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Plant Growth Promontory Effect on Cow Pea (*Vigna unguiculata* L.) Using Coir Pith Aqueous Extract Formulation of Cyanobacterium *phormidium*

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Abstract: This study describes the effect of a coir pith immobilized formulation of the cyanobacterium *Phormidium* and its aqueous extract on the germination and growth of cowpea. Evaluation of the various concentrations of the aqueous extract obtained from a 30 day old immobilized culture of the cyanobacterium revealed that a 5% aqueous extract significantly improved the germination of cow pea seeds under *in vitro* conditions. The basal application of the immobilized cyanobacterial formulation when combined with a foliar spray of the aqueous extract at 5 % concentration significantly increased the seed germination, plant height, plant weight, number of flowers, root nodules and biomass, over control. This treatment also significantly increased nodulation and plant growth on cowpea. The extracellular organic/bioactive compounds produced by the cyanobacterium are proposed to play a positive role in plant growth and establishment. In the present study showed on the basal and foliar application of cyanobacteria *Phormidium* sp-BDU5 + coir waste increased morphological and yield.

Key words: Aqueous extract · Immobilized cyanobacteria · Coir waste · Foliar spray · *Phormidium* Sp.

INTRODUCTION

Cyanobacteria are oxygenic, photosynthetic, free living and nitrogen fixing microorganisms commonly found in fresh water, marine water and soil. Cyanobacteria are being used as biofertilizer for plants, as food for human consumption and for the extraction of various products such as vitamins, drug compounds and human growth factors [1]. The role of N_2-fixing cyanobacteria in maintenance of rice fields has been well substantiated and documented all over the world. In India alone, the beneficial effects of cyanobacteria on yield of many rice varieties have been demonstrated in a number of field locations [2]. An additional benefit of cyanobacterial biofertilizers is their capacity to secrete bioactive substances such as auxins, gibberellins, cytokinins, vitamins, polypeptides, amino acids, which promote plant growth and development [3]. Kinetin lightened the influence of salinity on growth and production of plant growth regulators in *Vigna sinensis* and *Zea mays* [4]. Though cyanobacteria are commonly encountered in the aquatic environment, several reports are available on the beneficial effects of cyanobacterial biofertilizers on crops other than submerged rice.

Pulses form an integral part of the farming system and the vegetarian diet in the Indian sub-continent. Besides being a rich and cheap source of protein, they maintain soil fertility through biological nitrogen fixation and thus they play a vital role in sustainable agriculture. Among the grain legumes cow pea which is a native of central Africa, is grown mainly as for its seed and pods and to a lesser extent for its fodder value. Like most other pulse crops, cowpea fits well in a mixed cropping system. The nutrient value of cow pea has been estimated as 24% protein, 60% carbohydrate and 2 percent fat besides being a good source of vitamins and phosphorus. Hence there is an urgent need to increase productivity of this important pulse. Among the various alternatives that are

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available at present, cyanobacterial biofertilizers are an important bioresources that have not been extensively tried as biofertilizers for pulse production. In one of the available studies the hypersaline cyanobacterium *Phormidium tenue* was found to influence the overall growth performance and reproductive yield of *Vigna mungo,* when inoculated with a suitable *Bradyrhizobiam species* [5]. Cyanobacterial inoculation is also known to improve the stability soil due to excretion of polysaccharides, lipids which aid in enhancing aggregation [6]. Their influence on other crops besides rice, e.g. wheat, tomato and pulse and vegetable crops is also documented [7-9]

Coconut (*Cocus nucifera* L.) is one of the most useful and extensively cultivated palms in tropical countries. The outer husk of coconuts provide a valuable fiber called coir, which is used for manufacturing matting, mattress fillers and ropes in India, Sri Lanka, Bangladesh and other South Eastern Asian countries. As an alternative to peat or rock wool for plant culture it offers high moisture to air retention capacity, which enables easy growth and spread of the root system. It also contains 70% lignins and harbours several beneficial microbes. Considering the abundance and utility of this naturally available bio-degradable material several attempts have been made to utilize this as a carrier material for cyanobacterial biofertilizers by immobilizing cyanobacterial cells in the coir matrix.

The present study aims to determine the growth promoting effects of coir pith immobilized biomass of the cyanobacterium *Phormidium* and its aqueous extract on cowpea under *in vitro* and field conditions.

MATERIAL AND METHODS

Organism and Culture Conditions: The marine cyanobacteria *Phormidium* Sp. strain BDU-5 was obtained from the germplasm collection of National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The culture was grown and maintained in BG11 medium (g/L composition: $NaNO_3$ 1.5, K_2HPO_4 0.04, $MgSO_4.7H_2O$ 0.075, $CaCl_2$. $2H_2O$ 0.036, citric acid 0.006, ferric ammonium citrate 0.006, EDTA 0.001, Na_2CO_3 0.02. and trace metal mix solution 1ml. Trace metal solution contained (g/L) H_3BO_4 2.86, $MnCl_2.4H_2O$ 1.81, $ZnSO_4.7H_2O$ 0.222, $Na_2MoO_4.2H_2O$ 0.39, $CuSO_2.5H_2O$ 0.076, Co $(NO_3)_2.6H_2O$ 0.049 and adjust pH 7.1. Cyanobacterial culture was maintained in growth chambers at a constant light intensity of 1500 lux at 25°C [10].

Experimental Procedure: Lignocellosic material/coir waste was collected from a coir industry near Tiruchirappalli, Tamilnadu, India. For mass production, 500 g of a log phase cyanobacteria biomass inoculum was transferred to a water tank with 50 liter water. Cyanobacteria biomass and coir waste were added in the ratio of 1:50 and incubated for 15 to 20 d. The cyanobacterium was allowed to colonize the coir matrix and once sufficient colonization was ensured the culture aqueous extract was obtained by filtration. Various fold dilution ranging from 1:5-1:20 were prepared from the culture aqueous extract by appropriate dilution with distilled water [11]. The dried cyanobacterial biomass comprising of the cells that were immobilized in the coir matrix was used as a basal inoculum.

Effect of the Aqueous Extract on the Germination of Cow Pea Seeds under *In vitro* Conditions: The cowpea (*Vigna unguiculata* L.) seeds were collected from Regional Pulses Research Station, Tamil Nadu Agriculture University, Vamban, Pudukottai District, Tamil nadu, India. Seeds were surface sterilized (0.1% (v/v) $HgCl_2$, for 5 min, 70% (v/v) ethanol for 10 min) before plating. Five hundred viable seeds were tested for each concentration *viz.,* 5 % 10 % 15 % 20 % and 30 % of the aqueous extract. Each Petri dish contained thirty surface sterilized seeds that were placed on filter paper that was moistened with 10 ml of the aqueous extract of the desired concentration. The growth parameters including germination percentage, fresh and dry weight, shoot length (seedlings) were recorded on the 10 day after planting.

Pot Culture Experiments: The pot culture and field experiments were conducted at the National Facility for Marine Cyanobacteria Research Farm, Bharathidasan University, Tiruchirappalli, Tamil Nadu and India. Pots of 5 kg capacity were filled with amended soils, wetted with distilled water and incubated for 7 day before sowing. Five seeds per pot were sown at 0.5 cm depth. After emergence, seedlings were thinned to two plants per pot. The treatments along with control were arranged in a completely randomized block design with three replications. The aqueous extract was applied as a foliar spray at different stages crop growth viz., vegetative stage (15 DAS), growth stage (35 DAS) and flowering stage (50 DAS). Plant were harvested at 85 DAS and observations were recorded for various morphological and yield parameters observations, which included plant height, length of shoot, roots, nodule number and number of flowers/plant of cowpea.

Field Trials: The field experiments with cowpea conducted at the National Facility for Marine Cyanobacteria Research Farm, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. Plots were 5×5 m with eight rows spaced at 30×20 cm and arranged in a randomized complete block design with three replications, the different treatments are mentioned in (Table 3).

Biochemical Parameters: Biochemical parameters such as sugars [12] total phenolics [13] chlorophyll content [14] carotenoid [15] and protein content [16], were estimated in all treatments

Statistical Analyses: The data recorded in triplicate for the parameters in different treatments was analyzed using the statistical software [17]. Means were compared using least significant difference (LSD) at $P = 0.05$.

RESULTS

Coconut coir waste (pith plus short-to medium-length fibres) was evaluated as a lightweight material and showed a high total porosity, over 85% (vol). It exhibited very high air content together with fairly low easily-available water. pH was slightly acidic and salinity varied between 0.35 and 5.89 dS m^{-1}. Cation exchange capacity ranged from 34 to 92 m.e. /100 g and C/N ratios averaged 109. Coir waste contained more lignin (26.8%) and cellulose (34.6%), but less hemicellulose (6.55%). The other characteristics of coir waste include, an agriculture solid waste, light brown to dark brown in color, moisture content (on air dry sample)-12.1%, ash content-9.12%, carbon content-46.2% and water absorption-155%. The amount of naturally-occurring available nutrients was low, especially mineral nitrogen, calcium and magnesium (data not shown).

In the present study screened one efficient cyanobacterium from NFMC strains based on growth rate and coir waste degradation, *Phormidium* sp. strain BDU5 was selected, grows luxuriantly and utilizes the moisture content of coir pith as a substrate for their growth. The lignolytic activity of strain BDU5, 22% degradation within 30 days, enables it penetrate the coir pith thus making the transport to the field easier after drying. Rejuvenation being cyanobacteria biofertilizer comes in contact with water. The growth promoting ability of strain BDU5 was present both in the basal application and foliar which was substantiated by our previous field study in *Vigna radiata* and *Coriandrum sativum* [18]

At lower concentration 1:20 level of CWCE promote significantly higher seed germination (Table 1), on the hand seedling length and seedling dry weight of CWCE treated cowpea was higher when compared to control and other treatments. Among the treatment lower concentration 5 % showed significantly higher seedling length and seedling dry weight increased. A dose dependent effect from lower to higher was found among the treatment. The higher concentration to affect seed germination and plant growth *viz.,* seedling length, seedling fresh and dry weight leads to reduction of cowpea yield (Table 1).

In pot culture study, the basal and foliar application of 5 % concentration of CWCE significantly increased the growth and yield parameters (Table 2 and 3). The highest shoot length (62.75 cm/seedling), root length (16.42 cm/seedling), number of root nodules (8.29 /$plant^{-1}$), number of flowers (11.19/$plant^{-1}$), number of fruits (19.95/$plant^{-1}$), cluster (11.71/$plant^{-1}$) and fruit length (15.51 cm/plants) was recorded at 5% concentration of CWCE treated treatment when compared to other treatments/higher concentration of CWCE (or) uninoculated control. The same trend was observed under

Table 1: Effect of cyanobacteria *Phormidium* sp. BDU-5 + coir waste extract on seed germination of cowpea

Treatments	Germination (%)	Seedling length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Control	83.0 ± 5.20b	7.5 ± 0.35d	6.3 ± 0.46d	1.5 ± 0.28c
Phormidium sp.BDU-5+CWCF 5%	87.0 ± 4.62a	12.8 ± 2.19a	8.6 ± 0.81a	2.3 ± 0.29a
Phormidium sp.BDU-5+CWCF 10%	82.0 ± 4.04b	11.0 ± 1.15ba	8.1 ± 0.58b	1.9 ± 0.12b
Phormidium sp.BDU-5+CWCF 15%	78.0 ± 4.62c	10.0 ± 1.15bc	7.2 ± 0.62c	1.82 ± 0.15b
Phormidium sp.BDU-5+CWCF 20%	76.0 ± 3.46d	9.1± 0.36bcd	7.0 ± 0.46c	1.65 ± 0.12cb
Phormidium sp.BDU-5+CWCF 30%	67.0 ± 4.62e	8.3 ± 0.46cd	6.45 ± 0.43d	1.53 ± 0.11c
L.S.D	1.88	2.27	0.45	0.27

Each Values represents the mean ± SE of three replication. In the same column, significant differences at P = 0.05 levels are indicated by different letters. Data followed by same superscript letter in the same column are not significantly different from each other

Table 2: Effect of cyanobacteria *Phormidium* sp. BDU-5 + coir waste on growth and yield of cow pea under pot culture conditions

Treatments	Shoot length (cm)	Root length (cm)	Number of Branches	Number of lateral roots	No of root nodules
Control	19.77 ± 1.31d	8.6 ± 0.92d	5.2 ± 0.55d	9.35 ± 0.49c	3.18 ± 0.16e
Phormidium sp.BDU-5+CWCF 5%	62.75 ± 7.36a	16.42 ± 1.40a	26.6 ± 1.5 a	13.96 ± 1.71a	8.29± 0.60a
Phormidium sp.BDU-5+CWCF 10%	46.45 ± 4.30b	14.38 ± 0.80b	8.68 ± 0.97b	11.35 ± 1.36b	7.0± 0.29b
Phormidium sp.BDU-5+CWCF 15%	49.0 ± 5.20b	13.46 ± 0.84b	8.0 ± 0.58 cb	10.8 ± 0.75cb	7.18 ± 0.54b
Phormidium sp.BDU-5+CWCF 20%	32.85 ± 1.93c	10.92 ± 1.40c	7.1 ± 0.35 c	10.05 ± 0.55cb	4.24 ± 0.25d
Phormidium sp.BDU-5+CWCF 30%	28.23 ± 3.60c	10.98 ± 1.66c	6.72 ± 0.41c	9.94 ± 1.12cb	4.94 ± 0.54c
L.S.D	6.97	1.14	1.38	1.52	0.58

Each values represents the mean ± SE of three replication. In the same column, significant differences at P = 0.05 levels are indicated by different letter. Data followed by same superscript letter in the same column are not significantly different from each other

Table 3: Effect of cyanobacteria *Phormidium* sp. BDU-5 + coir waste on yield parameters of cowpea under pot culture conditions

Treatments	No of Flowers	No. of. fruits	No. of. Clusters	Fruits length (cm)
Control	4.71 ± 0.41d	5.27 ± 0.43e	4.37 ± 0.27e	11.39 ± 0.86c
Phormidium sp.BDU-5+CWCF 1%	11.19 ± 0.80a	19.95 ± 1.15a	11.71 ± 1.10b	15.51 ± 1.30a
Phormidium sp.BDU-5+CWCF 10%	7.52 ± 0.58b	9.7 ± 0.69cb	9.64 ± 0.66b	15.4 ± 1.24a
Phormidium sp.BDU-5+CWCF 15%	6.33 ± 0.42c	8.71± 0.70c	5.95 ± 0.61dc	14.28 ± 0.77b
Phormidium sp.BDU-5+CWCF 20%	5.23 ± 0.57de	10.42 ± 1.06b	6.86 ± 0.55c	14.85 ± 1.10ba
Phormidium sp.BDU-5+CWCF 30%	3.42 ± 0.28e	6.4 ± 0.35d	5.29 ± 0.23f	11.98 ± 1.23c
L.S.D	1.16	1.02	1.00	0.69

Each values represents the mean ± SE of three replication. In the same column, significant differences at P = 0.05 levels are indicated by different letter. Data followed by same superscript letter in the same column are not significantly different from each other

Table 4: Effect of cyanobacteria *Phormidium* sp. BDU-5 + coir waste culture filtrate on growth and yield parameters on cowpea under field conditions

Treatments	Plant height (cm)	Fresh weight (mg plant^{-1})	Biomass dry weight (mg plant^{-1})	No. of fruits / no plant^{-1}	No. of Nodule
Control	22.67 ± 1.54e	29.75 ± 5.05c	12.6 ± 1.50 bcd	5.3 ± 0.23e	5.74 ± 0.43c
Phormidium sp.BDU-5+CWCF 5%	66.85 ± 6.84a	37.95 ± 4.30a	15.93 ± 2.70a	9.7 ± 0.52a	10.42 ± 0.88a
Phormidium sp.BDU-5+CWCF 10%	51.74 ± 3.89b	34.35 ± 3.09b	13.09 ± 1.64bc	7.3 ± 0.23b	9.52 ± 0.42b
Phormidium sp.BDU-5+CWCF 15%	54.3 ± 4.21b	25.86 ± 3.67d	13.9 ± 1.96ba	6.6 ± 0.35c	9.45 ± 0.84b
Phormidium sp.BDU-5+CWCF 20%	42.75 ± 2.74c	25.02 ± 2.96d	11.36 ± 1.03cd	6.1 ± 0.35d	5.25 ± 0.20c
Phormidium sp.BDU 5+CWCF 30%	35.33 ± 3.08d	23.3 ± 1.91d	10.63 ± 0.80d	4.3 ± 0.23f	5.01 ± 0.28c
L.S.D	5.66	3.48	2.15	0.36	0.896

Each values represents the mean ± SE of three replication. In the same column, significant differences at P = 0.05 levels are indicated by different letter. Data followed by same superscript letter in the same column are not significantly different from each other

the field conditions (Table 4), the basal and foliar spray at 5% concentration of CWCE increased growth and yield parameters of cowpea. Plant height (66.85 cm /seedlings), fresh weight (37.97 mg g^{-1}), dry biomass (15.93 mg g^{-1}), number of fruits (9.7/plant^{+}) and number of nodule (10.42/plant^{-1}) were significantly increased at 5% concentration of CWCE when compared to other concentration and uninoculated control. The biochemical analysis treated plants at 5% concentration of CWCE was significant increased when compared to uninoculated control. The biochemical status of inoculated plants recorded highest amount of sugar (40.8%), phenol (39%), nitrate (27%), protein (228.5%), chlorophyll (107%) and carotenoid (20%) over uninoculated control plants or other treatments (data not shown).

DISCUSSION

Cyanobacteria are one of the potential organisms, which are useful to mankind in various ways vast potential resource in varied application such as mariculture, food, feed, fuel, fertilizer, medicine, industry and in combating pollution [19,20]. Degraded coir pith with cyanobacteria was used to promote plant growth and was found to enhance crop yield and especially soil productivity. The coir pith based cyanobacterial biofertilizer, plays a spectrum of remarkable roles in agriculture, especially in sustainable integrated agro ecosystems. Also, they add nutrients to soil, release growth promoting substances, increase soil organic content, improve soil structure and water holding capacity, reduce soil crusting problems, erosion from wind and water and improve buffering capacity against fluctuations in pH levels of soil. The release of micro and macro-nutrients from the cyanobacteria supports the plant growth and improves the quality and quantity of the crop yield.

The effect of coir waste with cyanobacteria culture aqueous extract with lower 5% concentration showed higher rates of seed germination, while as higher concentrations of extract inhibited the germination. he increased germination percentage it low concentrations may be due to presence of cyanobacteria is their capacity to synthesize and liberate bioactive substances such as auxins, gibberellins, cytokinins, vitamins, polypeptides, aminoacids, which promote plant growth and development[21].

Lignin, a key component of the stem and seed heads of plant material, is believed to be the main source of phenolic acids and quinones. These chemicals are released during the rotting process. A number of authors [22-24] stated that lignin degradation and the chemical release associated with this degradation, is the key component behind the inhibitory affects of barley on algae. Planas *et al.* [25] showed that phenols extracted from *Myriophyllum spicatum* inhibited growth of cyanobacteria and algae species *Anacystis nidulans* and *Selenastrum capricornutum*. Phenol besides being contaminants in water sources, are found in the environment in the form of lignocelluloses wastes which comprise of about 95% of the land biomass [26]. O hUallacháin *et al.*[27] Resent divergent results, e.g. with some studies resulting in inhibition associated with barley and other studies revealing that barley actually promoted growth of certain algal species. It was also found that the coir waste can be used as carrier for the blue green algae biofertilizer in view of its better attributes compared to soil as carrier and this proved to be the best way disposing the solid waste as it will be totally digested in the field [28].

The less investigated beneficial effects of cynanobacteria include of ammonia volatitization, suppressing weeds, reducing methane emission, transformation of P, Fe, Mn, Zn Cu, pesticide degradation and reclamation of wastelands /degraded soil [29-31].

The effect of basal and foliar application of coir waste based cyanobacterial biofertilizer. It was observed that 5% concentration of cyanobacterial with coir waste extract produce maximum percentage of the seed germination, seedling length, seedling fresh weight and dry weight when compared to control and other treatment significantly increased to the one present concentration. This could be due to the presence of growth promoting substance present in the cyanobacteria extract. But, higher concentration showed reduction in germination percentage and the same results obtained by number of studies [32, 33]. Morphological observation in control test plant of *B.ruba* demonstrated increase in stem circumference, branching, number of flowers, number of leaves and plant height over control [34].

Beneficial effects of cyanobacteria inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce [35]. The use of algae and cyanobacteria in waste treatment is beneficial in different ways since they can bring about oxygenation and mineralization, in addition to serving as food source for aquatic species. Studies at the NFMC have identified suitable cyanobacteria for treating a number of noxious effluents containing organophosphorus pesticides, detergents, antibiotics and other molecules [31], also for degradation of solid wastes like coir pith by their lignolytic action [23]. Cyanobacteria along with coir waste extract contain variety of organic substances like proteins, aminoacid, lpids and fatty acid and plant growth promoters. Kannaiyan *et al.* [12] showed that the combined use of fertilizer, nitrogen and inoculation of immobilized cyanobacterial gave the highest grain yield. Studies conducted by Venkataraman *et al.* [36] reported similar results with tomato yields, when coconut fiber substrate was compared to bark or rice husk. They also concluded that excess supply of nutrient solution is essential when coconut fiber substrate was used for the first time. Blom *et al.* [2] concluded with roses that during the first year coco coir produced about 15.6% more marketable stems as well as 18 % more fresh weight compared to granulated rockwool, while there were no significant differences between the substrates during the second year. In field experiments coir pith immobilized biomass of the cyanobacterium and its aqueous extract was tested on the growth of cow pea plants, It was found that the cow pea plants treated with 5 % aqueous extract showed maximum percentage of seed germination, length of shoot, root, number of leaves, flowers and seeds over other treatments and control. Addition of basal with foliar spray showed maximum growth and yield when compared to individual inoculation and uninoculated control plants. The reason behind this improvement may be due to action of plant growth regulators which cyanobacteria secretes such as vitamins, proteins, lipids and polysaccharides. This study revealed that cyanobacteria can act as bio-fertilizer and significantly involved to prevent the environment pollution and also helps the economically poor farmers and bring about the "green revolution"

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