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Research Article

EFFECT OF BIOFORMULATIONS OF *PSEUDOMONAS AERUGINOSA* PC1 ON GROWTH CHARACTERISTICS OF *TRITICUM AESTIVUM*

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ABSTRACT

Wheat (*Triticum aestivum)* is the major crop of India. Madhya Pradesh is one of the largest producers of wheat. The present study was conducted to develop new bioformulations and evaluate their efficacy in growth promotion of *Triticum aestivum* (Wheat). The interest is to use inorganic carrier *i.e.* talcum and bentonite based bioformulations of *Pseudomonas aeruginosa* PC1 to replace chemical agents in fertilizing soils because chemical agents harms the crops ultimately lowering its productivity and quality. The *Pseudomonas aeruginosa* possesses the ability of siderophore production and phosphate solubilization. The polybag experiment results of 60 days after sowing wheat seeds showed that both the Bentonite and Talcum based bioformulations of *Pseudomonas aeruginosa* PC1 significantly increased chlorophyll content, root and shoot length, number of tillers and fresh and dry weight of wheat plant compared to uninoculated controls. It has been suggested that this strain can be used for large scale production to prepare an effective bioinoculant for eco-friendly and sustainable production of wheat.

Keywords: Wheat, Bioformulations, *Pseudomonas aeruginosa,* Rhizobacteria, Bentonite, Talcum.

1. INTRODUCTION

Rhizosphere may be defined as the degree of soil influenced by roots or root tissues colonized by microorganisms and are considered as the hot spot of biodiversity [1]. Among these, the beneficial rhizo-bacterial strains have been shown to improve the plant growth after inoculation on to seeds or in soil and are therefore called Plant Growth Promoting Rhizobacteria (PGPR) [2]. Most common are the members of genera *Azotobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Klebsiella, Pseudomonas*, *Serratia* and *Xanthomonas*. Among these, pseudomonads are the major players and have been studied globally for both plant growth promotion and biological control of pathogens [3, 4].

Pseudomonas is gram negative rods, aerobic, flagellate, non-spore forming bacteria. Various bacterial strains, in particular the *Pseudomonassp.* are known to play a significant role in the control of different plant pathogens [5]. Owing to their physiological diversity and versatile nature, pseudomonads have received considerable attention across the globe for their promoting plant growth and biocontrol activities [6]. There are several mechanisms proposed for the biocontrol action of pseudomonads *viz.,* secretion of hydrolytic enzymes, competition for colonization sites and nutrients, antibiotic compounds production or induction of host plant defense system [7]. Most pseudomonads suppress soil-borne fungal pathogens by producing a diverse array of secondary metabolites with antifungal properties such as hydrogen cyanide, pyoluteorin, pyrrolnitrin, phenazine-1-carboxylic acid (PCA), 2, 4-diacetylphloro-glucinol (DAPG). Besides antibiotics, a large number of *Pseudomonas* species are known to suppress fungal pathogens through siderophore production [8]. Sidero-phore are compounds of lower molecular weight which are produced under iron deficient conditions and chelate iron from the surrounding environment. Indirectly, by making the pathogens starved of iron, siderophore producing pseudomonads suppress them and contribute to the biological control [9].

Among food crops, wheat occupies a prime position in the world. In India it is second most important food crop and contributes approx. 25 % of total grain production of the country. Thus, wheat (*Triticum aestivum*) plant has been selected as a source of rhizobacteria for the present study because among the different crops undergoing cultivation in Madhya Pradesh, it is one of the chief crops of this region. Like other crops, it is susceptible to variety of pathogens at various stages of plant growth, major being caused by soil borne pathogens *viz.*, root rot

by *Fusarium oxysporum*, *Sclerotium* wilt, *Alternaria* leaf blight etc. Yield losses have been reported up to 33% accounting for almost one third of total grain production thus affecting farmer's economy to a big extent [10]. Thus, it is necessary to protect the crop from pathogens in a way that it would minimize the crop loss in addition to being ecofriendly and the application of beneficial microorganisms or microbial products helps to meet out the above demands. The present study thus aimed to develop an inorganic carrier based bioformlations of *Pseudomonas aeruginosa* to enhance the growth of *Triticum aestivum* and control phytopathogens.

2. MATERIAL AND METHODS

2.1. Bacterial culture and growth condition

Pseudomonas aeruginosa strain PC1 (ACCN.MN883873) was isolated from the rhizosphere of *Triticum aestivum* from Bhopal, M.P., India and maintained as 40% glycerol stocks at -20°C in King's B medium.

2.2. Determination of plant growth promontory properties

2.2.1. Phosphate solubilisation

Qualitative estimation was conducted using petriplate assays on Pikovyskaya agar [11]. Test culture was spot inoculated using sterile needle on plates containing Pikovyskaya agar medium, the plates were then incubated at 28±2°C for 24 h. The clear zone formation around the bacteria indicated positive test for phosphate solubilization. Phosphate solubilization index was calculated by measuring the diameter of colony and the diameter of halo zone, using the following formula of Edi-Premono et al., 1996 [12].

Phosphate Solubilization Index (SI)=(Colony diameter+ Halo zone diameter)/Colony diameter

2.2.2. Siderophore production

Qualitative test was performed by spot inoculating the bacteria on Chrome Azurol Medium (Chrome Azurol Test by Schwyn and Neilands, 1987, [13]) the plates were then incubated at 28±2°C for 24 h. The formation of orange halos around the bacterial cultures indicated positive test for siderophore production.

Quantification of siderophoreproduction was measured in Luria Bertani (LB) medium using extinction coefficient of siderophore (E=16500) [14]. Pure culture of *Pseudomonas aeruginosa* PC1 was grown in LB broth for 24 h. One ml active culture was inoculated in 100 ml LB broth and flasks were incubated at 28±2°C (120) rpm. After 72 h of incubation, 5 ml of culture was withdrawn and centrifuged at 10,000 rpm for 10 min. The optical density of the supernatant was read at 400 nm and siderophore was quantified based on the extinction coefficient of pyoverdine as under:

Siderophore (mg /ml) = {(A400 x Mol weight of Pyoverdine)/Eλ x 10^{-3}}

Where, Mol weight of Pyoverdine=1,500 Da, Extinction coefficient (Eλ) = 16,500M^{-1} cm^{-1}

2.2.3. Inorganic carriers

Talcum and Bentonite [15] powders were used as inorganic carriers to prepare bioformulations. 100grams of the carrier powder was autoclaved at 121°C for 20 min. The sterilization procedure was repeated for two consecutive days. The sterilized powders were then used to prepare bioformulations.

2.3. Bioformulation preparation

The broth containing 3.8 X 10^{11} CFU ml^{-1} of *Pseudomonas aeruginosa* PC1 was used for the preparation of bioformulations. To 98 ml culture broth, 1 ml carboxymethylcellulose (1.0 g/L) and 1 ml glycerol, were added. Glycerol was used as a source of carbon to keep the cells viable, whereas carboxymethylcellulose was used as an adhesive. The culture broth, including the additives, was uniformly mixed. To prepare 100g of inorganic carrier-based bioformulation under sterile conditions, 80 g of sterilized carrier and 20 ml of culture broth were mixed [16]. The product was shade-dried following the method of Vidhyasekaran and Muthamilan [17] to reduce the moisture content and then they were packed in polythene bags which is UV-sterilized and sealed. The talcum powder formulations contained 2.3 X 10^{10} CFUg^{-1}, whereas bentonite-based formulations contained 2.8 X 10^{10} CFU $g^{-1}$of *P. aeruginosa* respectively. Aseptic conditions were maintained throughout the process.

2.4. Poly bag trial to assess the growth promontory activity of bioformulations on *Triticum aestivum*

2.4.1. Source of Seeds and sterilization

Seeds of wheat variety sharbati were procured from the local market of Bhopal. Surface sterilization of seeds was performed using 0.1% $HgCl_2$. Later, 0.5g of bioformulations was added to 50g of wheat seeds separately. The flasks were then kept in an orbital shaker at 150 rpm for 15 min for seeds coating with bioinoculant formulations. The average cell count per seed for talcum powder based formulation was calculated

as 1 X 10^5 cfu $seed^{-1}$ and for bentonite based formulations 1 X 10^4 cfu $seed^{-1}$ was calculated.

2.4.2. Experimental design for bioassay

Soil for the experiments was collected from agricultural land near Adharsheela, Bhopal and was analysed for various parameters like pH, electrical conductivity and soil texture. For plant bioassays, nonsterile soil was air dried and filled in plastic bags (2kg bag^{-1}). The sterilized soil was prepared by putting soil (sieved in mesh) in jute bag and autoclave at 15 psi for 2 hrs successively for three cycles with one day gap between the two autoclaving cycles. Two sets of control were used; one containing non-sterile soil and the other containing sterile soil. The treatments used were as Control C1 (Sterilized soil without bioformulation), C2 Control (Non Sterilized soil without bioformulation), T1 (Sterilized soil with bentonite based bioformulation), T2 (Non sterilized soil with bentonite based bioformulation), T3 (Sterilized soil with Talcum based bioformulation) and T4 (Non sterilized soil with Talcum based bioformulation). Six seeds were sown in each bag which were around 18 (4 treatments X 3 replicates + 2 controls X 3 replicates) and a completely randomized design was maintained for such bags. Sterile water for watering was used to soil where sterilized soil in used. Harvesting was done after 60 days. The plants were gently removed and thoroughly washed for analyzing various growth parameters such as estimation of chlorophyll content of leaves [18], root and shoot length, fresh and dry weight of root and shoot and numbers of tillers.

2.5. Statistical analysis

The experiments were carried out in a completely randomized design for *Triticum aestivum*. One-way ANOVA was performed with 0.5% as level of significance to enumerate significant difference between control and treatments.

3. RESULTS AND DISCUSSIONS

3.1. Plant growth promontory activity of *Pseudomonas aeruginosa* PC1

3.1.1. Phosphate solubilization

PGPR have been known to solubilize phosphates and increase phosphate availability to plants that represents a probable mechanism of plant growth promotion under field conditions [19]. In our study the *Pseudomonas aeruginosa* PC1 was found to be effective phosphate solubilizer showing a clear halo zone around its colony. After 48hrs of incubation, it showed 12.00 mm phosphate solubilizing halo zone. The solubilization index (SI) of the strain was also calculated at the end of the incubation period and it was observed as 2.45. The halo zone formation around the bacterial colonies might be due to the production of enzymes of phosphate solubilizing bacterial strains [20, 21].

3.1.2. Siderophore production

Under iron limiting conditions siderophore is one of the most prominent biocontrol mechanisms belonging to PGPR groups. PGPR produces a variety of siderophores which possesses a great affinity for iron. Therefore, the lower availability of iron in the environment results in the suppression of pathogenic organisms including phytopathogenic fungi [22]. In the present study *Pseudomonas aeruginosa* PC1 showed strong production (+++) of siderophore.

3.2. Soil Microcosm experiment for plant growth promontory activity

3.2.1. Analysis of Soil

The soil type is identified as clay loam. The pH of the soil was measured as 7.2. The electrical conductivity of the soil was found to be 0.78 dS m^{-1}

3.3. Efficacy of the carrier-based formulations of *Pseudomonas aeruginosa* PC1 on growth of *Triticum aestivum* in pot trials

The *Pseudomonas aeruginosa* PC1 was shown to improve the growth of wheat (*Triticum aestivum L.*) compared to the controls. The growth response of *Triticum aestivum* in terms of dry root weight, dry shoot weight etc. was determined after 60 days from germination. The PC1 bioformulations showed to improve the shoot weight, root weight, shoot length, root length, no. of tillers and chlorophyll content.

3.4. Effect of bioformulations on Shoot weight and Root weight

In comparison to control, all the treatments resulted in significant enhancement in fresh and dry weight of shoot with both the carriers. However, the degree of growth promotion varied among treatments and carriers. It was observed that the bio-formulations worked significantly well as compared to their respective control. Minimum shoot weight was found in sterilized soil inoculated with seeds without bioformulation (C1) in both fresh and dry weight *i.e.* 1.45±0.07g/plant and 0.51±0.05 g/plant respectively. Maximum shoot weight was found when non sterilized Talcum powder carrier-based bio-

formulation treatment (T4) was used. Maximum percent increase of 125.31% and 108.92% was observed in shoot fresh weight and shoot dry weight respectively with treatment T4 as compared to their respective control.

There was a significant difference between the control and the carrier based bio-formulations. The minimum root fresh weight and root dry weight was found to be 0.31±0.04 g/plant and 0.19±0.05g respectively in treatment C1 whereas the maximum root fresh weight and root dry weight *i.e.* 1.09±0.04 g/plant and 0.52±0.04 g/plant respectively was also observed with treatment T4. Percent increase of 109.61% and 132.14% was observed in root fresh weight and root dry weight respectively in Talcum based treatment T4 as compared to control.

3.5. Effect of bioformulations on Shoot length and Root length

The maximum shoot length (67.30%) was observed in treatment T3 followed by T4 (59.22%) and T2 (57.14%) as compared to the control while maximum root length was obtained with treatment T3 (43.84%). In a study by Pankaj et al. [10] maximum shoot and root length was observed with *Pseudomonas sp.* WBC10. Plant growth promoting rhizobacteria are capable of liberating molecules similar to plant growth regulators that are taken up by the plants causing enhancement of root surface and thereby enhancing uptake of nutrition [23]. Shaharoona et al. [24] reported that *Pseudomonas fluorescens* causes enhancement of total biomass of seedling, increased biocontrol efficiency, and decrease disease incidence under nursery conditions in cabbage.

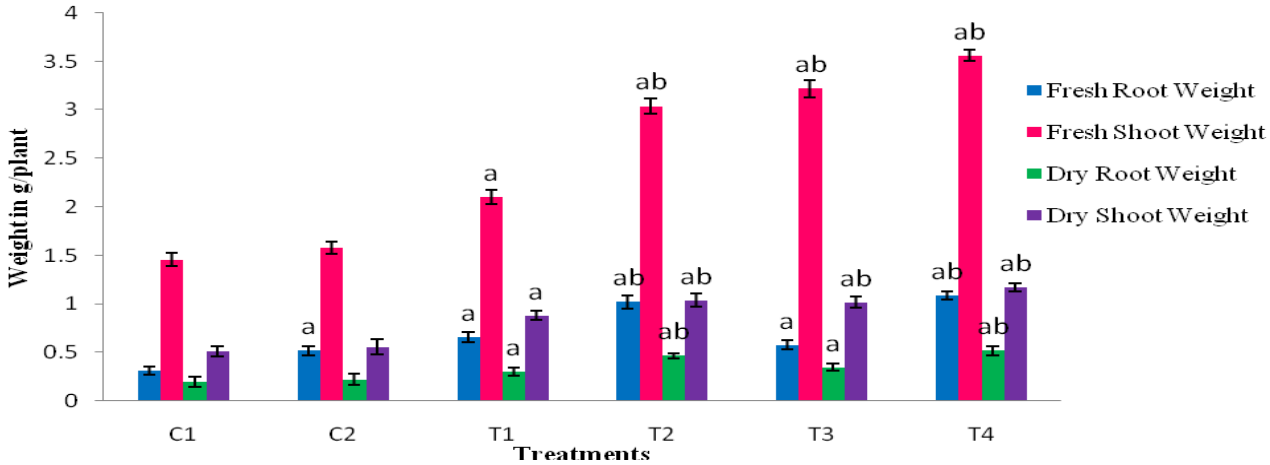

Fig. 1: Effect of inorganic carrier based bioformulations of *Pseudomonas aeruginosa*PC1 on root and shoot weight of wheat plant

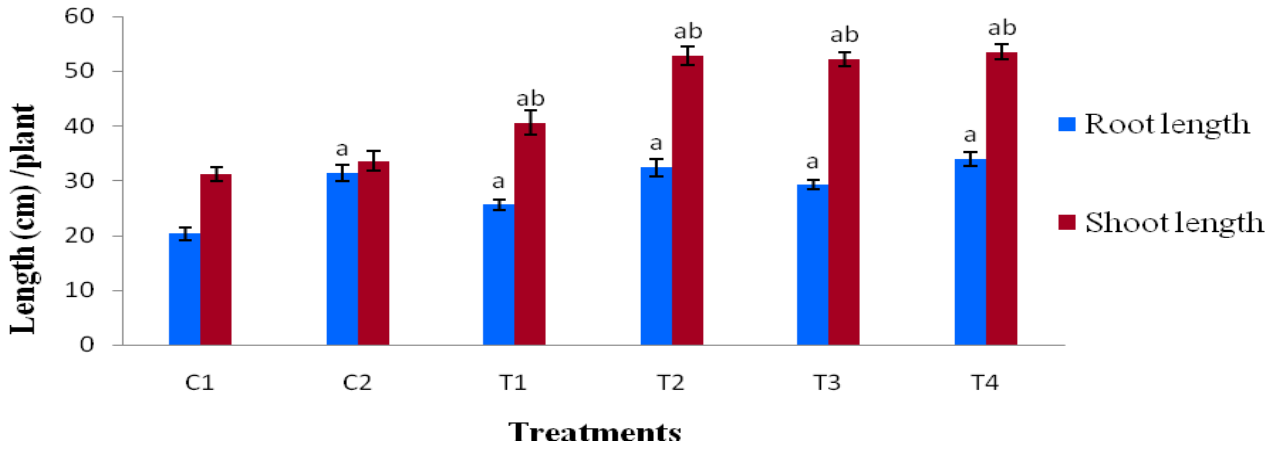

Fig. 2: Effect of inorganic carrier based bioformulations of *Pseudomonas aeruginosa*PC1 on root and shoot length of wheat plant

3.6. Effect of bioformulations on chlorophyll content of leaves

Chlorophyll content of wheat leaves was estimated *in vitro*. Results showed that all types of chlorophyll content (a, b and total) increased by all the treatments with bio-formulations of *Pseudomonasa aeruginosa*. The percent increase in chlorophyll content 'a' and 'b' was in the range of 8.39 to 24.54% and 10.29 to 31.56% respectively over control simultaneously. Similarly the total chlorophyll content of leaves 9.59 to 27.86 % increase over control. Maximum percent increase in total chlorophyll content was observed in T3 treatment (27.86 %) followed by T4 (24.31 %) and T2 (22.03%). Minimum percent increase in total chlorophyll content was observed in T1 (9.59 %). Sharma et al. [25] found that chlorophyll content in leaves was increased in mung bean due to *Pseudomonas* strain GRP3 or by supernatants in all the treatments, presumably due to microbial siderophore utilization which supports our findings where chlorophyll content was estimated at the end of the trial by collecting the fresh leaves.

3.7. Tillers

A study by Singh et. al. [26] showed high numbers of tillers are associated with high grain yields. *Pseudomonas fluorescens* biotype F (ACC3) was the most promising with 9% more tillers than the uninoculated control. Pinthus and Meiri [27] reported that tiller development and tillering can be markedly promoted in different wheat cultivar by a low day/high night temperature regime [28].

In our study the maximum tillers were obtained in treatment T4 *i.e.* 23±1.2 followed by T3 *i.e.* 21±0.7 whereas the lowest number was observed with treatment C1 *i.e.* 14.66±1.7. The maximum percent increase were observed in treatment T3 (43.24%) followed by treatment T4 (30.93%).

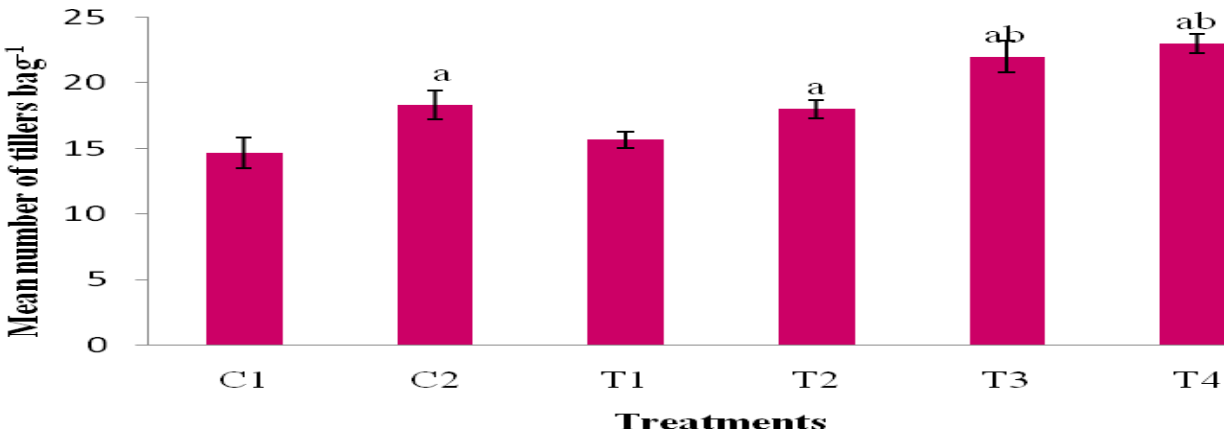

Fig. 3: Effect of different treatments on number of tillers bag^{-1}

4. CONCLUSION

The present study showed that the use of talcum and bentonite based bioformulations of *Pseudomonas aeruginosa* PC1 significantly increase the growth of Wheat plant by increasing the chlorophyll content, root and shoot fresh and dry weight, number of tillers and root and shoot length compared to control. In our study both the talcum and bentonite based bioinoculant formulation performed well. Thus, the *Pseudomonas aeruginosa* PC1 can be used as a potential biofertilizer which can replace the chemical fertilizer, pesticide in agriculture and protect the environment.

Conflict of interest

None declared

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