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Effect of biofertilizers on morphological and physiological parameters of cow pea *(Vigna unguiculata)*

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INTRODUCTION

"Biofertilizer" the term refers to preparations containing living cells of efficient strains of N_2 fixing, Phosphorus solubilizing or cellulolytic microorganisms which have the capacity to enrich soil fertility either as for living or in the association with the host plants. Simply the term bio fertilizer denotes all the nutrient input of biological origin for plant growth.(SubbaRao,1982).

Biofertilizers are the preparation containing living cells or spores, which can supply one or few plant food elements on inoculation. Now a days farming community shows much interest in the use of bioinoculants in a single packet as Biomix of *Azopus or Rhizopus, instead* of supplying it as individual organism. Several field studies on dual inoculation indicated the compatibility of N_2 fixing and 'P' solubilizing microorganisms (Subbarao, 1982). Brown (1974) reported the positive response of combined inoculation of N_2 fixing and P Solubilizing microorganisms. This shows the compatibility and synergistic action among the inoculants. Similarly, the compatibility of *Rhizobium* and *phosphobacteria* inoculants was reported earlier in cowpea. Hence, a preliminary attempt had been made to study the success of *Rhizopus* on nodulation of cowpea.

The chemical fertilizer no doubt increased the production but also produce many harmful effects. Therefore in developing countries like India, the use of biofertilizer is both economical and environmental friendly. Biofertilizers are "Microbial inoculants" used to improve the fertility of soil as suggested by Subba Rao (1982).

MATERIALS AND METHODS

Seeds of *Vigna unguiculata* variety (VBN3) were collected from the Tamilnadu Agriculture Department Pattukkottai and the biofertilizer used for this study were *Rhizobium*, *Azospirillum* & vermicompost obtained from the Amman biocare, Thirukanurpatti, Thanjavur (DT), Tamilnadu.

SEED TREATMENT WITH *RHIZOBIUM*

Rice starch was used for making the biofertilizer as slurry. The seeds were treated with the *Rhizobium* slurry and were kept overnight for germination.

SEED TREATMENT WITH *AZOSPIRILLUM*

Azospirillum was mixed with rice starch in a container to form slurry. Seeds were soaked in the slurry and kept overnight for germination.

SEED TREATMENT WITH VERMICOMPOST

Vermicompost was also mixed with rice starch in a container to form slurry. Seeds were soaked in the slurry and kept overnight for germination.

INOCULATION OF SEED TERATMENT WITH BIOFERTILIZER

Nearly, 300 undamaged healthy seeds were selected. After selection, the seeds were sown at equal depth in 24 pots with soil (clay soil, red soil and sandy soil with farmyard manure). Three control pots were also maintained by showing untreated seeds. The plants were watered at regular intervals and the growth parameters were studied periodically.

MORPHOLOGICAL PARAMETERS

The Percentage of germination, Number of shoots, Shoot length(cm), Plant height (cm), Number of branches, Branch length(cm), Root length(cm), Number of nodules, Leaves, Flowers and Fruits were studied. Estimation of total soluble sugar (Dubey's *et al.*,), total soluble protein (Lowry *et al.*,) and Chlorophyll (Horborne 1998) were analysed.

$$\frac{\text{Chlorophyll 'a'}}{\text{mg / g fresh leaves}} = \frac{(22.9 \text{xO.D.663}) - (2.69 \text{xO.D 645}) \text{ x V}}{\text{w x 1000}}$$

$$\frac{\text{Chlorophyll 'b'}}{\text{mg / g fresh leaves}} = \frac{(12.9 \text{xO.D.645}) - (4.68 \text{xO.D 663}) \text{ x V}}{\text{w x 1000}}$$

$$\text{Total Chlorophyll} = \frac{(8.02 \text{xO.D.663}) - (20.2 \text{xO.D 645}) \text{ x V}}{\text{w x 1000}}$$

V = Volume of the extract
W = Weight of the fresh leaves.

The chlorophyll contents estimated and the results were recorded.

RESULTS AND DISCUSSION

The *Azospirillum* treated in *Vigna unguiculata* plants were showed better performance than the control. The seeds were treated with different biofertilizers and the seed germination was observed in each 24 pots (Table-1). The root length was gradually increased in treated plants and the minimum level was observed in control. The shoot length was recorded. Maximum levels were observed in *Azospirillum* treated plants (Table2&3).

Maximum number of nodulation occurred in the root of 90th days old seedlings inoculated with *Azospirillum*. The number of nodule per plant was recorded 90th days old plants as follow. The control plants showed 42 nodules, *Rhizobium* treated plants had 86, the *Azospirillum* showed 92 and Vermicompost treated plants had 81(Table-3), nodules.

Table – 1: Percentage of seed germination in *Vigna unguiculata* using different biofertilizers

Days	Control	*Rhizobium*	*Azospirillum*	Vermicompost
	Germination (%)	**Germination (%)**	**Germination (%)**	**Germination (%)**
5	37.33	45	44.67	35
10	19	31.67	28	28
15	14.67	18.33	22.33	24.67
20	4	4	5	8.33
Total (%)	**75**	**99**	**100**	**96**

Table-2: Morphological parameters in *Vigna unguiculata* using biofertilizers
(15, 30, 45 days old seedlings)

Treatment	No of shoots			Shoot length (cm)			Plant height (cm)			No of branches			Branch length (cm)			Root length (cm)			No of nodules			No of leaves			No of flowers			No of fruits		
Days	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**
Control	1	3	5	2	14.6	16.9	5.4	15.6	20.7	0	1	3	0	5.2	18.3	5.9	7.2	8.6	7	15	22	3	18	42	0	0	10	0	0	15
Rhizobium	2	4	8	2.4	15.3	17	5.5	17.1	30.8	0	2	5	0	5.6	19.7	9.1	13.6	15.2	22	35	46	6	24	51	0	2	15	0	0	25
Azospirillum	**2**	**5**	**9**	**2.9**	**16.1**	**18**	**7**	**19.1**	**32.3**	**0**	**3**	**6**	**0**	**5.8**	**20.4**	**9.3**	**14.1**	**15.9**	**24**	**42**	**58**	**6**	**30**	**54**	**0**	**3**	**18**	**0**	**0**	**30**
vermicompost	2	4	7	2.7	15.1	16.8	5.5	15.7	30.4	0	2	4	0	5.5	19.5	9.1	13.5	14.8	20	32	44	6	24	51	0	2	13	0	0	20

Cm = Centimeter.

Table-3: Morphological parameters in *Vigna unguiculata* using biofertilizers
(60, 75, 90 days old seedlings)

Treatment	No of shoots			Shoot length (cm)			Plant height (cm)			No of branches			Branch length (cm)			Root length (cm)			No of nodules			No of leaves			No of flowers			No of fruits		
Days	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**	**60**	**75**	**90**
Control	7	8	9	19.2	19.8	20.1	34.6	39.1	41.6	5	6	6	30.3	39.2	41.3	9.1	13.2	14.4	30	38	42	69	98	115	12	23	25	30	40	52
Rhizobium	10	11	12	24.6	27.4	30.4	40.6	48.2	51.6	7	8	9	38.2	49	53.7	17.8	22.6	24.3	60	79	86	90	135	154	20	48	57	78	95	121
Azospirillum	**12**	**13**	**14**	**21.8**	**30.1**	**32.3**	**45.2**	**50.8**	**53.2**	**9**	**11**	**12**	**45.7**	**56**	**59.8**	**18.3**	**26.1**	**29.1**	**72**	**85**	**92**	**108**	**142**	**162**	**28**	**56**	**63**	**90**	**113**	**132**
vermicompost	9	10	11	21.3	26.9	29.5	39.1	46.5	49.5	6	7	8	37.8	46.5	48.9	17.5	21.3	22.7	54	74	81	87	126	146	18	40	48	65	89	112

Cm = Centimeter.

Table-4: *Chlorophyll content in leaves of Vigna unguiculata (biofertilizers treated)*
(mg/gm. Fresh weight)

Treatment	15th day			30thday			45th day			60th day			75th day			90th day		
	a	**b**	**Total**	**a**	**b**	**Total**	**a**	**b**	**Total**	**a**	**b**	**Total**	**a**	**b**	**Total**	**a**	**b**	**Total**
Control	0.14	0.20	0.34	0.15	0.27	0.42	0.11	0.26	0.37	0.09	0.20	0.29	0.014	0.16	0.174	0.011	0.08	0.019
Rhizobium	0.16	0.29	0.46	0.17	0.29	0.46	0.13	0.29	0.43	0.12	0.25	0.37	0.056	0.20	0.256	0.04	0.15	0.135
Azospirillum	**0.17**	**0.30**	**0.47**	**0.18**	**0.31**	**0.50**	**0.15**	**0.36**	**0.52**	**0.14**	**0.28**	**0.42**	**0.096**	**0.24**	**0.336**	**0.072**	**0.17**	**0.172**
Vermicompost	0.16	0.29	0.45	0.16	0.30	0.46	0.12	0.27	0.40	0.11	0.22	0.34	0.045	0.18	0.225	0.032	0.11	0.133

Mg = milligram, Gm = grams, A = Chlorophyll 'a', B = Chlorophyll 'b'

Table-5: *Protein and sugars of the leaves and seeds of Vigna unguiculata (biofertilizers treated)*
(mg/gm. Fresh weight)

Treatment	15th day				30thday				45th day				60th day				75th day				90th day			
	Protein		**Sugar**		**Protein**		**Sugar**		**Protein**		**Sugar**		**Protein**		**Sugar**		**Protein**		**Sugar**		**Protein**		**Sugar**	
	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed
Control	0.188	0	0.065	0	0.191	0	0.067	0	0.205	0.9	0.108	0.138	0.234	0.908	0.109	0.109	0.286	1.07	0.11	0.145	0.32	1.12	0.112	0.151
Rhizobium	0.194	0	0.113	0	0.325	0	0.119	0	0.43	1.069	0.125	0.450	0.469	1.105	0.129	0.129	0.472	1.12	0.133	0.512	0.529	1.247	0.137	0.549
Azospirillum	**0.4**	**0**	**0.124**	**0**	**0.514**	**0**	**0.129**	**0**	**0.547**	**1.214**	**0.132**	**0.510**	**0.638**	**1.256**	**0.137**	**0.137**	**0.699**	**1.305**	**0.142**	**0.532**	**0.714**	**1.428**	**0.149**	**0.558**
Vermicompost	0.286	0	0.112	0	0.341	0	0.116	0	0.217	1.08	0.118	0.395	0.285	1.147	0.124	0.124	0.547	1.27	0.127	0.51	0.562	1.286	0.131	0.542

Mg = milligram, Gm = grams.

The *Azospirillum, Rhizobium and* Vermicompost treated in *Vigna unguiculata* plants were showed better performance than the control. The root length was gradually increased in treated plants and the minimum level was observed in control.
Chlorophyll a, chlorophyll b and also total chlorophyll contents level of inoculated plants were also significantly higher than uninoculated control plants. Chlorophyll level was more in younger plant leaves, decreasing from mature plant leaves (Table4).

In general, all biofertilizers inoculated plants showed increased seed germination, Number of shoots, Shoot length(cm), Plant height(cm), Number of branches, Branch length(cm), Root length(cm), Number of nodules, Leaves, Flowers, Fruits and Chlorophyll content constituents when compared to uninoculated control plants. The soluble sugar contents were estimated from leaves and seeds. Soluble sugar content in the *Azospirillum* inoculated leaf and seeds were higher amount than other treated plants. The protein content of *Azospirillum* treated leaves and seeds showed higher amount than other treated plants. The lowest amount was recorded in control plants. (Table-5)

During the pot culture experiments, the association of *Azospirillum* showed better result than the other treatments and control. All the inoculated plants showed better growth than the uninoculated plants. Increase in morphometric, yield and attributing the parameters and also the suppression of leaf spot disease severity in the biofertilizer applied pots could be due to the ability of *Rhizobium* to produce plant growth promoting substance such as auxins, gibberellins and cytokinins resulting in higher plant vigour and growth. Similar observations have been made in plant growth promotion and suppression of pests and diseases in other agricultural crops (Chandra *et al.,* 1979; Rupach *et al.,* 1996 and Ramamoorthy *et al.,* 2001).

Soil microorganisms play an important role in soil biogeochemical processes which determine plant productivity. Successful functioning of introduced microbial bioinoculants and their influence on soil health. Exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats (Hill, 2000, and kanimozhi, 2011).

In the present investigation, even though the experimental plant belongs to a legume crop**,** *Azospirillum* influenced much for the maximum growth and yield than the other treatments which may be due to the efficient interaction between them.

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