So this way of using the bacterial inoculums for commercial crop production can be enhanced and applied in different agricultural fields. This will decrease soil pollution since all the materials are organic in nature and no chemical fertilizers are being used.

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