Original Article

DEVELOPMENT AND FORMULATION OF *Azospirillum lipoferum* AND *Pseudomonas fluorescens* AS EFFECTIVE BIOLOGICAL AGENTS FOR ENHANCED AGRO-PRODUCTIVITY

DESENVOLVIMENTO E FORMULAÇÃO DE Azospirillum lipoferum *E* Pseudomonas fluorescens *ENQUANTO AGENTES BIOLÓGICOS EFICAZES PARA UMA AGRO-PRODUTIVIDADE MELHORADA*

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ABSTRACT: Biofertilizer is a group of beneficial microorganisms used for improving the productivity of soil by fixing atmospheric nitrogen or by solubilizing soil phosphorus. They also stimulate plant growth through synthesis of growth promoting substances. In this present study, *Azospirillum lipoferum* is grown in Nitrogen free Bromothymol blue (Nfb) medium and *Pseudomonas fluorescens* in King's B medium. Bioprocess condition was optimized for both of the culture and found that *Pseudomonas fluorescens* has shown highest growth at 30^0C in pH 8 after 72 hours of incubation where as *Azospirillum lipoferum* showed highest cell concentration at 31^0C in pH 7, with incubation period of 72 hours. The optimized culture is mixed with different formulations of powder and liquid carrier such as Saw dust, Rice husk, Date seed powder, Matka khad, Jiwamrit and Beejamrit respectively. Shelf life study for 0, 30, 60, 90 and 120 days by cell counting and spread plate method showed that shelf life of the biofertilizer produced from Powder and liquid carriers had high amount of viable microbial population up to 120 days storage. Among biofertilizer based bio inoculants, Saw dust showed maximum population of $77x10^9$cfu/ml for *Azospirillum lipoferum* and 72 x 10^9 CFU/ml for *Pseudomonas* strain on 120th day and the liquid carrier Matka khad showed $85x10^9$ cfu/ml for *Azospirillum lipoferum* and 78 x 10^9 CFU/ml for *Pseudomonas fluorescens.*

KEYWORDS: Biofertilizers. *Azospirillum lipoferum. Pseudomonas fluorescens*. Powder carriers and liquid formulations.

INTRODUCTION

Biofertilizers are the bioinoculants of specific beneficial microorganisms that promote the growth of plant crops by converting the unavailable form of nutrients into available form. These biofertilizers also induce resistance in plants against pests, to improve soil fertility, to help plant growth by increasing the number and biological activity of desired microorganisms in the root surface (SIVASAKTHIVELAN et al., 2013). *Azospirillum* is a nitrogen fixing biofertilizer that colonizes in the root. Bacteria produces growth-promoting substances like indole acetic acid (IAA), gibberellins, pantothenic acid, thiamine and niacin and it also increases the rootlet density and root branching resulting in the increased uptake of mineral and water (VIJENDRAKUMAR et al., 2014). *Azospirillum* belongs to the family of Rhodospirillaceae and order Rhodospirillales associated with roots of monocots and can fix Nitrogen of 20-40 kg/ha, in addition to growth regulating substances. The *Azospirillum* form relationship with many plants mostly with those having the C4-dicarboxylic pathway of photosynthesis and Slack pathway, because they grow and fix nitrogen on salts of organic acids such as malic, aspartic acid (KAUSHAL et al.,2013). *Azospirillum* under stress conditions enhance plant growth by fixing atmospheric nitrogen. Production of growth promoting substances influence root development by increased uptake of nutrients from the land, and inhibiting pathogenic fungi and bacteria in the rhizosphere. (HOSSAIN ET al., 2015).

Pseudomonas fluorescence is a very common gram negative bacteria. It has antagonistic activity. It can produce some secondary metabolite or some antifungal compound such as fluorescent pigments, siderophores, hydrocyanic acid (HCN) and more important lytic enzymes. These lytic enzymes can degrade the chitin, β-1,3-glucan and protein components present on the fungal cell wall (RAMYASMRUTHI et al.,2012). It produces IAA and Promote enhancement of root length, shoot length, or number of lateral root (WAHYUDI et al., 2011). It is reported that *Pseudomonas fluorescence* is able to produce an antifungal metabolite called pyrrolnitrin, mainly used against *Rhizoctonia* Sp. and *Fusarium* Sp. (ANBUSELVI et al., 2010). It

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also has a potential of phosphate solubilisation, if it is placed on Pikovskaya media it forms a clear halo zone (FEKADU et al., 2013). So *Pseudomonas* acts as both biofertilizer and biopesticide.

A Bioinoculant can improve product stability, shelf life and also protect bacteria against different environmental conditions and provide initial food source. Application of PGPR either to increase crop health or to manage plant diseases depending on the development of bioformulations with suitable carriers that maintain the survival of bacteria for a considerable length of time (JAMBHULKAR et al., 2014).They can be applied for seed treatment, bio priming, seedling, foliar spray, fruit spray and sucker treatment (RITIKA et al., 2014)**.** Therefore, liquid inoculants formulation with good field performance characteristics that uses low cost materials and are easily attainable by small producers who could overcome many problems associated with processing solid carriers (SIVASAKTHIVELAN et al*.,* 2012).

In this study, *Azospirillum lipoferum* and *Pseudomonas fluorescence* were optimized for bioprocess conditions such as pH, temperature, incubation period and effective formulation were made by using liquid and powder carriers that promote the growth of the bacteria for the usage as an effective biological agent.

MATERIAL AND METHODS

Microorganisms

Azospirillum lipoferum (Nitrogen Fixer) and *Pseudomonas fluorescence* were procured from the Bannari Amman Sugars-BIOLAB, Sathyamangalam. *Azospirillum lipoferum* was maintained on Nitrogen Free Bromothymol blue Malate (Nfb) (Deshwal *et al*., 2013).

Optimization of the growth condition of *bacterial strains*

The growth conditions such as pH, temperature and incubation period of *Azospirillum lipoferum* and *Pseudomonas fluorescence* were optimized and the data were analyzed using MAT lab Version-8.1.0.604 (Prema *et al*., 2013).

Microbial analysis of different carriers

Formulations of Powder carriers

Various organic materials and agricultural wastes such as Saw dust, Rice husk and Date seeds powder were used for the mass multiplication of *Azospirillum* and *Pseudomonas* using the methodology of Marjan *et al.,* 2011.

Formulation of Liquid Carriers

Vedic krishi inputs such as Matkakhad, Beejamrit, Jiwamrit were the liquid carriers formulated using the methodology of Sanjay *et al*., 2012.

Shelf life of bioinoculants

The shelf lives of bioinoculants were checked for the different carriers'viz. powder and liquid carriers by spread plate technique. MATLAB Version-8.1.0.604 is used to analyse the shelf life of carriers.(Jorjani *et al.,* 2011).

Field study

Field performance of each formulations both liquid and powder has checked using Shallot (small onion, collected from Sathyamangalam). Different morphological characteristics for onion has scrutinized by ANOVA Agres Stat Version 3.1. Least Significant Difference test (LSD) at 1 % probability level was applied to compare the differences among treatment mean values.

RESULTS AND DISCUSSION

The culture of *Azopirllum lipoferum* and *Pseudomonas fluorescens* were maintained in Nitrogen free Bromothymol blue (Nfb) medium and King's B medium respectively

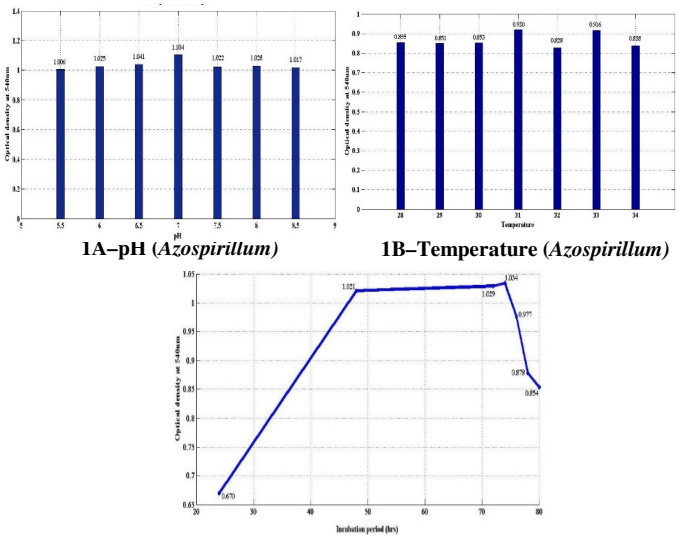
Optimization of growth condition for *Bacterial strains*

Azospirillum lipoferum maintained in Nfb medium showed maximum growth at pH 7, in 31^{o}C after 74 hours of incubation (Figure 1A, 1B, 1C).

Motiur et al., 2006 studied that the isolates of *Azospirillum,*MR-3, MR-4 and MR-8 showed maximum growth at 41°C and rest of the isolates showed maximum growth between 35° and 37°C. The isolates MR-1, MR-3, MR-4, MR-7, MR-8 and MR-13 showed optimum growth at pH 8.0 and the isolates MR-6, MR-11, MR-14, MR-15 and MR-16 showed optimum growth at pH 7.0, *A.amazonense* (MR-5) showed optimal growth at pH 6.5.

The optimal incubation period, pH, temperature for *Pseudomonas fluorescens* was found to be 8, 30^{0} C and 72 hours (Figure 2A, 2B, 2C) respectively.

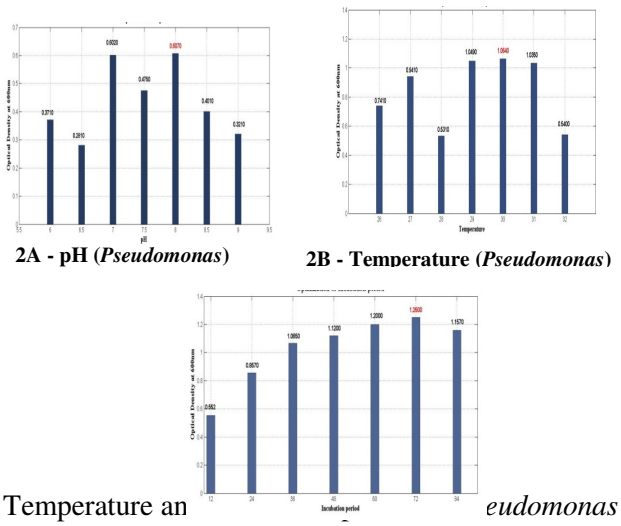
Prema et al., (2013) optimised *Pseudomonas* culture for the maximum production of siderophore with optimal medium composition such as 0.5 µM iron, 55 µM glucose, 30^{o}C, pH 7.0andincubation time of 72 hrs.



1A–pH (*Azospirillum*) **1B–Temperature (*Azospirillum*)**

1C- Incubation period (*Azospirillum*)

Figure 1. Optimization of pH, Temperature and Incubation period of *Azospirillum lipoferum*



2A - pH (*Pseudomonas*) **2B - Temperature (*Pseudomonas*)**

2C - Incubation period (*Pseudomonas*)

Figure 2. Optimization of pH, Temperature an *eudomonas fluorescens*

Shelf life study for Bioinoculants

Shelf life study of 120 days for *Azospirillum lipoferum* and *Pseudomonas fluorescence* in powder carrier's material is given in Figure 3-A, B. Among the three powder carriers used saw dust was proved to have highest cell count of 10^9cfu/ml for both the organisms.

A) *Azospirillum lipoferum* **B) *Pseudomonas flouroscences***

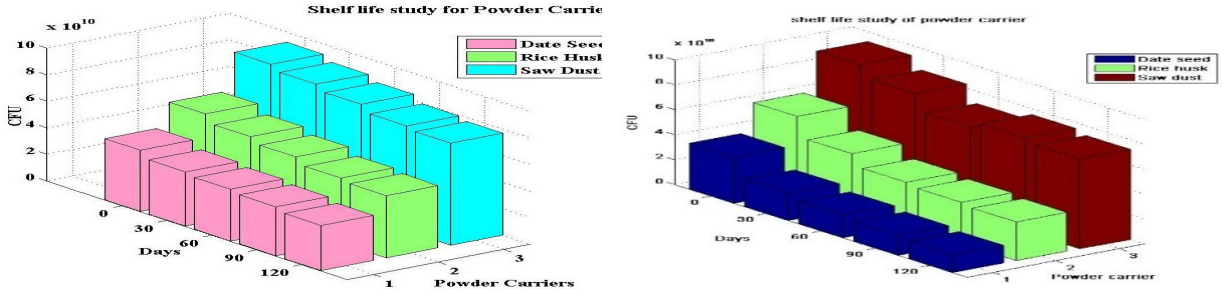


Figure 3. Shelf life study for powder carriers using MATLAB

Gandhi et al., 2009 reported the effect of vermicompost in maintaining the shelf life of bioinoculant such as *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescens* after 12 months in comparison with lignite carrier. Among Vermicompost based bioinoculants, *B.megaterium* showed maximum population of 7.60 x 10^8 cfu/g dry weight on 360th day followed by *Pseudomonas* 10^8 cfu/g dry weight respectively.

Liquid Formulations

Shelf life study of 120 days for *Azospirillum lipoferum* and *Pseudomonas fluorescence* in liquid carrier material is given in Figure 4-A, B. Among the three liquid carriers used Matka khad was proved to have highest cell count of 10^9cfu/ml for both the organisms.

A) *Azospirillum lipoferum* **B) *Pseudomonas flouroscences***

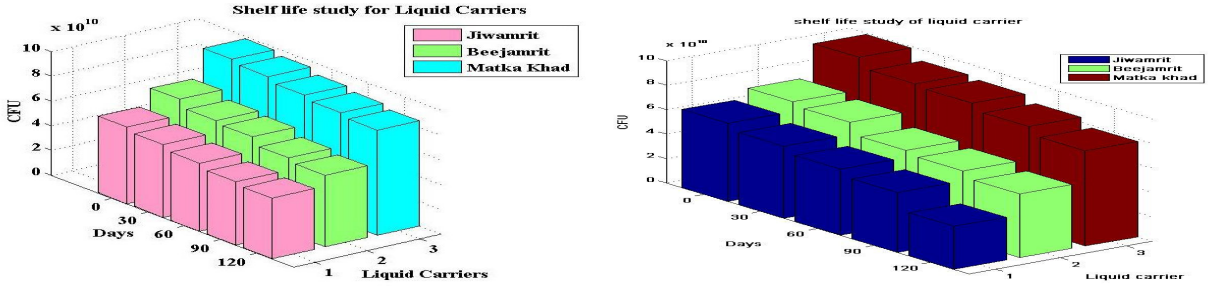


Figure 4. Shelf life study for liquid carriers using MATLAB

Kavi et al., 2014 reported the survival of these three PGPR strains in liquid formulations amended with additives PVP, trehalose and glycerol for a period of six months storage. The results revealed that the required population (1×10^8 cells /ml) of saline tolerant strains was maintained both in carriers and in liquid based formulation.

Morphological characters for onion

Powder carriers for *Azospirillum lipoferum* and *Pseudomonas fluorescence* (soil treatment).

The analysis of variance is presented in Table 1.

Table 1. Analysis of variance for powder carriers

	Azospirillum lipoferum(S)+Pseudomonas fluorescens(S)											
	Leaf length(cm)			**Leaf number**			**Plant height(cm)**			**Root length(cm)**		
P.C/ Vol.(g)	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**
C	32.9000	32.9000	32.9000	7.0000	7.0000	7.0000	44.3000	44.3000	44.3000	9.0000	9.0000	9.0000
0.5(C1)	28.1500	32.1000	25.1500	7.5000	12.5000	9.5000	51.4500	51.5500	42.3000	21.1000	17.2000	15.1500
1.0 (C2)	27.5500	35.0000	33.2000	10.0000	12.5000	13.5000	51.5500	55.7500	52.6000	21.4000	18.3500	17.1000
1.5 (C3)	30.3500	36.0000	34.8000	11.5000	13.0000	14.0000	54.8833	57.5000	58.6000	22.1500	19.0000	21.2000
2.0 (C4)	30.6500	36.9500	34.9000	12.0000	14.0000	16.0000	56.4000	59.9500	59.3500	23.2500	20.5000	23.2000
	SED= 2.15896			**SED=** 2.88675			**SED=** 5.34653			**SED=** 3.31937		
	CD(0.01)= 5.93714			**CD(0.01)=** 7.93857			**CD(0.01)=** 14.70295			**CD(0.01)=** 9.12827		

*Values are mean of the duplicate. P.C-Powder carriers, SD-Saw Dust, RH-Rice Husk, DSP-Date Seeds Powder; S-soil, C- Control.

Leaf length

Among the carriers, Rice husk has shown the best carrier treatment than saw dust and date seeds powder (Figure 5).

Leaf number

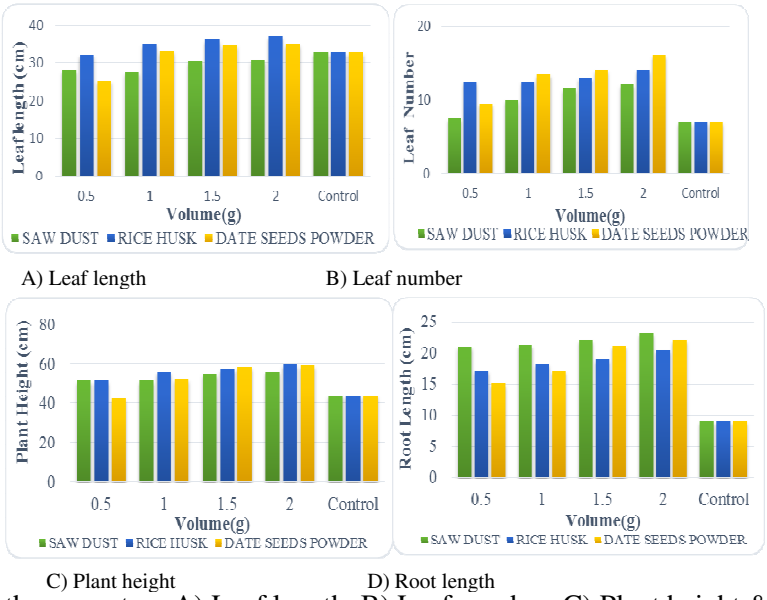
There is no significant difference among all the carriers and the concentration.

Plant height

Among the carriers, Rice husk has shown the best carrier treatment than saw dust and date seeds powder. C4 &C5 of the entire carrier has shown best treatmentsthanC1, C2 & C3.

Root length

There is no significant difference among all the carriers and the concentration (Table 2).



A) Leaf length B) Leaf number

C) Plant height D) Root length

Figure 5. Plant growth parameters-A) Leaf length, B) Leaf number, C) Plant height & D) Root length

Table 2. Analysis of variance for powder carriers

	Azospirillum lipoferum(S)+Pseudomonas fluorescens(S)								
	Bulb weight(g)			**Bulb diameter(cm)**			**Neck diameter(cm)**		
P.C /Vol. (g).	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**
C	2.2400	2.2400	2.2400	4.6000	4.6000	4.6000	3.0000	3.0000	3.0000
0.5 (C1)	5.6133	4.7333	3.1533	6.7500	6.2000	5.8500	3.7500	3.7000	2.6000
1.0 (C2)	5.7733	6.5300	9.9000	6.1333	6.8500	7.7000	3.8000	4.2000	4.6000
1.5 (C3)	10.0033	6.2100	10.9433	9.4000	7.4500	7.0500	5.8000	4.8500	4.9000
2.0 (C4)	10.4400	7.7933	11.2033	9.7000	7.7500	7.5433	6.7500	5.0500	5.0000
	SED= 1.47656 **CD(0.01)**=4.06054			**SED=**0.84977 **CD(0.01)**=2.33686			**SED=**0.83240 **CD(0.01)**=2.28910		

*Values are mean of the duplicate. P.C-Powder carriers, SD-Saw Dust, RH-Rice Husk, DSP-Date Seeds Powder, S-soil, C-Control

Bulb weight

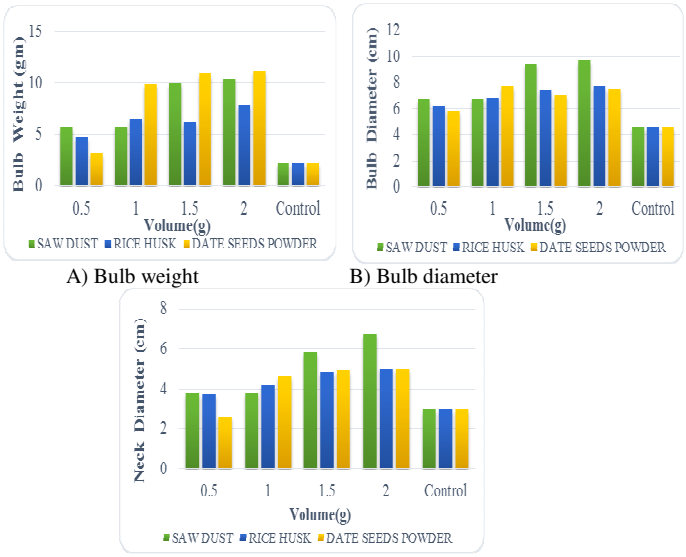
Among the carriers, Date seeds power has shown the best treatment than rice husk and saw dust.C4 & C5 have shown the best treatment than control (Figure 6).

Bulb diameter

There is no significant difference among all the carriers. C4 & C5 have shown best treatments than control.

Neck diameter

There is no significant difference among all the carriers. C4 & C5 have shown best treatments than control.



A) Bulb weight B) Bulb diameter

C) Neck diameter

Figure 6. Yield parameters are a) Bulb weight, b) Bulb diameter & c) Neck diameter

Powder carriers for *Azospirillum lipoferum* and *Pseudomonas fluorescence* (foliar spray treatment)

The results are summarized in Table 3.

Table 3. ANOVA of growth parameter for powder carrier

Azospirillum lipoferum(FS)+Pseudomonas fluorescens(FS)												
	Leaf length(cm)			**Leaf number**			**Plant height(cm)**			**Root length(cm)**		
P.C./Vol .(g)	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**
C	32.9000	32.9000	32.9000	7.0000	7.0000	7.0000	44.3000	44.3000	44.3000	9.0000	9.0000	9.0000
0.5 (C1)	28.8500	34.3167	25.1000	9.5000	9.5000	9.5000	49.7000	54.1000	44.1300	18.8000	20.2500	16.8800
1.0 (C2)	29.9500	33.1500	32.2500	10.5000	11.0000	14.0000	51.3000	56.3000	53.1700	18.9500	21.0500	18.4700
1.5 (C3)	30.3500	35.7500	35.0000	11.5000	13.5000	14.0000	54.7000	63.4000	58.1067	22.0500	25.0000	22.1733
2.0 (C4)	31.1833	38.6500	37.1000	14.0000	14.5000	16.0000	58.3500	67.8000	64.4500	23.4500	26.4500	24.0500
	SED= 2.83713			**SED=** 2.24846			**SED=** 3.90456			**SED=** 1.98247		
	CD(0.01)= 7.80210			**CD(0.01)=** 6.18325			**CD(0.01)=** 10.73754			**CD(0.01)=** 5.45180		

*Values are mean of the duplicate SD – Saw dust, RH – Rice husk, DSP – Date seed powder, FS-Foliar spray, C-Control

Leaf length

After analysis it has been found that among the carriers saw dust has shown the best treatment. C4, C5 (1.5 gm, 2 gm) of saw dust have shown the best result and C2,C3 (0.5 gm, 1 gm) have shown poor result. Date seed powder have shown the poor treatment among the entire carrier. C2,C1 (0.5 gm and control) of Date seed and control of rice husk have shown the poor treatment (Figure 7).

Leaf number

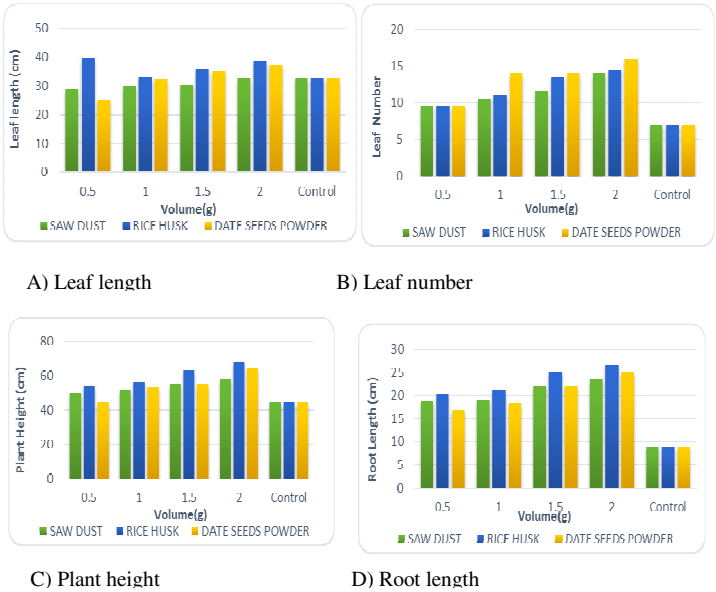
While considering leaf number among the carrier Rice husk and date seed powder have shown the best treatment and Saw dust has shown the poor treatment.C4,C5 (1.5 gm, 2 gm) of all the carrier have shown best result and control has shown the poor treatment.

Plant height

Similar to leaf length, plant height also high in Saw dust compared to rice husk and date seed carriers. Control of Rice husk, Date seed and Saw dust and C2 (0.5 gm) of Saw dust have shown the poor result.

Root length

While considering root length there is no significant difference among all the carriers. But among the concentration, C4 and C5 (1.5 gm, 2 gm) of all carriers have shown the best result than that of control.



A) Leaf length B) Leaf number

C) Plant height D) Root length

Figure 7- Plant growth parameters-A) Leaf length, B) Leaf number, C) Plant height & D) Root length

Bulb weight

After analyzing it has found that among the carrier Saw dust and Date seed have shown the best result while Rice husk has shown the poor result. Among the volume compared to control, C3, C4 and C5 (1.0 gm, 1.5 gm, 2 gm) have shown best treatment (Table 4 and Figure 8).

Bulb diameter

While considering bulb diameter there is no significant difference in the carrier but among the volume C3, C4 and C5 (1 gm, 1.5 gm, 2 gm) have shown best result than that of C2 (0.5 gm) and control.

Neck diameter

Date seed powder and Rice husk have shown highest neck diameter compared to saw dust carrier. Among the volume C5 (2 gm) of all carriers shown better result while control and C2 (0.5 gm) have reported to be poor treatment.

Table 4. ANOVA of growth parameter for powder carrier

Azospirillum lipoferum(FS)+Pseudomonas fluorescens(FS)									
	Bulb weight(g)			**Bulb diameter(cm)**			**Neck diameter(cm)**		
P.C / Vol. (g).	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**	**SD**	**RH**	**DSP**
C	2.2400	2.2400	2.2400	4.6000	4.6000	4.6000	3.0000	3.0000	3.0000
0.5 (C1)	4.1833	5.0633	4.8300	5.2500	7.4500	5.6500	4.0000	4.1000	2.8500
1.0 (C2)	4.7300	6.3833	10.2433	5.3500	8.2000	8.0500	4.5500	4.0000	4.4500
1.5 (C3)	5.4300	7.2833	10.2400	6.4000	8.3000	8.2000	4.6000	4.4000	4.6000
2.0 (C4)	6.7500	8.2233	12.9700	6.6000	9.3500	9.1000	5.9000	5.3167	4.7000
	SED= 1.77646			**SED=** 1.01336			**SED=** 0.96686		
	CD(0.01)= 4.88527			**CD(0.01)=** 2.78673			**CD(0.01)=** 2.65886		

*Values are mean of the duplicate. P.C-Powder carriers, SD-Saw Dust, RH-Rice Husk, DSP-Date Seeds Powder, FS- Foliar Spray , C-Control

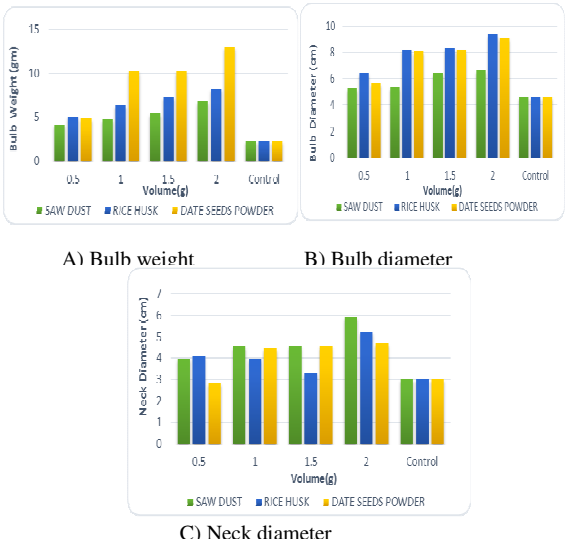


Figure 8. Yield parameters are a) Bulb weight, b) Bulb diameter & c) Neck diameter

Liquid Carriers for *Azospirillum lipoferum* and *Pseudomonas fluorescence* (soil treatment)

The results are presented in Table 5 and Figure 9.

Leaf length

Among the carriers, Rice husk has shown the best carrier treatment than saw dust and date seed powder.

Leaf number

There is no significant difference among all the carriers and the concentration.

Plant height

Among the carriers, Rice husk has shown the best carrier treatment than saw dust and date seed powder. C4 & C5 of all the carrier have shown best treatments than C1, C2 & C3.

Root length

There is no significant difference among all the carriers and the concentration.

Table 5. Analysis of variance for liquid carriers

Azospirillum lipoferum(S)+Pseudomonas fluorescens(S)												
	Leaf length(cm)			**Leaf number**			**Plant height(cm)**			**Root length(cm)**		
L.C./ Vol.(ml)	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**
C	31.8000	31.8000	31.8000	11.0000	11.0000	11.0000	50.9000	50.9000	50.9000	16.9000	16.9000	16.9000
0.5 (C1)	28.3500	32.4000	25.6500	12.5000	12.0000	7.5000	47.9500	51.4500	42.0500	17.3000	17.2000	14.5000
1.0 (C2)	28.5500	34.4000	26.7500	13.0000	13.5000	11.0000	48.5167	57.7333	44.4000	17.1000	21.1000	15.5000
1.5 (C3)	28.7000	37.1000	30.8500	15.5000	14.5000	12.0000	50.5500	62.6500	51.4500	19.9500	23.1500	18.3000
2.0 (C4)	31.6500	37.3500	33.4000	10.0000	15.0000	14.0000	53.8500	64.8000	58.7667	20.2000	18.6167	23.3800
SED= 2.93096				**SED=** 2.00278			**SED=** 4.33611			**SED=** 3.83832		
CD(0.01)= 8.06015				**CD(0.01)=** 5.50763			**CD(0.01)=** 11.92431			**CD(0.01)=** 10.55538		

*Values are mean of the duplicate. L.C.-Liquid carriers, MK-Matka khad, BM-Beejamrit, JM-Jiwamrit, C-Control, S-Soil

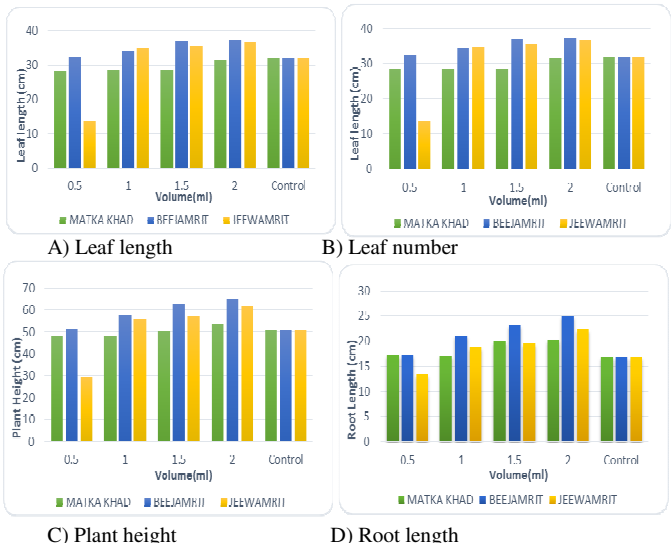


Figure 9. Plant growth parameters-A) leaf length, B) leaf number, C) plant height & D) Root length

Bulb weight

Among the carriers, C2, C3, C4 & C5 of saw dust and C4 & C5 of Date seeds power have shown the best treatment.C2 & C3of date seeds powder have shown the poor treatment (Table 6 and Figure 10).

Bulb diameter

C1, C3, C4 & C5 of saw dust and Control of Date seeds power and rice husk have shown the best treatment. C2 of rice huskand C2& C3 of date seeds powder have shown the poor treatment.

Neck diameter

C4 & C5 of saw dust have shown best treatments than C2 of date seeds powder.

Table 6. Analysis of variance for liquid carriers

Azospirillum lipoferum(S)+Pseudomonas fluorescens(S)									
	Bulb weight(g)			**Bulb diameter(cm)**			**Neck diameter(cm)**		
L.C./Vol .(ml)	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**
C	7.3500	7.3500	7.3500	8.5000	8.5000	8.5000	3.6000	3.6000	3.6000
0.5 (C1)	9.6733	4.7200	1.4500	7.3000	4.5333	3.3000	4.0500	2.7500	1.7000
1.0 (C2)	10.0300	5.3700	3.6833	7.4333	5.9500	4.4000	5.1500	3.2000	2.3500
1.5 (C3)	10.3733	6.2133	7.7300	7.7500	6.2500	7.0000	5.7000	3.3000	3.4000
2.0 (C4)	10.4100	6.2300	8.1800	8.6000	6.3000	7.0500	6.0500	3.6000	3.7500
SED= 1.39437 **CD(0.01)=** 3.83450				**SED=** 0.63584 **CD(0.01)=** 1.74857			**SED=** 0.17575 **CD(0.01)=** 0.48332		

*Values are mean of the duplicate. P.C-Powder carriers, SD-Saw Dust, RH-Rice Husk, DSP-Date Seeds Powder,C-Control

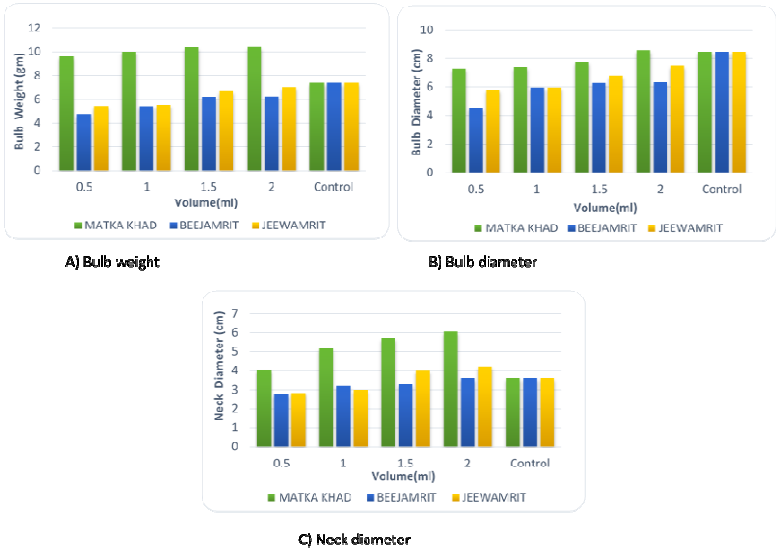


Figure 10. Yield parameters are a) Bulb weight, b) Bulb diameter & c) Neck diameter

Liquid Carriers for *Azospirillum lipoferum* and *Pseudomonas fluorescence* (foliar spray treatment)

Leaf length

There is no significant difference among the different liquid formulation. All the carriers shown similar results in this parameter. But among the volume C3,C4,C5 (1.0 ml, 1.5 ml, 2.0 ml) shows the best result and C2 (0.5 ml) have shown the poor result (Table 7 and Figure 11).

Leaf number

While considering this parameter, among the carrier Beejamrit has shown high significance and best result while Jeewamrit and Matkakhad have shown the poor result. Among the concentration C2, C3 that is 0.5 ml and 1.0 ml have shown poor treatment and C4, C5 (1.5 ml, 2.0 ml) has reported to be the best treatment.

Plant height

According to the analysis of the data it has been found that there is no significant difference among the carrier but among the concentration C4, C5 (1.0 ml, and 2.0 ml) have shown the best treatment and C2 (0.5 ml) has shown the poor treatment.

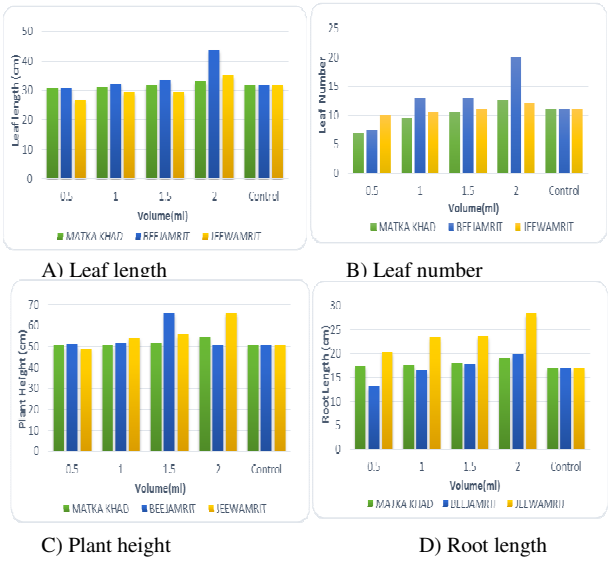
Root length

While considering root length, there is no significant difference among the carrier, but among the concentration C1,C2,C3 have (control, 0.5 ml, 1.0 ml) have shown poor treatment and C4, C5 (1.5 ml, 2.0 ml) has shown the best treatment.

Table 7. Analysis of variance for liquid carriers

Azospirillum lipoferum(FS)+Pseudomonas fluorescens(FS)												
	Leaf length(cm)			**Leaf number**			**Plant height(cm)**			**Root length(cm)**		
L.C./ Vol.(ml)	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**
C	31.8000	31.8000	31.8000	11.0000	11.0000	11.0000	50.9000	50.9000	50.9000	16.9000	16.9000	16.9000
0.5 (C1)	30.8500	30.8000	26.9333	7.0000	7.5000	10.0000	130.8500	45.7000	49.2000	17.4000	13.1000	20.5000
1.0 (C2)	31.0500	32.4000	29.2500	9.5000	13.0000	10.5000	31.0500	51.1000	54.4000	17.7000	16.4500	23.5500
1.5 (C3)	31.9500	33.6500	29.4500	10.5000	13.0000	11.6667	31.9500	51.5500	55.6000	18.0500	17.8000	23.6500
2.0 (C4)	33.1000	43.8500	35.1500	12.5000	20.0000	12.0000	33.1000	66.2000	66.1500	19.2667	19.9500	28.4500
SED= 3.24135 **CD(0.01)=** 8.91372				**SED=** 3.10972 **CD(0.01)=** 8.55174			**SED=** 37.91621 **CD(0.01)=** 104.26957			**SED=** 2.40137 **CD(0.01)=** 6.60378		

*Values are mean of the duplicate. L.C.-Liquid carriers, MK-Matka khad, BM-Beejamrit, JM-Jiwamrit, C-Control, FS-Foliar spray



A) Leaf length B) Leaf number

C) Plant height D) Root length

Figure 11. Plant growth parameters-A) leaf length, B) Leaf number, C) Plant height & D) Root length

Bulb weight

Among the carrier Beejamrit has shown significant difference and C5 (2ml) of Beejamrit has shown the best result. C2 and C3 (0.5 ml, and 1.0 ml) of Matka khad and C2 (0.5 ml) of jeewamrit has shown the poor result (Table 8 and Figure 12)

Bulb diameter

After the analysis it is found that among the carriers Beejamrit has shown good results. C3, C4 and C5 (1.0 ml, 1.5 ml, 2.0 ml) of Beejamrit have shown the best treatment and Matkakhad's C3 and C2 (1.0 ml, 0.5 ml) have shown the poor result.

Neck diameter

While considering this parameter, among the carrier Matkakhad has shown the poor treatment but Jeewamrit and Beejamrit have shown the best treatment. Among the volume C4 and C5 (1.5 ml, 2.0 ml) has shown good result and the C2 and C3 (0.5 ml, 1.0 ml) have shown poor result.

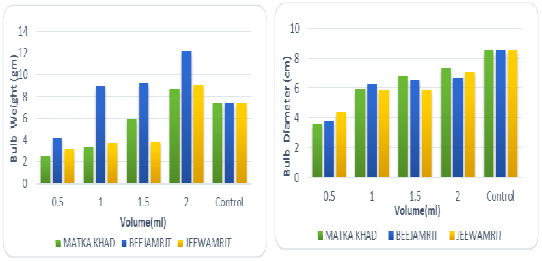
It is been reported that *Pseudomonas fluorescens* and other PGPRs induced a significant increase in root and shoot length, nodules, weight and even protein content in Mungbean plant (Dhanya *et al*., 2014, Heidari *et al*., 2014, Maiyappan et al., 2010).

It is also found that Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* have increased root and shoot elongation in canola, lettuce, tomato and also yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat (Brahmaprakash et al.,2012).

Table 8. ANOVA of yield parameters for liquid carrier

Azospirillum lipoferum(FS)+Pseudomonas fluorescens(FS)									
	Bulb weight(g)			**Bulb diameter(cm)**			**Neck diameter(cm)**		
L.C./Vol.(ml)	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**	**MK**	**BM**	**JM**
C	7.3500	7.3500	7.3500	8.5000	8.5000	8.5000	3.6000	3.6000	3.6000
0.5 (C1)	2.5400	4.1933	3.2133	3.6000	3.8500	4.4333	3.0000	3.0000	3.3000
1.0 (C2)	3.3100	8.9833	3.7833	5.9500	6.2500	5.9000	3.1500	4.3500	3.4333
1.5 (C3)	5.9200	9.2200	3.8400	6.7500	6.5500	5.9333	4.0000	4.5500	3.5000
2.0 (C4)	8.0267	12.1700	9.0833	7.3500	6.7000	7.1000	4.4833	4.9000	3.7500
SED= 2.00266				**SED=** 0.91364			**SED=** 0.67245		
CD(0.01)= 5.50730				**CD(0.01)=** 2.51251			**CD(0.01)=** 1.84923		

*Values are mean of the duplicate. L.C.-Liquid carriers, MK-Matka khad, BM-Beejamrit, JM-Jiwamrit, C-Control, FS- Foliar spray



A) Bulb weight B) Bulb diameter

C) Neck diameter

Figure 12. Analysis of yield parameter for liquid carrier

CONCLUSIONS

The highest cell concentration of 10^9 CFU was obtained for *A.lipoferum* at pH 7, in 31°C after 74 hours of incubation and for *Pseudomonas fluorescens* maximum cell concentration is obtained at pH 8, temperature 30° C and 72 hours of incubation respectively.

Different effective formulation of both powder and liquid carriers such as saw dust, rice husk, and date seed powder, Matka khad, Jiwamrit and Beejamrit respectively, were mixed with the optimised culture, which promotes the growth of the bacteria and produce an effective biofertilizer. Among the carriers (powder and liquid), highest cell viability was obtained in saw dust and Matka khad even after 120 days of storage.

For powder formulations, most of the carriers shown same result than control plant. Rice husk and date seeds powder have shown best result than saw dust.. Saw dust and Matka khad were the effective powder and liquid carrier formulations respectively with highest cell viability after 120 days of storage.

With respect to field performance such as yield and growth parameters, Saw dust and Beejamrit have proved be the best powder and liquid carriers. Thus biofertilizers has proven to be effective source than chemical fertilizers and was considered to be safe for practising agriculture naturally.

RESUMO: Biofertilizante é um grupo de microorganismos benéficos utilizados para melhorar a produtividade do solo através da fixação de azoto atmosférico ou por solubilização de fósforo no solo. Eles também estimulam o crescimento vegetal através de síntese de substâncias promotoras do crescimento. No presente estudo, Azospirillum lipoferum é cultivado em um meio de azul de bromotimol sem nitrogênio (Nfb) e Pseudomonas fluorescens num meio de King's B. A condição de bioprocesso foi optimizada para ambas as culturas e descobriram que Pseudomonas fluorescens mostraram maior crescimento a 300ºC em pH 8 após 72 horas de incubação, enquanto que Azospirillum lipoferum mostraram maior concentração de células a 310ºC em pH 7, com um período de incubação de 72 horas. A cultura optimizada é misturada com diferentes formulações de pó e veículo líquido tal como serragem, casca de arroz, pó de semente de tâmaras, Matka khad, Jiwamrit e Beejamrit respectivamente. O estudo do prazo de validade para 0, 30, 60, 90 e 120 dias por contagem celular e método de espalhamento em placa mostrou que o prazo de validade do biofertilizante produzido a partir do pó e veículos líquidos teve grande quantidade de população microbiana viável até 120 dias de armazenamento. Entre inoculantes biológicos de base biofertilizantes, a serragem mostrou população máxima de 77x109 CFU/ml para Azospirillum lipoferum e 72 x 109 CFU/ml para a estirpe Pseudomonas no 120º dia e um veículo líquido Matka khad mostrou 85x109 CFU/ml para Azospirillum lipoferum e 78x109 CFU/ml para Pseudomonas fluorescens.

PALAVRAS-CHAVE: Biofertilizantes. Azospirillumlipoferum. Pseudomonas fluorescens. Transportadores em pó e formulações líquidas

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