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Arthospira platensis and *Bacillus subtilis* Synergies for Sustainable Agriculture

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study is to analyze the biofertilizer value of *B. subtilis* by estimating its phosphate solubilizing potential and its antagonistic activity against pathogenic strains of *E. coli* and *Bacillus cereus*. Prolonged use of chemical fertilizers have adverse effects on the soil and through food chain they cause hazards to organisms including humans. Moreover pests and pathogenic microbes are becoming immune to the chemical pesticides and herbicides. Hence the need of biofertilizers that are ecofriendly and good biocontrol agents are gaining importance. Use of biofertilizers leads us towards the path of sustainable agriculture yielding better productivity without compromising the fertility of agricultural fields. *Bacillus subtilis* is a PGPR (Plant growth promoting rhizobacteria) capable of phosphate and zinc solubilizing and it increases the bioavailability of nutrients to the plants. Due to its antagonistic nature, *B. subtilis* can be utilized in biocontrol management practices. *Arthospira platensis* is a well explored microalgal species that is rich in micronutrients and a wide array of amino acids that has immense biofertilizer value. The methodology involves UV spectrometric estimation of bioavailable phosphate in Pikovskaya's broth at 690nm and percentage of inhibition of *B. subtilis* against *E. coli* and *Bacillus cereus* using

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OD600values. Evidences for utilizing mixed culture of *A. platensis* and *B. subtilis* as biofertilizer are explored from literature. *B. subtilis* strain AMLA5 isolated from oil contaminated soil from Coimbatore had phosphate solubilizing potential of 1.3ppm and showed good antagonistic effects against *E. coli* and *B. cereus.* The NCBI genebank accession number of the strain SUB13179211 AMLA5 is OQ874086 that showed 99.77% sequence identical to *Bacillus subtilis.* This strain was a surfactin producer that utilized *A. platensis* as its economical substrate. *A. platensis & B. subtilis* are GRAS level organisms that can be utilized in agriculture as biofertilizer as mixed culture. The synergism existing between these two organisms can be exploited in multiple sectors

Keywords: Synergism; phosphate solubiliser; antagonism; biofertilizer; sustainability.

1. INTRODUCTION

"Role of *B. subtilis* as a biofertilizer increasing the bioavailability of phosphorous to plants and its biocidal nature are well known from the literature. *Bacillus subtilis SNW3*, isolated from Fimkessar oil field, Chakwal, Pakistan produced surfactin suitable for bioremediation of crude oil (86%) and as potent plant growth-promoting agent that significantly increased seed germination and plant growth promotion of chili pepper, lettuce, tomato and pea maximum at concentration of (0.7 g/100 mL). The optimum parameters for production were obtained using substrates in combination (white beans powder (6% w/v) plus waste frying oil (1.5% w/v) and (0.1% w/v) urea) with surfactin production of about 1.17 g/L contributing 99% reduction in cost required for medium preparation. From this literature it is clear that *B. subtilis* can be exploited in both agricultural sector as PGPR and in bioremediation sector as degradation of crude oil, hydrocarbons and toxic pollutants" [1]**. "***Bacillus subtilis* when used with Phosphorus fertilizer gave better response compared to when used individually. In case of wheat, it increases the plant height by 8%, and number of branches by 1.74%" [2]. "Phosphate solubilising microbes (PSM) were isolated from the rhizosphere of *Piper nigrum. L* plants grown in the Western Ghats of Karnataka. The potential microorganisms were chosen and identified as *Bacillus subtilis* and fungus *Aspergillus* in 16S rDNA sequencing analysis. The phosphatase activity of the isolates were found to be (5.33 U mL-1) and (11.5 U mL-1) respectively" [3]. "The potential isolate MRB4 identified as Bacillus subtilis by 16S rRNA sequencing was isolated from the agricultural fields of Mahbubnagar, district of Telangana State, India. It solubilized tri calcium phosphate under various abiotic stress such as temperature 45°C (193 µg/ml), pH 9 (181 µg/ml), salt 8% (124 µg/ml), and Drought −0.49 matric potential (90 µg/ml). Isolate MRB4, produced highest concentration of indole acetic acid i.e. 65 micro gram (µg)/ml, showed positive results for ammonia, hydrogen cyanide, cellulase, protease and exhibited promising antifungal activity" [4].

"Role of *A. platensis* as biofertilizer can be viewed in many aspects. *A.platensis* is a blue green algae(BGA) rich in micro and macro nutrients and capable of Nitrogen(N) fixing. The *A. platensis* lyophilized biomass is rich in free amino acid content that is related to the bioactivity found at bioassays like chlorophyll synthesis and amino acid and protein increments on seedlings leaves. The growth promotion and metabolic changes found on seedlings of red beet hypocotyls and consequent yield gains are mainly due to surplus protein content of *A.platensis"* [5]. "Hence *A.platensis* can be utilized as an efficient biofertiliser. The results above show that the application of spirulina (*A. platensis*) foliar spray has a positive effect on the growth performance and biochemical properties of lettuce (L. sativa) grown in an aquaponic system. Application of 8g/lit of *A. platensis* increased leaf length, width, and leaf area and number of leaves of the plant. Supplying 12g/lit spirulina in water once in a week, increased the dry weight of the plant and total phenols and antioxidants of *L. sativa"* [6]**. "**Obtained results proved that spirulina extracts have high potential to be applied in modern agriculture. The use of *Spirulina*-based products is consistent with the idea of sustainable agriculture that could help to ensure production of sufficient human food to meet the needs of rising population and protection of the environment by the following techniques. The filtrates obtained from *A. platensis* applied as foliar spray, seed coat and seed soaking increased the length of radhish plant compared to control. The chlorophyll content and micro and macro nutrients content reached maximum value. Thus spirulina biofertiliser results in production of biofortified vegetables beneficial to human health eradicating micro hunger or malnutrition" [7]**. "**To

reduce the usage of harmful chemical fertilizers algal bio fertilizers can be used as an alternative source. Chlorella vulgaris and *A. platensis* extracts are treated with plant green gram *Vigna radiate*. The maximum number of fresh weights of root nodules, plant heights, plant branches, plant leaves, and leaf area index were recorded as 100% with treatment I of *Chlorella vulgaris* and treatment II of *Spirulina platensis* in treated plants. The leaf chemical composition such as N, P, and K, total carbohydrates, Indole, and phenol evidenced with good results in plants treated with *Chlorella vulgaris* and *Spirulina platensis.* Growth parameters like root length, shoot length, weight of green gram were high at the flowering stage" [8]. **"**The possibility of replacing 40 to 90 % of chemical N fertilizers Florida prince peach orchards by using humic acid at 40 to 90 ml/ tree/ year and the biofertilizer *Spirulina platensis* algae at 5 to 30 ml/ tree/ year was investigated during 2010 and 2011 season. Results reveal that decreasing percentages of inorganic N from 100 to 50 % out of the suitable N and at the same time increasing levels of humic acid from 40 to 80 ml/ tree/ year and *S. platensis* algae biofertilizer from 5 to 25 ml/ tree/ year resulted in major promotion on the leaf area and its content of N, P and K, yield and fruit quality For promoting yield of Florida prince peach trees as well as replacing of 50 % inorganic N, it is advised to fertilize the trees with the suitable N (500 g N/ tree/ year) via 50 % inorganic N source + humic acid at 80 ml/ tree/ year + *S.platensis* algae biofertilizer at 25 ml/ tree/ year" [9]**. "***S. platensis* is used as a biofortification agent to enhance zinc levels in cultivars of *Amaranthus gangeticus, Phaseolus aureus* and Tomato. Different methods like soaking seeds in different concentrations of Spirulina (5, 10, 15, 20, 25 and 30 g in 100 ml of water); soaking seeds in Spirulina hydrolysate at different time intervals (1, 2, 3, 4, 5 hrs and overnight); and foliar spray with different concentrations of Spirulina (25, 50, 75, and 100g in 5 litres of water) & Spirulina in combination with biofertilizers, chemical fertilizer, organic fertilizer and vermicompost in various proportions (25:75; 50:50; 75:25) were applied and the results were found positive" [10]**. "***S.platensis* liquid extract (2%) was used in *Triticum aestivum* and treated with sea water10% and 25%. The general characteristics of a good biofertiliser are not only increasing the bioavailabilty of nutrients but also increasing the stress tolerance of plants. *S. platensis* liquid extract (SLE) not only enhanced the phytochemical contents and growth of *T. aestivum* L. but also increased the tolerance of *T. aestivum* L. against salinity" [11]. "The microalgae *S.platensis* and *Chlorella vulgaris* were mixed with cow dung and applied to onion plants. The total soluble sugars, total phenols free amino acids and total indoles were observed to be maximum in treated plants and the anti-nutritional factors of onion and mineral analysis of onion were observed in high quantities and maximum growth were observed in algal biofertilizer treated onion plants" [12]**. "**In Okra plant,pod length, pod breadth, pod weight, yield/hectare increased on applying Oscillatoria and spirulina as biofertiliser. The yield per hectare increased significantly when compared to the control. Hence the use of spirulina and oscillatoria are recommended for organic crop production" [13]. "The research was undertaken to investigate the influence of soil drench application method of marine microalgae (*Chlorella vulgaris, Spirulina platensis*) in rice plant in clay loamy soil. The results showed increase in rice yield up to 7–20.9% .The mechanism involved is nitrogen fixing by the microalgae. These microalgal species can be used as ecofriendly biofertiliser thus minimizing the use of chemical nitrogen fertilisers" [14].

From the above literature we can come to the conclusion that *A.platensis* which is a blue green algae can improve plant health when added in required concentrations. There are various methods of applying like liquid culture by spraying in plant parts and rhizosphere; In seed coats and by soaking seeds in spirulina liquid culture for required time and then allowing it to germinate; *A. platensis* improves health in vegetable crops, pulses, fruit trees like peach, okra plant. It supplies micro and macro nutrients and acts as a source of nitrogen as it is rich in aminoacids and proteins. It is applied as single culture or as mixed culture with *Chlorella vulgaris*, *Azospirillum* and cow dung. Hence it is clear that the combination of *A.platensis* and *B.subtilis* can serve agricultural fields in an efficient manner as mixed biofertilizer.

2. OBJECTIVES

- To analyze *B .subtilis* as an efficient phosphate solubilizer and biocontrol agent using UVspectrometric assays.
- To obtain molecular identification of the most potential phosphate solubilizing *B. subtilis* strain AMLA5 through 16s rRNA sequencing.
- To analyze the biofertilizer value of *A.platensis,* a BGA fertilser and to study its efficiency through literature.

- To analyze the evidences for *A.platensis- B .subtilis* synergetic effects in sustainable agriculture.

3. METHODOLOGY

- Soil sample collection from oil contaminated sites of Coimbatore.
- Isolation and identification of five strains of *B.subtilis* in Luria Bertani agar and Bacillus differentiation agar.
- The strains that showed highest clearance zone in Pikovskaya's agar was selected for molecular identification using 16S rRNA sequencing.
- Quantification of bioavailable phosphate in Pikovskaya's broth in UV spectrometric assay using ammonium molybdate method.
- The antagonistic activity of *B.subtilis* was tested against *E.coli MTCC50* and *B.cereus* strain to test its biocidal nature.

4. *B.subtilis* AS THE POTENTIAL PHOSPHATE SOLUBILISER

Out of the five strains isolated from pure culture obtained from soil samples collected from Rathinapuri,Coimbatore, isolate AMLA5 was found to be potential phosphate solubilizer based upon the clearance zone in Pikovskaya's agar. The bioavailable phosphate was calculated in UV spectrometer at 690nm in ammonium molybdate method.

Composition of Pikovskaya's agar (For 1000ml):

Glucose-10g
Calcium tri phosphate-5g
Ammonium sulphate-0.5g
Potassium chloride-0.2g
Magnesium sulphate-0.1g
Manganese sulphate-trace(0.0001)
Ferrous sulphate-trace(0.0001)
Yeast extract-0.5g
Distilled water-1000ml
Agar-15g

Qualitative method: All the suspected colonies were screened for phosphate solubilization on pikovskayas medium. Isolates showing phosphate solubilizing ability were spot inoculated at the centre of pikovskaya's plate and incubated at 37 ^{0}C.Diameter of clearance zone was measured successively after 24 hours, upto 7 days. The phosphate solubilization efficiency (PSE) is the ratio of total diameter. i.e. clearance zone including bacterial growth(Z)and the colony diameter ,(C), multiplied by 100 [15]**.**

$$\mathrm{PSE} = \frac{\mathrm{Z} \times 100}{\mathrm{C}}$$

Solubilizing Efficiency (SE) was calculated by the following formula:

$$\mathrm{SE} = \frac{\text{Solubilization diameter} \times 100}{\text{Growth diameter}}$$

After incubation, growth diameter and solubilization diameter were measured and SE was calculated and it was found to be 164 [16].

Quantitative method: 100 ml of pikovskaya's broth medium with 250mg of Tricalcium phosphate was prepared and sterilized,1ml of each isolates was inoculated into the broth medium. Then the inoculated sample were incubated for 14 days on rotatory shaker 370C,after incubation culture broth was centrifuged at 10,000 rpm for 30min,pH of all the isolates were measured.

Phosphate estimation-The amount of phosphorus present in the isolates was determined by Indian standard methods. 10ml of sample was taken in 2 test tubes and volume was made upto 25ml with distilled water.1ml of Ammonium molybdate was added to the sample. Three drops of stannous chloride was added to the tubes and vortexed. The blue color intensity was read out within 10 min at 690nm.Concentration of phosphorus in the sample was calculated [ISI standard estimation methods].

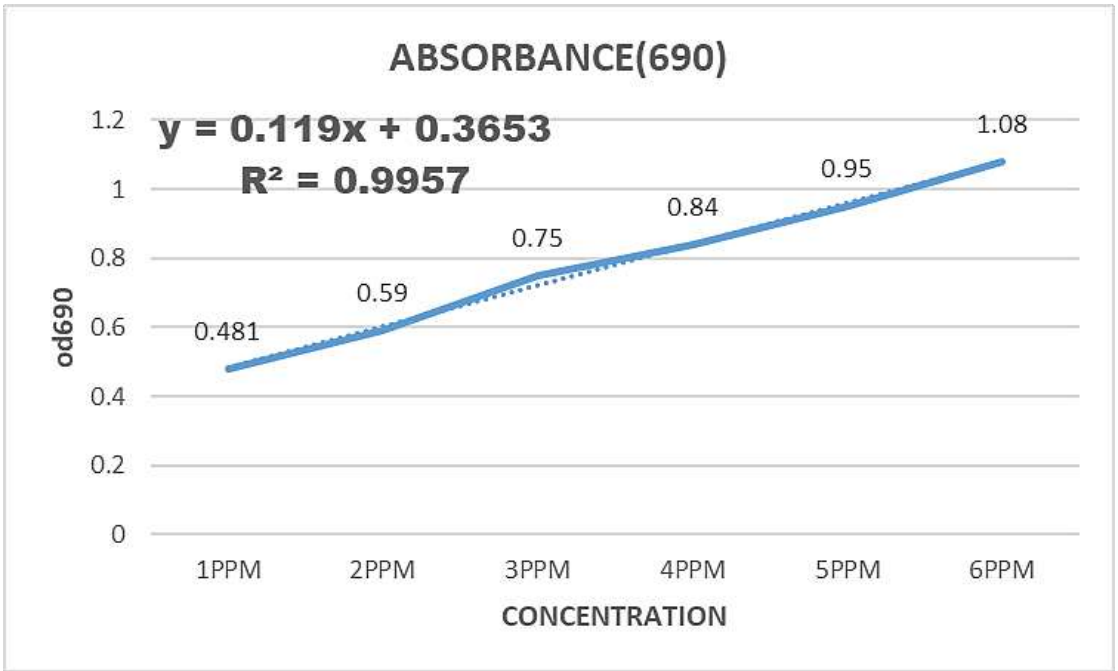
Table 1. Phosphate standard values

Concentration	Absorbance (690)
1PPM	0.481
2PPM	0.59
3PPM	0.75
4PPM	0.84
5PPM	0.95
6PPM	1.08

Table 2. Phosphate sample values

Strain no	Value1	Value2	Value3	Std dev	Mean	Conc(ppm)
S1	0.452	0.422	0.46	0.020033	0.444	0.73
S2	0.411	0.433	0.457	0.023007	0.434	0.577
S3	0.401	0.422	0.433	0.016258	0.419	0.451
S4	0.444	0.458	0.486	0.021385	0.462	0.812
S5	**0.52**	**0.535**	**0.512**	**0.011676**	**0.522**	**1.3**

Fig. 1. Pikovskaya's agar plating of *B.subtilis*



Graph 1. Showing R^2 value

5. RESULTS

5.1 Result- 1

Phoshphate solubilisation by *Bacillus subtilis* strain was found to be 0.649mg/Lit in the study [17]. "Among the 8 potent isolates, 3 strains showed maximum PSI of psm1, psm2 and psm6 in agar plates along with high soluble phosphate production of 0.37 mgL -1, 0.30 mgL -1 and 0.28 mgL -1 in broth culture. These isolates psm1, psm2 and psm6 belong to genus *Pseudomonas, Bacillus* and *Rhizobium* as identified by their morphological and biochemical properties respectively" [18]**.**

5.1.1 The results obtained in the study show that isolate *B.subtilis* AMLA5 solubilizes phosphate efficiently to an extent of 1.3ppm

5.1.1.1 Biocidal Nature of B.subtilis

Agar well diffusion method for Antibacterial activity

Antibacterial activity of *Bacillus subtilis* strains was determined by agar well diffusion method according to Bergey's manual. Inoculum containing pathogenic bacterial culture to be tested was spread on nutrient agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 8 mm diameter were punched into the agar medium and filled with 100 µl of *B**acillus subtilis*** strains and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37° for 24 h. After incubation, the diameters of the growth inhibition zones were measured in mm.

Antibacterial activity can be tested by OD 600 values in UV spectrometer by comparing culture growth of indicator strains (10ml) taken as control in three sterile test tubes and another set of three test tubes with 10ml control along with 1 ml test organism *Bacillus subtilis* isolated from oil contaminated soil samples. The indicator organisms were *Escherichia coli MTCC50* forming pink colonies in Macconkey agar and *Bacillus cereus* isolated from soil sample through bacillus cereus agar forming blue colonies.

Five strains were used in the study. From the zone of inhibition, isolate AMLA5 was found to be more efficient and the results are tabulated.

Table 3. Inhibition against *E.coli & B.cereus*

E.coli	**Triplicates**	
Name	OD600(24hrs)	OD600(48hrs)
control1	1.02	1.48
control2	1.22	1.52
control3	1.18	1.44
test1		0.622
test2		0.636
test3		0.629
Bacillus cereus		
Name	OD600(24hrs)	OD600(48hrs)
control1*	1.56	1.788
control2*	1.444	1.755
control3*	1.428	1.728
test1*		0.722
test2*		0.734
test3*		0.728

Fig 2. *E. coli* zone of inhibition

Fig. 3. B. c*ereus* zone of inhibition

The activity of inhibition was calculated using the formula:

$$\% \text{ of inhibition} = \frac{\text{Growth control OD} - \text{Treatment OD} \times 100}{\text{Growth control OD}}$$

For E.coli % of inhibition was found to be 58%,58.1%,56.3% and the mean value was **57.5%**

For *Bacillus cereus*,% of inhibition was found to be 59.6%,58.1%,57.8% and the mean value was found to be **58.5**%.

5.2 Result-2

5.2.1 (*B.subtilis* as a PGPR)

The results obtained in the study show that isolate *B.subtilis* AMLA5 solubilizes phosphate efficiently to an extent of 1.3ppm and its biocidal nature against pathogenic *E.coli* was found to be 57.5% and against *B.cereus* was found to be 58.5%. These two properties of this strain, increasing the bioavailability of phosphate to plants from soil and antagonistic against harmful bacteria and fungi make it suitable for its application as a PGPR. There are lots of literature available proving the phosphate solubilizing nature of *B.subtilis* as well as *B.subtilis* as a biocontrol agent improving crop growth.

"124 samples were collected from the intestine of broiler chickens, piglet faeces, fermented foods, soils and Chinese herbs. The six bacilli with the largest inhibition zones against the four indicator bacteria *Escherichia coli* K88, *E. coli* K99, *Salmonella typhimurium* and *Staphylococcus aureus* were chosen. The strain *Bacillus subtilis* MA139 showed full resistance and exhibited the highest antimicrobial activity. Based on these results, *B. subtilis* MA139 was selected as a potential probiotic and fed to piglets" [19]**.**

"An effective substance was isolated from *Bacillus subtilis* SC-8, which was obtained from fermented soybean paste, *cheonggukjang*. The substance was purified by HPLC, and its properties were analyzed. It had a strong antagonistic effect on *Bacillus cereus*, and its spectrum of activity was narrow. Spores of *B. cereus* did not grow at all in the presence of 5 µg/mL of the purified antagonistic substance which was a lipo peptide" [20]. The phosphate solubilizing activity and biocidal nature of *B.subtilis* upgrades its value as a PGPR and its GRAS level position makes it important as a biofertiliser.

6. MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS

After screening, the potential biosurfactant producing isolate ***Bacillus subtilis* AMLA5** that was an efficient phosphate solubilizer was selected for molecular identification using 16S

rDNA gene sequencing homology. The pure bacterial culture was sent for commercial amplification and 16S rDNA gene sequencing to the company (YAAZH XENOMICS, COIMBATORE). Obtained nucleotide sequences were subjected to Basic Local Alignment Search Tool (BLAST) analysis against closely associated taxa available in the GenBank sequence database of National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). Phylogenetic analysis was conducted using MEGA X. The obtained 16S rRNA gene sequences of the present study were submitted to NCBI GeneBank for accession number. NCBI GeneBank for accession number for SUB13179211 AMLA5 is OQ874086 that showed 99.77% sequence identical to *Bacillus subtilis* .

7. Evidences FROM LITERATURE FOR SYNERGISM BETWEEN *A. platensis* & *B. subtilis*

Some literature state that *A.platensis* has antagonistic properties against *B.subtilis*. This antagonistic property seem to be strain specific. The following literatures provide various circumstances using *A.platensis* and *B.subtilis* together in their study.

This study used *Bacillus subtilis* G 2 for lipase production under optimized parameters from agro industrial residues. “Among the seven nitrogen sources examined for cellulase production from *B. subtilis G2,* spirulina powder (2.276 IU/ml) and cotton seed cake (2.228 IU/ml) enhanced cellulase production by 33.09 and 30.29 %, respectively, as compared to control” [21].

In this study, the mixed fermentation of *Spirulina platensis a food supplement* with *Lactobacillus plantarum* and *Bacillus subtilis* was investigated using random-centroid optimization. “Fermentation with *B. subtilis* and *L. plantarum* effectively improved the odor and protein availability of *Spirulina*. Fermented *Spirulina* with the maximum total viable counts of both organisms achieved best sensory characteristics and degree of proteolysis. The mixed fermentation noticeably reduced the volatile compounds of *Spirulina*, and yielded the highest relative contents of acetoin and other odorants collectively producing a creamy aroma. Approximately one-third of the *Spirulina* proteins were hydrolyzed, yielding over 16% polypeptides and increasing the ratio of essential amino acids to total free amino acids to 1.5-fold compared with unfermented *Spirulina***”** [22]**.**

A.platensis and panchagavya as mixed biofertilizer

The synergistic effects of employing Plant Growth Promoters *S. platensis* and organic fertilizer (Panchagavya)] in a waste land soil bag containing *Vigna radiata* seeds was studied. The application of consortium - biofertilizer is recommendable to boost the productivity of mungbean in alkaline waste soils of Cheyyar. The productivity of mungbean almost doubled [23]. There are various literature showing evidence for presence of *B.subtilis* as an important segment of the microbial flora in panchagavya [24,25,26].

This study speaks about the probiotic value of *A.platensis* extracts and *Bacillus subtilis* PB6(BSPB) in broiler chicken. “Individual use of *Spirulina* and BSPB improved bodyweight gain and feed conversion ratio of broiler chickens. Co-supplementation with 0.1% *Spirulina* and BSPB increased VH and the VH : crypt depth ratio in the duodenum compared with diets supplemented, with 0.1% *Spirulina* and BSPB alone. The results show that supplementation with *Spirulina* and BSPB had a positive effect on performance and carcass quality of broiler chickens. The synergistic interaction between these supplements leads to enhancement of epithelial morphology in the small intestine. Hence this combo can be recommended for dietary additives” [27]. **The synergism existing between these two organisms can be exploited in multiple sectors.**

8. *A. platensis- B. subtilis* MIXED CULTURE AS BIOFERTILIZER

The synergistic effects of employing Plant Growth Promoters *S. platensis* and organic fertilizer (Panchagavya)] in a waste land soil bag containing *Vigna radiata* seeds was studied. The application of consortium - biofertilizer is recommendable to boost the productivity of mungbean in alkaline waste soils of Cheyyar. The productivity of mungbean almost doubled.

Group A - Preparation of microalgal extract [23]

Dried biomass of Spirulina platensis obtained from SANAT industry, Kodai road. Homogenate was prepared by a suspension of dry Spirulina platensis in deionized water and mixing at 37 ◦C

for 40 min. The obtained solution was centrifuged for 20 min. (4600 rpm). The supernatant was separated and treated as an algal filtrate 25 gram mixed with water at 100ml of water concentration, w/v.

Group B - Panchagavya

Requirements of ingredients added for the preparation of Panchagavya
Fresh cow's dung – 3kg
Fresh cow's urine – 5L
Cow's milk – 1L Cow's curd – 1L
Cow's ghee – 500mg
Tender coconut water – 2L
Sugarcane juice – 1L
Well Ripe banana fruit – 5
Water – 5L

Mode of Preparation Panchagavya liquid fertilizer was prepared according to the method of Pal and Patel et. [28] After 30 days, the panchagavya was filtered properly sieving through a fine cloth. The filtrates were kept as 100% stock of panchagavya and 3% solution was prepared with mixing of appropriate distilled water and used for further treatment.

Group C - Preparation of Consortium

Equal proportion of the biofertilizers in group A and B were mixed to form consortium [1:1]

The authors isolated beneficial microbes from panchakavya that were confirmed as *Bacillus* sp., *Pseudomonas* sp., *Lactobacillus* sp. and *Azotobacter* sp. and the fungal isolates were confirmed as *Aspergillus* sp. and *Saccharomyces* sp. (yeast). The enzymes released by bacteria convert the complex insoluble organic compounds to simpler soluble form. Thus they are helpful for plants to uptake nutrients through the mechanism of enzymes [24]. This study revealed the potential of the microbes isolated from cow based manures for nitrogen fixing, IAA production, P, K, Zn solubilisation. Isolated microbes showed inhibition properties against C. *gloeosporioides, C. fimbriota, P. aphanidermatum* and *F. oxysporum*. Therefore, these formulations can be effectively used for nutrient and insect pest management in organic production of food crops [25]. *Bacillus subtilis* (BD2) was one among the seven isolates obtained from panchakavya and cow pit manure samples. CPP manure contained the highest bacterial load (4.8×10^6 cfu g^{-1}) and *Bacillus subtilis* was predominant in CPP manure which can be exploited in industrial production of biofertilizer and bio-control agents [26].

9. CONCLUSION

From the above literature we can see that *S.platensis* extracts and panchakavya biomanure that is rich in *Bacillus* sp., *Pseudomonas* sp., *Lactobacillus* sp. and *Azotobacter* sp. and the fungal isolates *Aspergillus* sp. and *Saccharomyces* sp. (yeast) are used as biofertiliser combo in agricultural fields. Hence isolate *B.subtilis* AMLA5 can be used as biofertiliser combo together with *A.platensis* extract that can be prepared in industrial scale and marketed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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