Efficacy of agro-industry by-products as biocarriers: A green strategy for PGPR bioformulations

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Abstract- The current study is focused and designed to formulate a suitable carrier for PGPR bioformulations based on the strategy theme i.e., a product of land for the land with minimum environmental disturbance. On the basis of physiochemical properties, nutrient composition and cost-effectiveness, two agro-industry by-products i.e., wheat bran and rice husk were selected to be tested as a potential biocarrier for PGPR bioformulation preparations. For bioformulations preparation, 100g of sterilized materials were separately inoculated with 15ml (106 cfu/ml) of four PGPR isolates i.e., *Achromobacter* sp. (P1), *Bacillus* sp. (P2), *Enterobacter* sp. (P3) and *Pseudomonas* sp. (P4) to prepare wheat bran based bioformulations (WB1, WB2, WB3 and WB4) and rice husk based bioformulations (RH1, RH2, RH3 and RH4) separately. A plant-microbe interaction assay was conducted with Zea mays as test crop under three different treatments i.e, T1, T2 and T3 to observe the efficiency of these PGPR bioformulations based upon the survivability of bio-inoculants. Significant increments upto 150% in shoot length, number of leaves, fresh weight, protein content and total chlorophyll content and root architectural modifications of treated plants especially with treatment T2 illustrated wheat bran and rice husk as potential cost-effective and viable biocarriers for eco-friendly PGPR bioformulations. These agricultural by-products can be efficiently utilized as environment friendly and low-cost organic carriers for bioformulation preparations which enables the applications of PGPR biofertilizers a success.

Index Terms- *Achromobacter* sp., *Bacillus* sp., Bioformulations, *Enterobacter* sp., *Pseudomonas* sp., PGPR, Rice husk, Wheat bran

I. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR), a term coined by Klopper and Schroth, in 1980's are only about 2–5% of rhizospheric bacteria [1]. This diverse list of genera includes, *Achromobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas and Serratia* etc as PGPR, which can be used as single strain or in consortia. PGPR should be eco-friendly, rapid colonization capability, compatible with other rhizobia, abiotic stress tolerant (heat, desiccation, radiations, and oxidants) and have diverse-action spectrum [2]. Plant growth promoting rhizobacteria (PGPR) are the heterogeneous group of beneficial bacteria that stimulate plant growth through various mechanisms. PGPR function as biofertilizers, phytostimulators, biopesticides and rhizoremediators [3], [4]. A wide range of secondary metabolites are secreted by PGPR i.e., siderophores, antibiotics, VOCs, HCN, several hydrolytic enzymes (cellulases, proteinases and chitinases), defense proteins, mobilization and solubilization of nutrients to mitigate stress conditions to ensure growth enhancement [1]. PGPR enhance crop yield and growth by two basic process (a) direct mechanisms i.e., nitrogen fixation, phosphate, zinc and potassium solubilization, nutrient mobilization and by the production of siderophores (Fe uptake and accumulation), phytohormones (indole acetic acid, gibberellins, ethylene, abscisic acid and cytokinins) organic acids, volatile organic compounds and signaling molecules, (b) indirect mechanisms i.e., production of several hydrolytic enzymes (cellulases, proteinases and chitinases), secondary metabolites, HCN and defense proteins, alongwith biocontrol of pests and bioremediation[5], [6], [7], [8], [9]. PGPR enhance plant growth and yield through various mechanisms like nitrogen fixation, phytohormone production, phosphate solubilization, siderophore production, zinc solubilization and disease control [10], [11]. Application of PGPR formulations either on seeds or soil improve seed germination, seedling vigor, shoot and root growth, plant's biomass, grains, seeds weight and fruits yield via various mechanisms including nitrogen fixation, phosphate solubilization etc [12].

The term bioformulation is defined as preparation of bacterial isolates using suitable carrier material to provide optimum micro-environment for mass multiplication and survival of inoculants. The carrier material helps to retain beneficial bacteria in addition to commercial facilitation of the product. There are two types of bioformulations i.e., solid and liquid bioformulations [13], [14]. In sustainable agricultural practices of green revolution era, PGPR are applied to plants under field condition with suitable carrier to effectively deliver them to soil, which effectively reduce dependence upon expensive chemicals fertilizers. An ideal bioformulation should have prolong viability, water-soluble, non-phytotoxic nature, disease-resistance, cost-effectiveness and eco-friendly [15]. Carriers enhance bacterial survivability during unfavorable conditions. A number of carrier varieties are reported on the base of physical form and properties i.e., compost, charcoal, saw-dust, peat, turf, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, press mud, talc and vermiculite [2]. For bioinoculants based-bioformulations, strain and carrier selection, storage condition, environmental competition, quality control and application method are the influencing methods for their efficacy [6], [15].

From the numerous carriers available in the market, the choice of most appropriate one is major concern regarding the shelf-life and effective delivery of bioformulations to the fields. The appropriate selection of carrier for PGPR directly concerned with cost, biosafety, release rate, shelf-life of product, while essential nutrients and organic contents of carrier relates with the compatibility and survivability of microbes during marketing [6], [7]. To ensure PGPR stability during preparation, distribution and storage of bioformulations, suitable micro-environment

should be prepared by mixing PGPR with carrier [16], [17]. Due to the fluctuations in the demand and price of biofertilizers, by-products of agro-industry wheat bran and rice husk, were explored as alternative carrier for cost-effective and eco-friendly microbial bioformulations preparations. So, the current study focused on agriculture-based carriers i.e., wheat bran and rice husk due to their low cost, bulk availability, stability during storage and non-toxic nature for plants [15], [18].

The major challenges of bioformulations commercialization are the strain stability, standardization issues, cost competiveness and consumers acceptance. The quality control during formulations' preparations, inappropriate storage and application methods and multiple environmental factors i.e., temperature, pH, nutrient content disturbs the survivability and effectiveness of products. The current bioformulations market of >2.5 billion USD, which is growing exponentially with an estimate of 10-15% increase till 2026 [7], [8].

From the cereal crops i.e., wheat, rice, maize, barely and sorghum, numerous PGPR are reported to enhance maize production especially PGPR of genus *Bacillus*, *Pseudomonas* and *Enterobacter* [1]. Due to its ample concentration of starch (72%), maize serves as livestock fodder as well as staple food of almost 4.5 billion people including Pakistan. It is third most cultivated crop besides wheat and rice in all provinces of Pakistan. Maize production stabilize Pakistan's economy with the annual production of more than 5 million tons and 2,864 kg/ha of average yield of grain, in 12-15% of total cultivated area and 30-35% of total annual productivity [19], [20]. Moreover, maize cultivation requires ample nutrients like nitrates and ammonia and the PGPR present in bioformulations convert atmospheric nitrogen into organic and in mineral nitrogen for plants [21], [22].

In the current experimental study, four already isolated and identified PGPR strains i.e., *Achromobacter* sp. (HS4), *Bacillus* sp. (AB8), *Enterobacter* sp. (A7B) and *Pseudomonas* sp. (AH2) were used as bio-inoculants in by-product of agro-industry i.e., wheat bran and rice husk as carrier, for microbial bioformulations' preparations. Due to economic instability, consistent climatic changes, extreme use of chemical fertilizers, increased food demand, biosafety of consumers health required the use of cost-effective and eco-friendly techniques for sustainable agriculture practices. The basic aim of this study is to evaluate the agro-organic carriers for bio-inoculants based bioformulations preparations, their shelf-life and ultimate growth promotion effects of PGPR on plants. For this purpose, an experiment on Plant-Microbe interaction was performed by using bioformulations in different concentration treatments with Zea mays L. as test crop under laboratory conditions.

II. METHODOLOGY

2.1 PGPR selection

In the preparation of bioformulation, selection of appropriate microbes is essential step, as it is our ultimate source for plant growth promotion. For current experimental work, four already isolated and characterized indigenous PGPR strains i.e., *Achromobacter* sp. (HS4), *Bacillus* sp. (AB8), *Enterobacter* sp. (A7B) and *Pseudomonas* sp. (AH2) by [23] were collected from Microbiology Research Laboratory, Institute of Botany, University of the Punjab, Lahore, Pakistan. All these strains were grown on L-Agar and L-Broth medium [24] and incubate at 37±2oC before further usage.

2.2 Carriers' selection & physiochemical properties

In this study, two agro-industry byproducts were selected as an organic carrier due to their cost-effective and eco-friendly nature i.e., wheat bran and rice husk. The nature of these materials was evaluated as an effective and safe carrier of PGPR for bioformulations by measuring several physiochemical properties by following Pandey and Maheshwari [25], which are enlisted below.

pH

To evaluate the nature of carriers, a paste was prepared separately by using autoclaved distilled water and pH was determined by using pH meter.

Water holding capacity

In 100g oven dried wheat bran and rice husk, water was added until saturation followed by water drainage for overnight with the sieve of 0.25m, to measure the percent water holding capacity with leftover mass of materials.

Inherent moisture content

About 10g of wheat bran and rice husk were placed individually in drying oven for 24 hours 70oC to get the endpoint of moisture loss. The inherent moisture content was calculated by using the following formula:

M= [(m1-m2)/m2] *100

Where, M=moisture content, m1=mass of material before drying and m2=mass of material after drying.

2.3 Bioformulation preparations and storage

For bioformulations preparation, 100 grams of air-dried, grounded and sterilized carriers i.e, wheat bran and rice husk were packed in separate polythene bags with 65-75% vacant space for aeration. For bio-inoculations of carriers, the CFU/ml of PGPR strains grown on Lauria-Broth media were adjusted to 106 and then centrifuged at 600rpm to get pellets, which were redissolved in autoclaved distilled water, to be used as inoculants. Under laboratory conditions, these polythene bags of wheat bran and rice were bio-inoculated with 15ml of four selected PGPR strains (i) P1 i.e., *Achromobacter* sp. (HS4) (ii) P2 i.e., *Bacillus* sp. (AB8), (iii) P3 i.e., *Enterobacter* sp. (A7B) and (iv) P4 i.e., *Pseudomonas* sp. (AH2) separately. Based on carriers bioformulations were divided into two groups, which were further categorized on the base of bio-inoculations (P1, P2, P3 and P4) i.e., wheat bran bioformulations as WB1, WB2, WB3 and WB4 and rice husk bioformulations as RH1, RH2, RH3 and RH4. While the un-inoculated carriers (wheat bran and rice husk) were termed as WB0 and RH0, respectively. To facilitate optimum growth, inoculated carriers were thoroughly mixed for proper PGPR distribution and stored at room temperature for 30 days to estimate the viability of carriers, survivability of PGPR and the shelf-life of bioformulations.

2.4 Plant-Microbe interaction (PMI) assay

A certified variety MAALIKA-16 of Zea mays obtained from Federal Seed Certification and Registration Department, Lahore was used as test crop. A plant-microbe interaction was performed under laboratory conditions in randomized complete block design (RCBD), by using different concentration treatments, i.e., the treatment T1, soil was inoculated with PGPR strains individually (P1, P2, P3 and P4) and T1P0 regarded as un-inoculated soil. In the treatment T2, 20% of bioformulations used

with 80% of soil per pot, (i) in case of wheat bran bioformulations (WB), i.e., T2 with WB1, WB2, WB3 and WB4 independently with soil, (ii) in case of rice husk bioformulations (RH), i.e., T2 with RB1, RB2, RB3 and RB4 independently with soil and T2P0 regarded as 20% of un-inoculated carriers (wheat bran and rice husk) independently with soil. In the treatment T3, 40% of bioformulations used with 60% of soil per pot, (i) in case of wheat bran bioformulations (WB), i.e., T3 with WB1, WB2, WB3 and WB4 independently with soil, (ii) in case of rice husk bioformulations (RH), i.e., T3 with RB1, RB2, RB3 and RB4 independently with soil and T3P0 regarded as 40% of un-inoculated carriers (wheat bran and rice husk) independently with soil.

All these treatments were applied in triplicates on pots arranged in randomized complete block design. Before sowing, seeds were surface sterilized by using mercuric chloride (0.1%) to avoid any contamination. After harvesting, growth promotion effects of different treatments on Zea mays seedlings were measured to estimate the efficacy of bioformulations, survivability of PGPRs and viability of wheat bran and rice husk as suitable carriers. For this purpose, different growth parameters i.e., shoots length, root length, leaves number and fresh weight of seedlings and biochemical parameters i.e., protein content Lowry et al. [26] and chlorophyll content Wellburn [27] were measured.

Statistical analysis

The data obtained for growth and biochemical parameters of harvested seedlings was statistically analyzed by applying Duncans multiple-range test in SPSS v.16.

III. Results

3.1 Physiochemical properties of carriers

The results of physiochemical properties of wheat bran and rice husk showed the viability of these material as suitable carriers for PGPR bioformulations, because of their non-toxic nature, inherent moisture and water holding capacity.

pH

The pH of wheat bran and rice husk was observed as 6.9-7.2 and 6.7-6.9 respectively, which is a normal for any crop and soil.

Water holding capacity

The water holding capacity of wheat bran and rice husk were in the range of 65 to 75% and 45 to 55%, respectively.

Inherent moisture content

The inherent moisture content of wheat bran and rice husk was recorded after 24 hours as 5.4 and 4.7%, respectively.

3.2 Bioformulations preparations

In case of wheat bran, four different types of bioformulations were prepared based upon PGPR inoculations i.e., WB1 with *Achromobacter* sp. (P1), WB2 with *Bacillus* sp. (P2), WB3 with *Enterobacter* sp. (P3) and WB4 with *Pseudomonas* sp. (P4). While in case of rice husk, four different types of bioformulations were also prepared based upon PGPR inoculations i.e., RB1 with *Achromobacter* sp. (P1), RB2 with *Bacillus* sp. (P2), RB3 with *Enterobacter* sp. (P3) and RB4 with *Pseudomonas* sp. (P4).

3.3 Plant-Microbe interaction assay

The viability of wheat bran and rice husk as carriers, survivability of PGPR in carriers and the efficacy as well as the shelf-life of bioformulations were estimated on the base of results of growth and biochemical parameters of harvested plants.

3.3.1 Growth parameters

The effects of all the treatments on the growth parameters of harvested plants were measured as enlisted below.

Shoot length (cm)

In case of shoot length, the treatment T1 showed 62, 44, 43 and 35% increment in P3, P1, P2 and P4 treated plants respectively, as compared to control P0. For wheat bran, the treatment T2 showed significant increase of 107, 103, 86 and 83% in WB2, WB1, WB4 and WB3 treated plants respectively, as compared to control. While the treatment T3 recorded 31, 9 and 4% increment in WB3, WB1 and WB4 treated plants, while WB2 recorded a decrement of 12% as compared to control. For rice husk, the treatment T2 recorded significant increment of 62, 57, 56 and 45% in RH2, RH3, RH4 and RH1 treated plants respectively, as compared to control. While the treatment T3 recorded 89, 65 and 2% increment with RH1, RH4, RH3 and 15% decrement with RH2 treated plants respectively, as compared to control (Fig 1. A).

Root length (cm)

The roots of harvested plants showed root architectural modifications towards more branching. In case of root length, the treatment T1 showed a 15, 7, 2 and 1% decrement with P1, P4, P2 and P3 treated plants as compared to control. For wheat bran, the treatment T2 recorded a decrement of 12, 8, 3 and 2% in root length of plants treated with WB3, WB1, WB4 and WB2 respectively, as compared to control. While the treatment T3 recorded a decrement of 25, 23, 21 and 8% in WB2, WB4, WB1 and WB3 treated plants respectively, as compared to control. For rice husk, the treatment T2 illustrated a decrement of 7, 6, 5 and 1% in RH3, RH2, RH4 and RH1 treated plants respectively, as compared to control. While the treatment T3 recorded a decrease of 10, 5 and 1% with RH2, RH3 and RH4 treated plants and exceptionally an increase of 16% with RH1 treated plants as compared to control (Fig 1. A).

Number of leaves

In case of number of leaves, the treatment T1 recorded an increase of 33, 32, 30 and 28% with P2, P4, P1 and P3 treated plants as compared to control. For wheat bran, the treatment T2 recorded a significant increase of 76, 54, 53 and 51% with WB1, WB2, WB4 and WB3 treated plants as compared to control. While the treatment T3 showed an increase of 15, 11, 10 and 6% in WB3, WB1, WB4 and WB2 treated plants respectively, as compared to control. For rice husk, the treatment T2 illustrated a consistent increase of 50, 45, 42 and 40% in RH4, RH2, RH3 and RH1 treated plants respectively, as compared to control. While the treatment T3 illustrated an increase of 47, 30, 24 and 12% in RH2, RH4, RH1 and RH3 treated plants as compared to control (Fig 1. B).

Fresh weight (g)

In case of fresh weight, the treatment T1 showed a significant enhancement of 40, 35, 29 and 25% with P3, P3, P1 and P2 treated plants as compared to control. For wheat bran, the treatment T2 recorded a significant increment of 74, 69, 60 and 55% in WB1, WB3, WB4 and WB2 treated plants respectively, as compared to control. While the treatment T3 recorded an increase of 79, 39, 38 and 28% in WB1, WB3, WB4 and WB2 treated plants respectively, as compared to control. For rice husk,

the treatment T2 showed an increment of 71, 63, 61 and 42% in RH4, RH2, RH3 and RH1 treated plants respectively as compared to control. While the treatment T3 showed an increment of 81, 45, 25 and 23% in RH4, RH1, RH2 and RH3 treated plants respectively as compared to control (Fig 1. B).

3.3.2 Biochemical parameters

The effectiveness of different treatments was estimated by measuring biochemical parameters of treated plants.

Protein content (µg. g^{-1})

In case of protein content (µg. g-1), the treatment T1 showed an increment of 48, 44, 25 and 8% in P3, P4, P2 and P1 treated plants as compared to control. For wheat bran, the treatment T2 recorded a significant increment of 113, 106, 105 and 98% in WB3, WB1, WB4 and WB2 treated plants respectively as compared to control. While the treatment T3 recorded an increment of 91, 81, 69 and 60% in WB2, WB1, WB3 and WB4 treated plants respectively as compared to control. For rice husk, the treatment T2 illustrated a significant increment of 171, 135, 77 and 72% in RH3, RH2, RH4 and RH1 treated plants respectively, as compared to control. While the treatment T3 illustrated an increment of 48, 46, 42 and 37% in RH4, RH2, RH3 and RH1 treated plants respectively, as compared to control (Fig 1. C).

Total chlorophyl content (µg. g^{-1})

In case of total chlorophyll content (µg. g-1), the treatment T1 recorded an increment of 70, 60, 56 and 45% in P1, P4, P2 and P3 treated plants as compared to control. For wheat bran, the treatment T2 illustrated an extraordinary increment of 188, 111, 103 and 97% in WB1, WB4, WB2 and WB3 treated plants respectively as compared to control. While the treatment T3 illustrated an increment of 70, 56, 29 and 3% in WB4, WB3, WB1 and WB2 treated plants respectively as compared to control. For rice husk, the treatment T2 recorded a significant increment of 89, 56, 37 and 12% in RH4, RH3, RH2 and RH1 treated plants respectively, as compared to control. While the treatment T3 recorded an increment of 23, 15, 7% in RH4, RH1 and RH2 and a decrement of 4% in RH3 treated plants respectively, as compared to control (Fig 1. D).

IV. Discussion

In the current era, the consistent use of synthetic fertilizers in agriculture degraded soil texture and fertility which adversely affected plant health and yield. The severe environmental degradation and high percentage of human health deterioration, shifting agriculture industry towards eco-friendly and cost-effective techniques i.e., biofertilizers or bioformulations. In the current study, four plant growth promoting rhizobacteria were used as bio-inoculant to prepare bioformulations. The basic aim of this study is to check the potential of two agro-industry by-products i.e., wheat bran and rice husk as suitable carrier for the survivability of plant growth promoting rhizobacteria (PGPR) from laboratory to fields. Due to ample production, high nutrient content, cost, non-toxic nature of these by-products for humans and environment, these were selected as a carrier for bio-inoculants. The physiochemical properties of these organic solid carriers also illustrated the effectiveness of these materials as viable vehicle for PGPR. The shelf-life of PGPR depends upon the bacterial genera, nutritional content and particle size of carriers. The high surface area due to small particle size enhanced the desiccation resistance of bio-inoculants [28]. The use of low-cost organic substrates with several nutritional amendments, help to prepared cost-effective biofertilizers. The use of cereal brans i.e., wheat and rice also effect the growth and shelf-life of biofertilizers [29], [30]. The nutrient content of wheat bran i.e., fats (5-10%), dietary fibers (30-45%), vitamins (A, B, E and K), minerals (Mg, Ca, Mn, Cu, Fe and Zn) and for rice husk, the nutrient composition i.e., fats (10-20%), fibers (5-15%), proteins (10-15%), vitamins (E, thiamin, niacin) and minerals (N, P, K, Fe, Mn, Ca, Cl, Zn etc) illustrate both material as suitable carrier for PGPR survivability during shelf-life [31], [32]. For wheat bran the composition of macroelements recorded as ±20mg/g N, ±17mg/g P and microelements as ±190µg/g Fe, ±24µg/g Zn, ±5µg/g Cu, ±3µg/g Mn, ±4mg/g total soluble sugars (TSS) and ±3.5mg/g total soluble proteins (TSP). While for rice husk the composition of macroelements recorded as ±13mg/g N, 4mg/g P and microelements as ±150µg/g Fe, ±25µg/g Zn, ±4µg/g Cu, ±3µg/g Mn, ±3.5mg/g total soluble sugars (TSS) and ±3mg/g total soluble proteins (TSP) respectively [33].

The application of PGPR on soil cause significant enhancement in the shoot length, but the increment of upto 107% was observed with bioformulations' treatment with soil. This increment indicates survivability of PGPR with optimum shelf-life in agro-organic carriers. The increment in shoot length is assisted by the mineralization of insoluble nutrient by secondary metabolites produced by PGPR [34]. Moreover, several studies also reported the increase in phytohormones production i.e., auxins and gibberellins increased the production of several enzymes, which ensure the nutritional uptake and metabolism during early stages of germination i.e., starch [35], [36]. The production of phytohormones [37], hydrolytic enzymes [38], and phytohormones-like substances i.e., brassinosteriods and jasmonates and their antagonistic role against pathogens by the interaction of PGPR to plants, plays a significant role in shoot growth enhancement [39].

The root architectural modification of plants treated with PGPR were a notable change in plant-microbe interaction. A considerable decrease of upto 15% in root length and increased roots' branching with different treatments of bioformulations was due to high production of phytohormones i.e., gibberellic acid and indole-acetic-acid (IAA) which increased surface area for absorption with immense roots' branching. The use of tryptophan from the root exudates by PGPR, enhance the IAA production for plants, which directly increase lateral roots, root hairs and surface area to improved nutrients uptake. The mineralization of nutrients i.e., phosphate improves roots biomass and plants growth [40], [41], [42].

A significant increment of upto 70% and upto 80% in number of leaves and fresh weight of treated plants was observed by different treatments of bioformulations respectively. These increments were due to the increased mineral solubilization (phosphate and zinc) and uptake by plants [39]. The interaction of PGPR with plants improve the bioavailability of carbon via its degradation and phosphate solubilization [43]. Moreover, the increased production of phytohormones i.e., IAA and GA due to utilization of amino acid and mineral alter the physiology and growth rate and fresh weight of treated plants [44]

In case of biochemical parameters, the protein content of plants treated with bioformulations showed an exceptional increment of

upto 135% due to high nutrients and mineral absorption by plants which directly increased food production [45]. A significant increase of upto 110% in total chlorophyl content of treated plants with bioformulations was due to absorption of essential elements i.e., nitrogen which directly increased the chlorophyl content and photosynthesis. The ample availability of essential elements also activates ribulose-bisphosphate carboxylase's precursor and accumulate Fe for high chlorophyll content [46].

V. Conclusion

The significant increments in growth and biochemical parameters of plants treated with bioformulations, illustrate the viability of wheat bran and rice husk as effective carriers for PGPR bioformulations. The particle size of these materials also effective for the survival of bio-inoculants during storage conditions and efficiency of these bioformulations in fields. The above results indicated that wheat bran is suitable carrier for the bio-inoculations in the order of *Enterobacter* sp. (P3) > *Bacillus* sp. (P2) > *Pseudomonas* sp. (P4) > *Achromobacter* sp. (P1) in bioformulations preparations. While the rice husk is more effective carrier for the bio-inoculations in the order of *Pseudomonas* sp. (P4) > *Achromobacter* sp. (P1) > *Enterobacter* sp. (P3) > *Bacillus* sp. (P2) for bioformulations preparations. As far as the treatments are concerned, treatment T2 is more effective than treatment T3, which illustrate that soil is an essential medium for plants to overcome basic nutrient requirements. The growth promotion effects of bioformulations indicate the importance of wheat bran and rice husk as potential, eco-friendly and cost-effective carriers for bioformulations. Now, the further studies should be done by focusing on the amendments of these carriers with several ingredients to improve the shelf-life of product to overcome one of the major loopholes of bioformulations marketing and commercialization.

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VII. References

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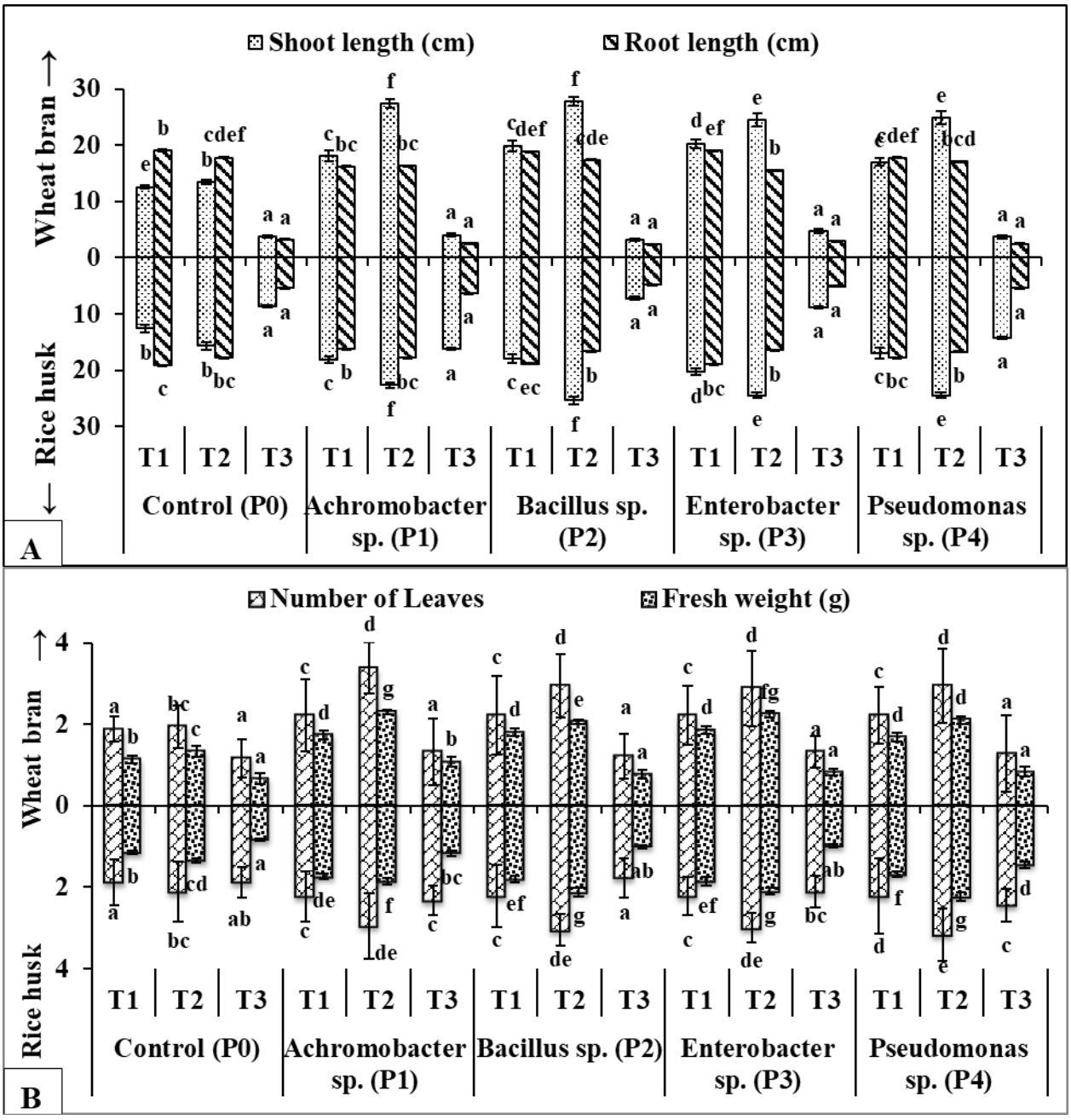
Shoot length (cm)
Root length (cm)
Wheat bran →
← Rice husk
30
20
10
0
10
20
30
T1 T2 T3 T1 T2 T3 T1 T2 T3 T1 T2 T3 T1 T2 T3
Control (P0)
Achromobacter sp. (P1)
Bacillus sp. (P2)
Enterobacter sp. (P3)
Pseudomonas sp. (P4)
A
Number of Leaves
Fresh weight (g)
Wheat bran
Rice husk
4
2
0
2
4
T1 T2 T3 T1 T2 T3 T1 T2 T3 T1 T2 T3 T1 T2 T3
Control (P0)
Achromobacter sp. (P1)
Bacillus sp. (P2)
Enterobacter sp. (P3)
Pseudomonas sp. (P4)
B

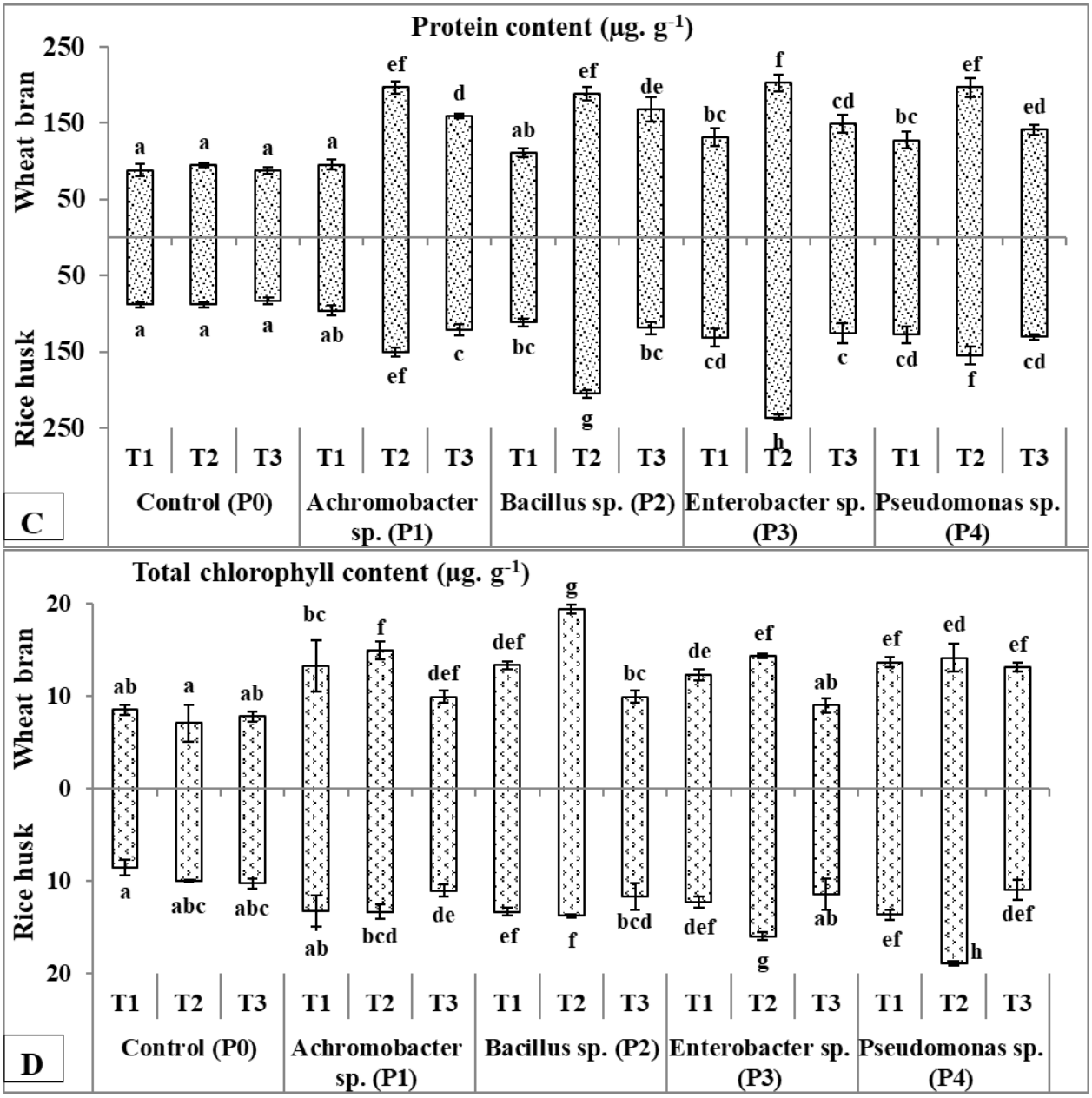

Fig. 1: Impact of different treatments of plant-microbe interaction (A) on shoot and root length, (B) number of leaves and fresh weight, (C) protein content and (D) total chlorophyl content of Zea may plants. Treatment T1; inoculated soil with P1, P2, P3 and P4, Treatment T2; 20% of bioformulations (wheat bran and rice husk separately) with 80% soil per pot and Treatment T3; 40% of bioformulations (wheat bran and rice husk separately) with 60% soil per pot. The T1 (Blank soil), T2 (20% un-inoculated wheat bran and rice husk) and T3 (40% un-inoculated wheat bran and rice husk) of P0 used as control for T1, T2 and T3 of P1, P2, P3 and P4 analyzed by Duncan's multiple range test ($P \leq 0.05$).