



## Research Article

# ENHANCEMENT OF GERMINATION IN BHENDI SEEDS BY A NEWLY FORMULATED SEAWEED BASED LIQUID BIOINOCULANT

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**Abstract-** The GC-MS studies revealed the presence of antifungal bioactive compounds and antioxidant compounds especially phenols in the seaweed extracts of *Gracilaria cervicornis* and *G. gracilis*. Hence the extract of these two were utilized for the liquid bioinoculant production in combination with the 3 compatible Phosphate Solubilising Bacterial cultures (Pd 1, Pd3) and Zinc Solubilising Bacterial cultures (Zd1). As the microbial load of these bacterial isolates were not affected by the addition of seaweed extract upto 5% level, the final liquid bioinoculant was prepared by growing Pd 1, Pd3 and Zd1 cultures in liquid broth containing 5% seaweed extract. The influence of the prepared 'seaweed based liquid bioinoculant' on the germination of bhendi was observed for the bhendi crop (Hybrid: Green Gold Plus) by imposing 5 treatments and one control. The treatments imposed were T1 - *Azospirillum* + PB + KRB, T2- PSB + ZSB, T3- PSB + ZSB + 1 % Seaweed Extract, T4- PSB + ZSB + 3 % Seaweed Extract, T5- PSB + ZSB + 5 % Seaweed Extract and Control. T2 (78.9%) recorded a germination percent lesser than T1 (80.3%) which is the existing recommendation of bioinoculants. T3 (83.5%) and T4 (83.7) were statistically on par. Germination % observed in T5 was statistically significant than all other treatments. The % increase over control showed that influence of T2 treatment was lesser than the existing recommended bioinoculants treatment (T1). But the newly formed 'seaweed based liquid bioinoculant' at 1%, 3% and 5% increased the germination percent. T5 treatment recorded 15.27 % increase over control. When compared the % increase over T1 (existing bioinoculant recommendation) it was 6.23, 4.23 and 4.0 for T5, T4 and T3 respectively. This study proved that this newly formulated seaweed based liquid bioinoculant enhances germination of bhendi crop.

**Keywords-** Seaweed, Phosphate Solubilising Bacteria, Zinc Solubilising Bacteria, Liquid bioinoculant, Bhendi, Germination

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## Introduction

Marine environment is a major source of metabolites such as polyunsaturated fatty acids, lipids, sterols, carotenoids, proteins, polysaccharides, essential minerals, vitamins, antioxidants, enzymes, bioactive peptides and many other fine chemicals. Seaweeds are the source of major metabolites and are an extensive source of secondary metabolites. More than 600 secondary metabolites have been isolated from marine algae. Brown seaweeds are the second most abundant group comprising about 2000 species and are most used in agriculture. Brown algae like *A.nodosum*, *Fucus* spp., *Laminaria* spp., *Sargassum* spp., and *Turbinaria* spp. are used as biofertilizers in agriculture [1].

As sources of organic matter and fertilizers they are used as soil conditioners for centuries [2-4]. Studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress [5-7]. Study has been made to prepare seaweed based liquid bioinoculant with mineral solubilizing bacteria for improving the performance of vegetable crops.

## Materials and Methods

### Isolation and screening of mineral solubilizing bacteria

Bacterial cultures capable of solubilizing minerals especially phosphate and zinc were isolated from the soil samples of Farmland of Agricultural College and Research Institute, Kudumiyamalai using Katznelson & Bose agar and Aleksandrov agar plates respectively.

The diameter of clearing zones was measured in mm and the isolates with appreciable clearing zone around the colonies were screened for further study.

### Compatibility study for screened ZSB and PSB

Determination of the compatibility among the selected mineral solubilising bacteria was performed by modified cross-streak method (MCSM). Muller-Hinton agar plates were prepared and inoculated with ZSB cultures Zd1, Zd4 separately by a single streak in the centre of the Petri dish and incubated at 32°C for 3 days. This was done to provide enough time for the active organism to produce the antimicrobial substances if any, which will diffuse into the agar medium. The cross-streak method (CSM) was modified [8] by keeping the distance between the test streak fixed as 1 cm; length of the streak 7 cm; streak width, 0.5 cm; and length of tested PSB streak, 3 cm and width of 0.5 cm. The plates were seeded with test organisms by streaking perpendicular to the line of ZSB growth. The plates containing active organisms were kept for 3 more days to observe antagonism based on the inhibitory interaction between the ZSB and test strains of PSB.

### Development of seaweed based Liquid bioinoculant

The compatible PSB and ZSB cultures screened for compatibility were grown in liquid broth containing insoluble sources of both phosphate and zinc along with seaweed extract @ 1%, 3% and 5% levels separately.

The microbial load of both PSB and ZSB were enumerated after 7 days of incubation by drop plate technique. The compatibility of this newly prepared liquid bioinoculant was also tested with beneficial bioinoculants viz., Phosphobacteria (*B. megatherium*), Azotobacter (*A. chroococcum*), Azospirillum (*A. lipoferum*) and Rhizobium (*R. leguminosorum*) and KRB (*B. amyloliquefaciens*) by cross streak assay.

#### Influence of Seaweed based Liquid bioinoculant on germination of Bhendi seeds

The influence of the prepared 'seaweed based liquid bioinoculant' on the germination of bhendi was tested by performing germination test. The seeds were placed on the germination paper impregnated with the seaweed based liquid bioinoculants, kept in sterile petriplates. Germination percent was observed for the bhendi crop (Hybrid: Green Gold Plus) by imposing 5 treatments and one control in 4 replicates. The treatments included, T1 - Azospirillum + PB +KRB, T2- PSB + ZSB, T3- PSB + ZSB + 1 % Seaweed Extract, T4- PSB + ZSB + 3 % Seaweed Extract, T5- PSB + ZSB + 5 % Seaweed Extract and control.

#### Results and Discussion

##### Collection of Seaweeds from seashore of Manamelgudi village, Avudayar block of Pudukottai District

Seaweeds were collected from seashore of Manalmelkudi, Pudukkottai district (Latitude 10.0396°N, Longitude 79.2318°E). Totally 12 types of seaweeds were collected from 5 locations. Among the 12 seaweeds collected 3 were identified based on the morphological characters & cross section under microscope (Marine seaweed Manual, 2018) and confirmed by Botanical Survey of India, Coimbatore as *Gracilaria cervicornis*, *Gracilaria gracilis* and *Endocladia muricata*.

Table-1 Diameter of halo zones around the mineral solubilizing bacteria on agar plates

Isolate	Diameter of Halo zone (mm)
Phosphate Solubilising Bacteria (PSB)	
Pd1	19
Pd3	25
Pd5	8
Pd6	5
Zinc Solubilising Bacteria (ZSB)	
Zd1	15
Zd4	19

Table-2 Influence of 'Seaweed based Liquid bioinoculant' on germination of Bhendi seeds

Treatment	Germination %*	% increase over control (C)	% increase over existing recommendation of bioinoculants (T1)
C- Control	74.0 ± 1.82 <sup>a</sup>	-	-
T1 - Azospirillum + PB +KRB	80.3 ± 1.34 <sup>c</sup>	8.51	-
T2- PSB + ZSB	78.9 ± 1.1 <sup>cd</sup>	5.68	-2.62
T3- PSB + ZSB + 1 % Seaweed Extract	83.5 ± 2.01 <sup>b</sup>	12.84	4.00
T4- PSB + ZSB + 3 % Seaweed Extract	83.7 ± 1.92 <sup>b</sup>	13.11	4.23
T5- PSB + ZSB + 5 % Seaweed Extract	85.3 ± 2.08 <sup>a</sup>	15.27	6.23
LSD	1.62		

\*Mean (n=4) ± SE; Values followed by superscript letters within the column differ significantly

#### Screening of Mineral solubilising microbial isolates

Isolation of P and Zn mineralizing bacteria was done using the soil samples collected from farm of AC&RI, Kudumiyannalai. Totally 23 isolates of P solubilising and 17 isolates of Zn solubilising bacteria were isolated and morphologically characterized for shape, colour, colony characters and biochemically for Gram reaction, Catalase, Oxidase, IMViC, Starch hydrolysis, Urease activity and Nitrate Reductase activity. They were screened based on their P and Zn solubilisation efficiency [Table-1]. Four isolates of P solubilising bacteria

Pd1(19 mm), Pd3 (25mm), Pd5 (8 mm), Pd6 (5 mm), two isolates of Zn solubilising bacteria Zd1(15 mm), Zd4 (19 mm) were selected as they had appreciable amount of halo zone around their colonies.

#### Compatibility studies and development of Seaweed based Liquid bioinoculant

PSB cultures Pd 1, Pd3 and ZSB cultures Zd1 were compatible with each other. Hence these 3 isolates were used for preparation of Mineral solubilising microbial consortium. Since the GC-MS studies revealed the presence of antifungal bioactive compounds and antioxidant compounds especially phenols in *G. cervicornis* and *G. gracilis*, the extract of these two were utilized for the liquid bioinoculant production in combination with the 3 selected PSB (Pd 1, Pd3) and ZSB cultures (Zd1). As the microbial load of these bacterial isolates were not affected by the addition of seaweed extract upto 5% level, the final liquid bioinoculant was prepared by growing Pd 1, Pd3 and Zd1 cultures in liquid broth containing 5% seaweed extract. It was found that this newly prepared liquid bioinoculant was also compatible with other bioinoculants of general recommendation viz., Phosphobacteria (*B. megatherium*), Azotobacter (*A. chroococcum*), Azospirillum (*A. lipoferum*) and Rhizobium (*R. leguminosorum*) and KRB (*B. amyloliquefaciens*).



Fig-1 Seaweeds collected from seashore of Manalmelkudi, Pudukkottai district (Latitude 10.0396°N, Longitude 79.2318°E)

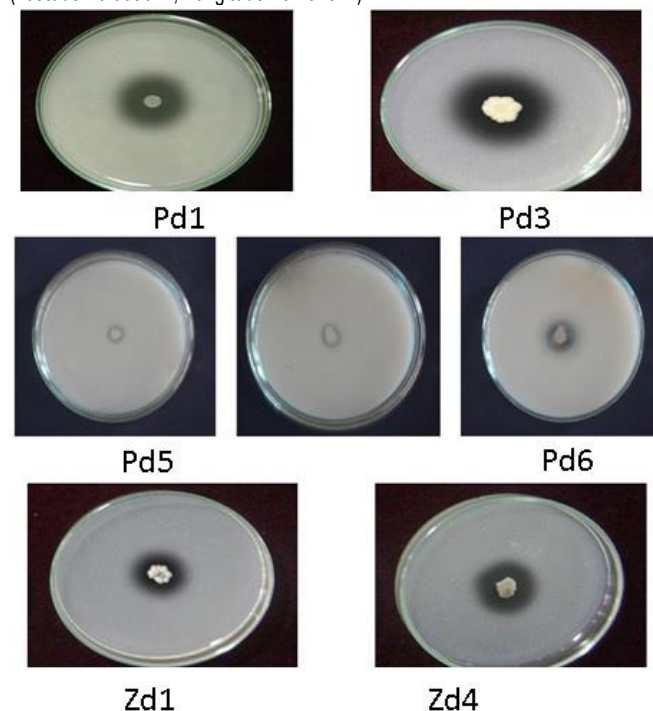


Fig-2 Screened Phosphate Solubilising Bacteria (PSB-Pd1, Pd3, Pd5, Pd6) and Zinc solubilizing Bacteria (ZSB-Zd1, Zd4) showing clearing zones in agar plates

### Influence of Seaweed based Liquid bioinoculant on germination of Bhendi seeds

Among the treatments, T2 (78.9%) recorded less than T1 (80.3%) which is the existing recommendation of bioinoculants. T3 (83.5%) and T4 (83.7) were statistically on par [Table-2]. Germination % observed in T5 was statistically significant than all other treatments. The % increase over control showed that influence of T2 treatment was less than the existing recommended bioinoculants treatment (T1). But the newly formed 'seaweed based liquid bioinoculant' at 1%, 3% and 5% increased the germination percent. T5 treatment recorded 15.27 % increase over control. When compared the % increase over T1 (existing bioinoculant recommendation) it was 6.23, 4.23 and 4.0 for T5, T4 and T3 respectively.

It has been proved that Seaweed components such as macro- and micro element nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA) like growth substances affect cellular metabolism in treated plants leading to enhanced growth and crop yield [9,10,11]. The synergistic activity of the various chemical components of seaweed extracts are appreciated even though their modes of action are yet to be explored [12].

### Conclusion

Seaweeds collected from Manamelgudi village, Avudayarkoil block of Pudukottai District were identified as *Gracilaria gracilis*, *Gracilaria cervicornis* showed for the presence of appreciable amounts of Phenols, Fatty Acids, Pentanoic acid, Phytol, Heptanoic acid, Ascorbic acid, Cholesterol, vitamin A, Vitamin D3 by GC-MS studies (Data not shown). The Liquid biofertilizer formulation containing PSB and ZSB cultures developed with 5% seaweed extracts of *Gracilaria gracilis* and *G. cervicornis* has increased the seed germination in Bhendi by 15.27 % than control and 6.23 % than existing bioinoculant recommendation.

**Application of research:** The presence of high amounts of phenols and other fatty acids impart the antifungal activity and antioxidant activity to this newly formulated seaweed based liquid bioinoculant. These compounds along with the PSB and ZSB have enhanced seed germination in bhendi seeds. Hence the newly formed 'seaweed based liquid bioinoculant' could be further explored for recommendation in vegetable crops.

**Research Category:** Agricultural Microbiology

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**Study area / Sample Collection:** Farmland of Agricultural College and Research Institute, Kudumiyamalai

**Cultivar / Variety / Breed name:** Nil

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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