Journal of Bioprocessing & Biotechniques

Mishra and Sundari, J Bioproces Biotech 2015, 5:2
DOI: 10.4172/2155-9821.1000202

Research Article Open Access

Native PGPM Consortium: A Beneficial Solution to Support Plant Growth in the Presence of Phytopathogens and Residual Organophosphate Pesticides

Nivedita Mishra and Krishna Sundari S*

Plant & Microbial Technology (PMT) Group, Biotechnology Department, Jaypee Institute of Information Technology, Sector-62, NOIDA, 201307, Uttar Pradesh, India

Abstract

Modern sustainable agricultural practices prefer to use biological agents for plant growth promotion, biocontrol and bioremediation as these are cost effective and eco-friendly. Our present study aims to investigate the effects of direct inoculation of selected consortia on plants to study its effects on supporting plant growth in the presence of root pathogen *Sclerotium rolfsii* and organophosphate pesticides Malathion (ML) and Methyl Parathion (MP). Candidate Plant Growth Promoting Microbial (PGPM) isolates chosen for the study are two bacterial isolates (PGPM2 a diazotrophic bacterium, PGPM9 a fluorescent Pseudomonad) and one fungal species (T103 a biocontrol fungus), originating from native agricultural fields of western U.P., India. Host plants inoculated with individual species showed a distributed growth enhancement pattern i.e., while isolate T103 improved root biomass, isolate PGPM9 enriched photosynthetic pigment content and isolate PGPM2 expanded root and shoot lengths. It appeared as though individual isolates showed a preference to enhance certain parameters over the other rather than exhibiting a uniform increment in all growth parameters. This preference to specific growth parameter over the other waned off in consortium studies where *Sorghum bicolor* inoculated with the consortium registered almost 2-fold increase in all parameters *viz*., root length, shoot length and overall biomass (root, shoot and total biomass) along with 23% rise in total chlorophyll content as compared to un-inoculated control. Selected consortia combination was able to provide better growth promotion in presence of pathogen *Sclerotium rolfsii* registering 58% increase in total biomass content while individual inoculation of biocontrol fungus T103 showed only 36% improvement. Selected consortia were also effective in plant growth promotion in presence of organophosphate pesticides ML and MP. More than two fold amplification was registered in all roots and shoot growth parameters studied when consortia was provided with ML and 28% increase was recorded when MP treatment was countered with consortia inoculation. All these results affirms our hypothesis that synergistic action of carefully selected PGPMs can escalate the benefits of plant growth promotion even in presence of pathogen and pesticide, hence this consortia may be a valuable option for sustained plant growth in modern agriculture systems.

Keywords: Plant growth promoting microbes (PGPM); Biocontrol; Bioremediation; Organo phosphate pesticides; Malathion; Methyl parathion

Introduction

Present agricultural practices depend upon chemical fertilizers, pesticides and other chemicals for plant growth promotion and pathogen control with an intention to increase crop yield. Chemical residues left in soil after cropping are accumulative, difficult to degrade and harmful to animals, plants and human health in general and to soil health in particular. They decrease soil fertility by gradually altering its chemical composition and rendering it non-fertile. Integrated agriculture management system needs to focus on plant nutrient management for increasing productivity by providing better nutritional support as well as better control of pathogens while maintaining and improving the soil nutrient pool and removing deleterious chemicals to increase its productivity. Modern sustainable agricultural practices prefer to use biological agents for plant growth promotion and biocontrol as these are cost effective and eco-friendly.

Many rhizosphere bacteria are known to have beneficial effects upon plant growth since long. It is scientifically proven also that inoculation of specific microorganisms in the rhizosphere and other bio-augmentation efforts leads to higher microbial diversity in the soil and play a significant role in maintaining soil health [1,2]. Scientific literature endorses positive effects of microbial inoculation on plant growth promotion and attributes this growth enhancement to various reasons like improved nutrient acquisition, improved levels of phytohormones and other growth enhancing metabolites, suppression of plant diseases etc. [3,4]. A number of such studies confirmed that these microbial bio-inoculants develop close association with host plants. An increasing number of Plant Growth Promoting Rhizobacteria (PGPRs) had been studied and a few have been developed as commercial biofertilizers for crop improvement [5-7]. Plant Growth Promoting Microbes (PGPMs) when applied as biofertilizers affect the plant growth directly via nutrient mobilization, providing growth metabolites as hormones etc. or indirectly through their anti-pathogenic activities [8]. Various microbes providing isolated benefits are well represented in literature as plant growth promoter or biocontrol agent [9-13] but still no chosen biofertilizer/biocontrol agent has been tested to provide protection against residual pesticide contamination which is common in agriculture soils. Commercial biofertilizers are mostly single species inoculants catering for isolated benefits such as providing either macronutrient (NPK) or biocontrol and acting with host specific bias which often results in non-consistent field performances.

Recently, the emphasis has been shifted towards microbial consortia studies and its effects upon plant's growth [14-18]. These studies on consortia combination inoculation promote mixed impact picture about their growth promotional effects. Some reported significant positive impact of consortia probably due to cumulative synergistic effects of consortia inoculation over individual inoculation [15,17] while others reported no statistically significant impact of consortia over single species inoculation [19]. Some studies have even reported inconsistent and contradictory impact of consortia inoculation under greenhouse and field conditions [18].

***Corresponding author:** Krishna Sundari S, Plant & Microbial Technology (PMT) Group, Biotechnology Department, Jaypee Institute of Information Technology, Sector-62, Noida, 201307, Uttar Pradesh, India, Tel: 0120-2594206; Fax: 0120-2400986; E-mail: krishna.sundari@jiit.ac.in

Received December 02, 2014; **Accepted** January 16, 2015; **Published** January 21, 2015

Citation: Mishra N, Sundari SK (2015) Native PGPM Consortium: A Beneficial Solution to Support Plant Growth in the Presence of Phytopathogens and Residual Organophosphate Pesticides. J Bioprocess Biotech 5: 202 doi:10.4172/2155-9821.1000202

Biodegradation or bioremediation, a significant area of modern day biotechnology is mostly attempted for detoxification of oil spills, toxic chemical spills and industrial effluent remediation. At present presence of residual agricultural chemicals in agrarian soil is posing a big problem impacting plant, soil and human health all. Thus need of hour is availability of a system which not only provides plant growth and biocontrol benefits but also supports plant growth and development in agricultural soils contaminated with pesticide residues. Individual bacterial and fungal species showing pollutant degrading properties are long known [20-22]. Studies on bioremediation of petroleum oil, metal pollutants and organic pesticide are also widely available [23-28]. From time to time, few reports on pollutant and pesticide degradation capabilities of PGPMs have emerged [20,21,23]. Most of these earlier reports delve upon pollutant tolerance abilities of individual species studied under *in vitro* conditions. It would be excessive to expect that a single microbe or every PGPM should have all such qualities and substantiate for the entire spectrum of benefits i.e., plant growth promotion, biocontrol, shield plants from damage due to pollutants and also contribute to soil quality by removing such harmful chemical residues. Thus it is logical to design and device consortia studies to provide holistic and integrated benefits to the tripartite association between plant, soil and microbes. Consortia studies for their biodegradation abilities are very little explored [29]. Further the potential of such microbes having biodegradation capabilities of pollutants/pesticides has rarely been tested on plant growth promotion using direct plant inoculation methods [30].

Studies correlating bioremediating potential along with plant growth promotion and biocontrol properties needs to be explored. Direct plant inoculation to study plant growth protection abilities of suggested consortia in organophosphate contaminated environment has not received much attention earlier. Keeping all these gaps in mind, our present work aims at developing a consortium supporting plant growth in the presence of pesticides and retention of their plant growth promoting effects even in conditions of pathogen attack and is reported in our publication for first time.

Candidate PGPMs chosen for the study are two bacterial (PGPM2, PGPM9) and one fungal species (T103) originating from native agricultural fields of western U.P., India which have been carefully selected based on extensive *in vitro* lab tests as potential candidates for designing the consortium. All these potential candidates were tested for their growth promoting, biocontrol and pesticide remediation abilities *in vivo* on *Sorghum* plant. Candidate microbes selected were: one diazotrophic microbe (PGPM2) providing growth promotion without specific host bias [31], one fluorescent pseudomonad (PGPM9) with phosphate metabolizing tendencies [32] and one fungus (T103), providing biocontrol over a range of phytopathogen. This native *Trichoderma* isolate T103 is a-priori tested for its biocontrol abilities *in vitro* before exploring its ability to protect plant from pathogen attack in current *in vivo* experimentation [33].

Cereal crop Sorghum (*Sorghum bicolor*) selected for present study is a globally important crop used as food, feed, fodder and fuel. Pathogen *Sclerotium rolfsii* is a root pathogen reported to cause considerable economic losses worldwide. *S. rolfsii* causes leaf sheath blight in sorghum infecting lower stems near soil surface causing wilting and yellowing of leaves and plant death resulting in crop damages and yield losses [34,35]. ML and MP are some of the most wildly used organophosphate pesticides in Indian agriculture [36] and thus selected here to study their effects on plant growth and to check any protecting and growth promoting effects of consortia on host plant in presence of these pesticides.

This study is a design to establish our hypothesis that cumulative synergistic action of carefully selected PGPMs may improve fertility to escalate the benefits of plant growth promotion even in presence of pathogens and help in reclamation of soil health as well. As these bio-products do not disturb chemical composition of soil they prove to be a valuable option for achieving sustainability in modern agriculture systems.

Material and Methods

Fungal and bacterial isolates

Nine bacterial and fungal cultures have been isolated from agricultural fields of NOIDA (Western U.P., India) in our lab and tested for their plant growth promoting and biocontrol tendencies *in vitro* [31-33]. Among them three microbes, one a Pseudomonad (termed PGPM9) that yielded the highest chlorophyll content and one diazotroph (termed PGPM2) that showed greater impact on root and shoot growth along with fungal isolate with potential for biocontrol *i.e.*, *Trichoderma* spp. (T103) were chosen for consortium studies.

Preparation of bacterial and fungal inocula

Bacterial cultures (PGPM2 and PGPM9) were grown overnight in nutrient broth at 30°C and at 180 rpm in incubator shaker to obtain culture suspension of 10^8 cfu/ml. Pathogen *Sclerotium rolfsii* was grown on Malt Dextrose Agar (MDA) plates for seven days at 30°C. T103 was grown on MDA plates for five days at 30°C.

To obtain fungal culture suspension, 50 ml sterile water was added to the agar plates and spores were scraped to obtain a suspension of 10^8 viable counts/ml. Bacterial and fungal inoculums so prepared were used for all individual treatments in further experiments. For consortia preparation, fungal suspension and bacterial suspensions were mixed in equal-volume just before treatments were applied. A total of five treatments namely, uninoculated control, single inoculations of PGPM2, PGPM9 and T103 respectively and consortia inoculation comprising all three isolates: PGPM2, PGPM9 and T103 in equal proportion were tested in all of the following experiments except in case of pesticide challenge experiments.

Germination assay

Seeds of *Sorghum bicolor* cultivar: CSH –16 procured from The Directorate of Sorghum Research (DSR) (formerly, National Research Centre for Sorghum (NRCS)), Hyderabad, India have been used in this study. The seeds were surface-sterilized with 0.1% H_2O_2 for 30 seconds; rinsed five times with Sterile Distilled Water (SDW) followed by similar sterilization cycle twice with 70% ethanol and soaked in SDW overnight before germination. Sterilized seeds were immersed for 30 min in respective treatment suspensions under sterilized conditions. Treated seeds were placed for germination in petri plates on sterilized cotton @ 25 seeds per plate and were inoculated in triplicate with all respective treatments. Petri dishes were watered with SDW and incubated at 30°C for three days. Number of germinated seeds was recorded per plate after three days post incubation and percentage germination calculated [37] using formula given below:

Percentage Germination = (Number of seeds germinated/Total number of seeds placed in petri plates) × 100

Further seedling length of all germinated seeds was recorded at same time for calculating Seed Vigor Index (SVI). Seed vigor index was calculated using following formula:

SVI = Mean seedling length (cm) × Percentage germination

Effect of various treatments on germination percentage and seed vigor index was analyzed by calculating percentage increase or decrease observed in specific treatment/s as compared to uninoculated control and tabulated (Tables 1and 2).

Effect of consortia on plant growth promotion

Seeds were prepared for germination as described earlier and soaked for 30 minutes in respective treatment suspensions. Uninoculated control seeds were soaked in SDW for same duration. Three days after germination, all post incubation parameters were recorded and germination percentage and seed vigor index was calculated.

Such germinated seeds were placed singly in individual cells of 100 ml Root Trainers (RTs) containing peat moss and vermiculite mixture (1:2 V/V). The experiment was arranged in a randomized block design with eight replicates per treatment. RTs were watered on alternate days to meet watering requirements [38]. Two weeks after sowing, the plantlets were harvested and root length, shoot length were measured (represented in cms, Figure 1). Plantlets were dried and dry weights of roots and shoots were recorded (presented in grams, Figure 1). All the results were subjected to ANOVA and post-ANOVA analysis (p=0.01) to analyze significance of various treatments (Figure 1).

For determining the photosynthetic pigments, 500 mg fresh weight of leaves from each of the treatments was homogenized in 80% acetone and the homogenate centrifuged at 10,000 g for 10 minutes. The optical density of the supernatant was measured spectrophotometrically at 645, 663 and 750 nm [39]. Effect of various treatments on the photosynthetic pigments, namely chlorophyll a, chlorophyll b and total chlorophyll, were estimated according to Lichtenthaler formula [40] as given below:-

Chlorophyll a = ((13.36 × A663 − 5.19 × A645) ×8.1)/weight of plant tissue [mg/g]

Chlorophyll b = ((27.43 × A645 − 8.12 × A663) ×8.1)/weight of plant tissue [mg/g]

Total chlorophyll = ((5.24 × A663+ 22.24 × A645) × 8.1)/weight of plant tissue [mg/g]

Where A645 = Absorbance at 645 nm and A663 = Absorbance at 663 nm

Effect of various treatments was analyzed by calculating percentage increase or decrease as tabulated in Tables 1 and 2.

Effect of consortia in presence of phytopathogen

Phytopathogen *Sclerotium rolfsii* used in present studies was procured from The Central Research Institute for Dry land Agriculture (CRIDA), Hyderabad, India. To study shielding effects of consortia on plant growth against pathogen, three treatments only pathogen inoculated, pathogen inoculation with single species T103 and pathogen inoculation with consortia were compared. Germinated seeds were prepared as described earlier with 30 minute soaking in respective treatment suspensions and placed in RTs in eight replications arranged in a randomized block design. Two weeks after sowing, the plantlets were harvested and root- shoot lengths and root-shoot dry weights were measured as described earlier. ANOVA and post-ANOVA analysis (p=0.01) were carried out to compare impact of various treatments on plant growth promotion. Results represented in graphical form (Figure 2).

Effect of consortia in presence of pesticides

Selected pesticides under study were organophosphate Methyl Parathion (MP) and Malathion (ML) employed at 10 ppm concentration each separately. In this study a total of four treatments were tested representing: 10 ppm ML, 10 ppm MP, 10 ppm ML along with consortium inoculation and 10 ppm MP with consortium inoculation. Seeds were soaked for 30 min in respective treatments and placed for germination as described earlier. After three days, all post germination parameters were recorded and germination percentage and seed vigor index calculated and analyzed as described earlier (Table 2).

RT experiment was placed with these germinated seeds as described earlier in eight replicates using randomized block design. Two weeks later the plantlets were harvested and all growth promoting parameters including chlorophyll pigment content were recorded and analyzed as described in earlier sections (Figure 3).

All the experiments were conducted under constant environmental conditions in eight replicates. Data of the same treatments were pooled together for all the parameters measured and subjected to analysis of variance (ANOVA) and post-ANOVA multiple analysis tests as required. The difference among treatment means was tested at 1% probability level (p=0.01).

Results and Discussion

The present study establishes that a consortium of PGPMs can offer cumulative synergistic efforts over single species inoculation even in presence of organophosphate pollutants and pathogen species though exhaustive studies will be needed to understand exact mechanism of this synergism.

Effects of single specie inoculation vs. consortium on plant growth promotion

In our present study, individual inoculation results by PGPM when compared with results from consortia inoculation experiments have proved that synergistic impact of consortia has better impact on plant growth promotion compared to distributed impact of individual inoculation. Each of the PGPM inoculation contributed to the plant growth but effects were different showing enhancement in a different growth parameter for different PGPM. The results of present study reported that T103 inoculation resulted in maximum root length (20.34 ± 1.53 cm) followed by PGPM2 (18.78 ± 1.85 cm) and PGPM9 (14.98 ± 1.4 cm) inoculation. PGPM2 showed best impact on shoot length increase (13.78 ± 1.32 cm) followed by T103, and PGPM9 (11.91 ± 0.93 cm and 11.15 ± 1.71 cm respectively) as presented in Figure 1. Growth enhancement showed by diazotrophic PGPM2 in *Sorghum bicolor*, is in accordance to the earlier studies by the authors [31] where this microbe has proven plant growth promoting effects on Moong (*Vigna radiata*), Gram (*Cicer arientum*) and Wheat (*Triticum vulgare*). Such growth promotion properties may be probably due to its ability to produce catechol type siderophore, hormone IAA and other enzymes involved in nutrient mobilization and increasing nutrient availability to host plants [31,32,41]. The fact that PGPM2 is showing no host specific bias makes it an ideal broad host range biofertilizer candidate. T103 isolate also posted significant improvement in root dry weight along with increase in shoot biomass (>50%), total chlorophyll (15%) including chlorophyll a and chlorophyll b compared to control (Table 1). Though PGPM2 showed significant increase (70%) in total biomass and root length (93%), its impact on shoot length enhancement (7%) and on chlorophyll pigmentation was non-significant compared to control (Figure 1) as evident from ANOVA analysis followed by

Treatments	Percentage Germination	Seed Vigor Index	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Chlorophyll Total (mg/g fresh weight)
Control	76%	481.65	187.200	78.969	266.169
Consortia	64% (↓15.8%)	1224.80 (↑154.3)	206.603 (↑10.4)	120.738 (↑52.9)	327.341 (↑23.0)
PGPM 9	72% (↓5.3%)	1078.20 (↑123.9)	236.407 (↑26.3)	335.072 (↑324.3)	571.480 (↑114.7)
PGPM 2	60% (↓21.1%)	1126.50 (↑133.9)	139.516 (↓25.5)	63.067 (↓20.1)	202.583 (↓23.9)
T103	72% (↓5.3%)	1464.30 (↑204.0)	223.391 (↑19.3)	81.323 (↑3.0)	304.714 (↑14.5)

Values in parentheses indicate % decrease/increase compared to control

All values indicate mean values ± SD of all eight replicates and analyzed by one-way ANOVA at p=0.01

Table 1: Effect of plant growth promotion properties of single specie inoculation vs. consortium inoculation

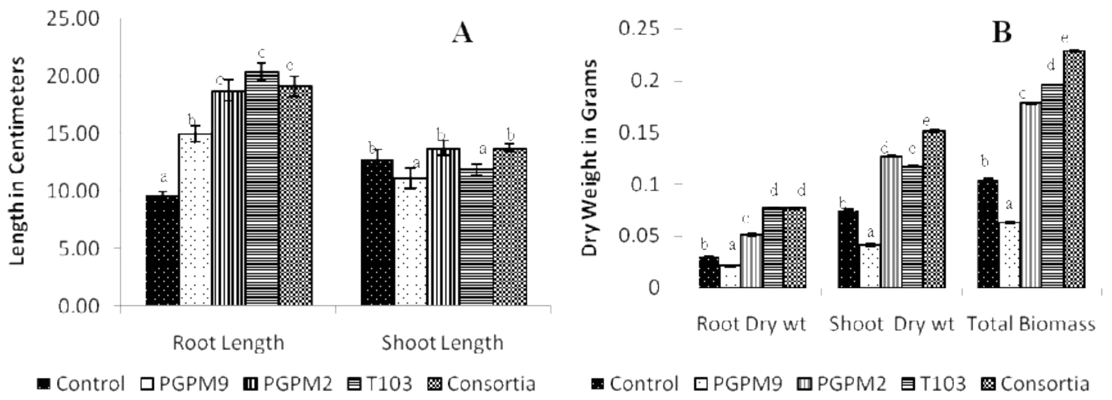
Treatments	Percentage Germination	Seed Vigor Index	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Chlorophyll Total (mg/g fresh weight)
Malathion	44%	167.75	39.650	72.664	112.314
Malathion+Consortia	48% (↑9.9a)	239.40 (↑42.7a)	68.213 (↑72.1a)	125.011 (↑72.1a)	193.224 (↑72.1a)
Methyl Parathion	48%	378.60	29.913	85.897	115.810
Methyl Parathion+Consortia	38% (↓20.8b)	369.87 (↓2.3b)	79.204 (↑164.8b)	68.018 (↓20.8b)	147.222 (↑27.1b)

Values in parentheses followed by 'a' indicate % decrease/increase compared to ML treatment.

Values in parentheses followed by 'b' indicate % decrease/increase compared to MP treatment

All values indicate mean values ± SD of eight replicates analyzed by one-way ANOVA (p=0.01)

Table 2: Effect of consortia on plant growth promotion in presence of pesticides



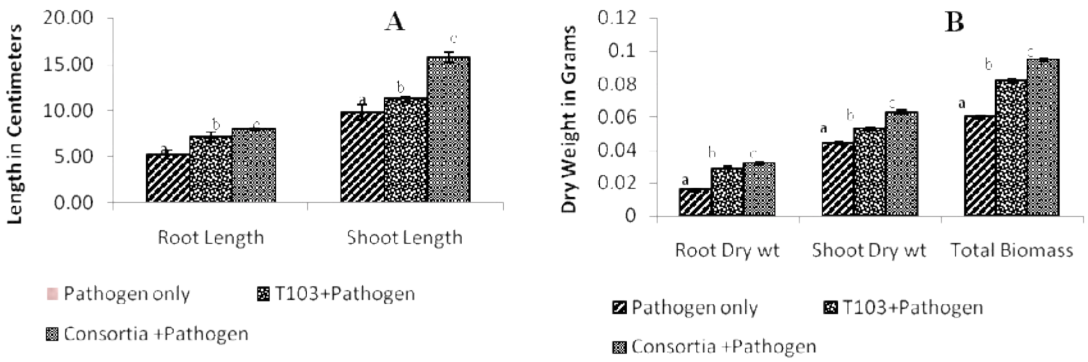
(A) Effect of Consortia on Root and Shoot Length, (B) Effect of Consortia on Root, Shoot Dry Weight and Total Biomass.

All values indicate mean values ± SD; One-way ANOVA applied followed by DMRT. Different letter above bars indicate significant difference among treatments (p=0.01)

Figure 1: Effect of single specie inoculation vs. consortium inoculation on plant growth promotion

DMRT (p=0.01). PGPM9 showed positive impact on % germination (72%) and seed vigor index (1078.2) (Table 1). PGPM2 and T 103 registered comparative efficiency in terms of % germination (60% by PGPM2 and 72% by T 103 respectively) and seed vigor index (1126.5 by PGPM2 and 1464 by T 103 respectively) while un-inoculated control could reach only 481.65 index for seed vigor (Table 1). This showed that PGPM9 inoculation has improved seed vigor 2.5 fold compared to un-inoculated control however its inoculation had most pronounced impact on chlorophyll pigmentation (2.5 fold increases in total chlorophyll and 7 fold increase chlorophyll b). PGPM9 produced IAA and also grew on nitrogen free media [33]. Hence we speculate that this suggestive role in nitrogen fixation along with IAA production and phosphate metabolizing tendencies leads to better chlorophyll development. With individual PGPM9 inoculation improvement in root length and shoot length was significant whereas overall impact on biomass was statistically not significant as evident from Table 1. PGPM9 from our collection is a fluorescent *Pseudomonad* with phosphate metabolizing trait. The noticeable growth promoting impact during early developmental stages of seed germination and also during late developmental stages could have been due to the significant increase in phytosynthetic pigment content (Table 1). Literature suggests essentiality of including microbes showing phosphorus supplementation trait in biofertilizers and proposes an association between phosphate utilization ability and photosynthetic efficiency. Therefore, we can speculate that phosphate metabolizing ability of T103 isolate may have contributed to the stimulation it has provided in plant growth [3,4,16,19]. Slight non-significant decrease reported in root, shoot and total biomass with PGPM9 individual inoculation is in accordance with earlier reports of *Pseudomonas* species on plant growth and yield of chickpea where dual-inoculation of two species had a synergistic effect and increase in plant dry biomass [15].

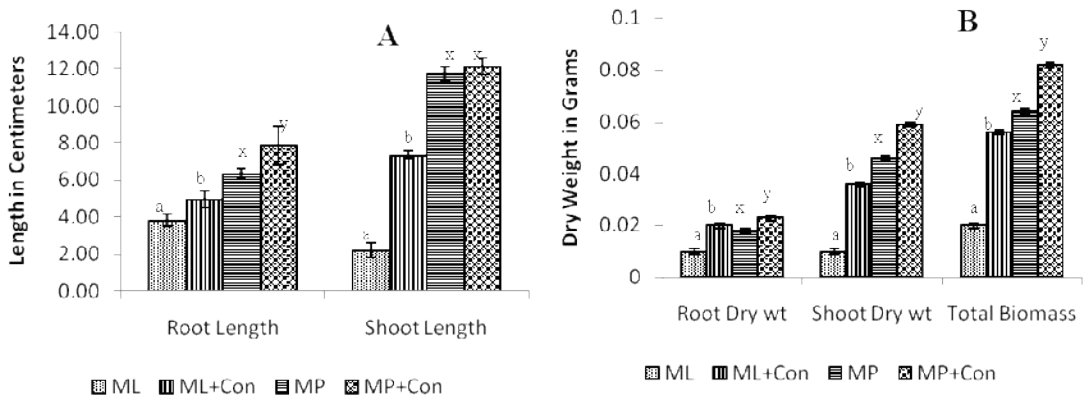
To find out whether a consortia application is better than individual inoculation, comparative study was conducted including un-inoculated control, single species inoculation with either PGPM2/PGPM9/T103 respectively and consortia inoculation with all three members PGPM2, PGPM9 and T103 together. Host plants *Sorghum bicolor* inoculated with the consortium recorded significant improvement in root length (19.1 ± 1.77 cm) and shoot length (13.76 ± 0.7 cm) over un-inoculated



(A) Effect of Consortia on Root and Shoot Length, (B) Effect of Consortia on Root and Shoot Dry Weight and Total Biomass.

All values indicate mean values ± SD; One-way ANOVA applied followed by DMRT. Different letter above bars indicate significant difference among treatments (p=0.01)

Figure 2: Biocontrol efficiency of single PGPM vs. consortium



t(A) Effect of Consortia (Con) on Root and Shoot Length in presence of pesticide Malathion (ML) and Methyl Parathion (MP), (B) Effect of Consortia on Root and Shoot Dry Weight and Total Biomass in presence of pesticide Malathion (ML) and Methyl Parathion (MP)

Letter series a, b represent difference among Malathion (ML) treatments

Letter series x, y represent difference among Methyl Parathion (MP) treatments

All values indicate mean values ± SD; One-way ANOVA applied followed by DMRT. Different letter above bars indicate significant difference among treatments (p=0.01)

Figure 3: Consortium's ability to tolerate organophosphate pesticides

control (9.73 ± 0.43 cm and 12.78 ± 1.66 cm respectively) as represented in Figure 1. Similarly, consortia inoculated plants fared well in total biomass production also (229 mg with consortia against 105 mg in un-inoculated control) as depicted in Figure 1. In fact, consortia inoculation has registered almost 2-fold increase in root length (97%) and total biomass (118%). Two fold augmentations in shoot dry weight (102%) with consortia inoculation indicated prominent positive impact on plant growth promotion however improvement in shoot length (8%) was not significant compared to un-inoculated control (Figure 1). Though both single species and consortium inoculation exhibited slight reduction in percentage germination as presented in Table 1; these differences were found to be statistically non-significant when subjected to analysis of variance at 1% probability level. This marginal reduction in percentage germination could have been a biotic stress response of microbes competing for available nutrients during initial establishment however; improvement in growth parameters supplemented with PGPMs have overcome this lag during later stages of plant growth. These studies prove that the preference of individual microbes to affect specific growth parameter over the other has faded off when applied as a consortium because, the consortium treated *Sorghum bicolor* were superior with respect to every growth parameter studied as compared to any of the individual inoculation effects and much prominent in growth over un-inoculated controls.

Effect of single inoculations vs. consortia in presence of phytopathogen

In this experiment, we compared growth effects between treatments: pathogen inoculation only, T103+pathogen and consortium+pathogen to check pathogen control tendencies of consortia over individual inoculation of T103.When studied for protection against pathogen *Sclerotium rolfsii* on host *Sorghum bicolor*, single specie inoculation with T103 showed statistically significant improvement in root length, shoot length, root dry weight and shoot dry weight over pathogen challenged plants (Figure 2). *Trichoderma* are well known biocontrol agents that produces some common cell wall lytic enzymes as protease, cellulase, pectinase, laccase, gelatinase and lipase along with volatiles and many other metabolites which all are involved in biocontrol [33,42-45]. The efficiency of our native T103 isolate in supporting plant growth and resisting disease could be a result of such enzyme driven metabolic reactions. When consortium inoculated host plants were challenged with pathogens, they exhibited statistically significant improvement in all parameters i.e., root length, shoot length, root dry weight and shoot dry weight as compared to single species inoculation with T 103 only as evident from Figure 2. While T103 single inoculation gave an increment of 36% in root length, 81% in root dry weight and 37% in total biomass in the presence of pathogen, inoculation with consortia provided >50% increase in root length and doubled the root biomass compared to plants inoculated with pathogen only showing significantly better cumulative effect of consortia over individual inoculation. The consortium partner T103 showing not a major influence on seed germination and photosynthetic pigment is in accordance with literature reports [46]. Effect of individual T103 inoculation on shoot length and shoot dry weight recorded 14% and 21% increase respectively. However, consortia combination showed >50% increase in shoot length, shoot dry weight and total biomass. Consortia inoculation provided 12% improvement in root length and 63% increase in shoot length and overall increase in root, shoot and total biomass was statistically significant over individual T103 inoculation when both were treated with pathogen (Figure 2).

Results from the experiment confirm better protection ability of consortia over individual inoculation, clearly indicating a complementary mechanism occurring amongst microbes of consortia thus providing quantifiable improvements in plant performance in its presence. There are studies where two species consortia were tested and reported improved yield parameters for combined inoculation over single inoculation [3,4,15-17,47]. Benefits accrued through consortium inoculation may be due to synergistic output amongst different biocontrol mechanisms adapted by different microbial partners involved.

Effect of consortia in presence of pesticides

The present manuscript brings to fore retention of plant growth promotion abilities in consortium inoculated plants even in presence of organophosphate pesticide contamination. ML and MP (at a concentration of 10 ppm) were tested for their effect on plant growth. This concentration is much higher than maximal allowable concentration for ML (0.5 ppm) and for MP (4 ppm) beyond which concentrations can be lethal to growth promoting bacteria [48,49]. It was observed that pesticide inoculations reduced % germination to 44 and 48 and seed vigor index to 167.7 and 378 only with ML and MP treatments respectively compared to control data recorded as 78% germination and 481.7 seed vigor index (Tables 1 and 2). Consortia treatment recorded an improvement in seed germination by 9% and seed vigor by 43% in ML treated seeds but no significant improvement was registered with consortia treatment in presence of MP (Table 2). Consortia treatment showed improvement on all aspects on plant growth studied: percentage germination, seed vigor index, root dry weight, shoot dry weight, total biomass, root length, shoot length and chlorophyll content as compiled in Table 2. In the presence of ML, consortia inoculation showed significant improvement of 72% in all chlorophyll contents. In presence of MP, consortia inoculation exhibited 3 fold increases in chlorophyll a content while total chlorophyll content was increased by 27% only (Table 2). These differences in controlling the damaging impact of different pesticide may be due to the differential effect of pesticides on photosynthetic process and apparatus. ML and MP have different composition, structure, degradation pathways and degradation products and by products [36] which have different impact on plants thus the extent and loci of damage to chloroplast also vary. Thus we speculate that consortia having similar synergistic action in both cases have different impact on damage control which is evident from chlorophyll studies.

Root length, shoot length and total biomass of ML treated plants were 3.81 ± 0.66 cm, 2.23 ± 0.72 cm and 20 mg (root dry weight 10 mg and shoot dry weight 10 mg) respectively as represented in Figure 3. Significant growth improvement was observed when inoculated with consortium in all measured parameters both in the presence of ML and MP as compared to uninoculated control plants (Figure 3). Statistically significant improvement in total biomass was recorded (180% with ML and 28% with MP respectively) when pesticide treatments were supplemented with consortia. Consortia treatment in presence of ML showed doubling of root dry weight and approximately five fold increase in shoot length and shoot dry weight with little impact (30% increase) on root length (Figure 3). This is probably due to better availability of nutrients and other metabolites required for plant growth augmented by synergistic interaction of microbes in consortia aiding in reducing pesticide induced stress. It is also possible that these PGPM might be utilizing pesticides as carbon/nitrogen/phosphorus sources accelerating degradation of pesticide leading to better growth to microbes and in term better growth of plants. However more experimentation is required to elucidate upon exact process/es affecting plant growth promotion and pesticide degradation in presence of consortium members.

Conclusion

In the past, studies related to plant growth supporting potential of PGP microbes were majorly confined to understand either biocontrol or plant growth benefits [46,50]. None of these studies explored building a consortium with an ability to provide growth promotion, disease resistance and also mitigate negative impact of pesticide residues. Very limited studies are available about pesticide degradation by consortia [29] and even the studies where available are mostly *in vitro* studies where only tolerance and biodegradation potential of individual species were tested [21,51-53]. No study is available where plant inoculation of consortia was tested to study growth protection and pesticide mitigation abilities on plant. Consortia combination presented in the manuscript endowed the plant with significant growth enhancement as compared to individual inoculation indicating better impact of consortia on overall plant growth, pathogen protection and in mitigation of negative impact of presence of pesticide. The cumulative impact of consortia might be due to various hormones and other metabolites contributed by each participating member of the consortia acting in synergistic way and manifesting as overall growth improvement both in the presence of phytopathogen and residual pesticide. We thus summarize that carefully selected combination of microbes show a complementary effect on all aspects of plant development compared to individual inoculation. This is first ever plant inoculation study to prove biocontrol and bioremediating properties of a plant growth promoting consortia. A detail further research is required to fine tune combination's potential and to understand the exact mechanism/s involved however, this study clearly proves that suggested plant growth promoting consortia can be effectively developed as biocontrol and soil cleaning formulation.

Acknowledgements

The authors acknowledge The Directorate of Sorghum Research (DSR) (formerly, National Research Centre for Sorghum (NRCS)), Hyderabad, India, for providing us *Sorghum bicolor* cultivar; CSH 16 seeds and The Central Research Institute for Dryland Agriculture (CRIDA, Hyderabad, India) for providing *Sclerotium rolfsii* culture. The authors thankfully acknowledge the support by Department of

Biotechnology (DBT), Govt. of India for the research grant and Jaypee Institute of Information Technology (JIIT, NOIDA) for providing all necessary support to conduct this study.

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