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PHYSIOLOGY OF *ROSA* GRUSS-AN-TEPLITZ FOLLOWING TREATMENT WITH RHIZOBACTERIA (PGPR), SALICYLIC ACID (SA) AND ZINC SULPHATE

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Abstract

Fresh cuttings from 2-year-old rose cultivar *Rose gruss-an-teplitz* was treated with 2 PGPR used as seed soaking treatment and with Zinc sulphate added to the rhizosphere soil or treated with Salicylic acid (SA) as foliar spray was sown both in the field and in pots (under sterilized condition). Physiological parameters were recorded at flowering.

All the treatments promoted growth parameters and improved the quality of rose water. However, greater increase in all growth parameters were recorded in *Pseudomonas putida* + Salicylic acid treatment. Potted grown plants also showed significant stimulation in growth and reproductive parameters; *Bacillus cereus* being highly effective. The treatment effects were greater in summer but in winter the % increase was also significantly greater over control. Organoleptic analysis of rose water revealed that treatment with *Pseudomonas putida* + Salicylic acid (SA) imparted maximum aroma and sweetness in field grown plants while *Bacillus cereus* inoculation proved most effective in potted plants. Plant was more responsive to treatments in summer than that of winter. Rose petal water from potted plants of winter revealed higher aroma in winter as compared to summer. Similar trend was noted in case of field grown plants.

The incidence of disease was not recorded in treated plants particularly in presence of SA. Both the PGPR and SA act synergistically and can be applied to boost growth, yield and quality of roses.

This is the first report regarding the effect of PGPR, defense hormone SA and Zinc sulphate alone and in combination on roses. The investigation revealed that PGPR can augment the effectivity of Zinc sulphate and SA on growth and reproductivity of rose as well as on its quality e.g., flavor and sweetness of rose water. The combined treatments may be implicated to enhance productivity and quality of roses.

Key words: *Pseudomonas putida*, *Bacillus cereus*, *Rosa gruss-an-teplitz*, Salicylic acid (SA) and Zinc sulphate.

Introduction

The roses have significant value in every culture and region due to their widespread use in celebrations, welcome parties, medical needs, cosmetic uses, and food tonic supplements (rose petal jam) (Jour & Leghari *et al*., 2016).

Arqe-golab, the traditional name for rose water (hydrosol), has been used in religious ceremonies for centuries (Haghighi *et al*., 2008); well-liked in the food industry as an additive for specialized foods (Nikbakht & Kafi, 2008). Rose water has been previously reported for eye and mouth wash, gastrointestinal problems and throat and bacterial infections (Safia *et al*., 2019; Wati *et al*., 2021). *Rosa gruss-an-teplitz* belongs to the botanical group of Chinensis, Locally called surkha or red rose its origin is Asia and middle East. It is used in making perfume and rose water called arq-e-gulab.(Younis *et al*., 2006).The quality and quantity of rose production may vary from species to species and contributes significantly in the horticulture sector of Pakistan.

Use of Plant growth promoting rhizobacteria (PGPR) present in the rhizosphere as well as in association with host plant root is an ecofriendly approach to boost plant growth and productivity (Ejaz *et al.,* 2020). Their effect is mediated by making the nutrient availability, producing phytohormones and through root proliferation (Alori *et al*., 2019).

Salicylic acid is a plant growth promoting hormone having a well-defined defense role (Chen *et al*., 2020). Improvement in yield and quality of flowers apart from time of planting invariably depends on the nutrition enrichment of the plants. Nutrients applied in the form of mineral and organic fertilization are supplied to plants by root and foliar application. Foliar nitrogen application increases essential oil content in some plants and affects essential oil composition (Mehdi *et al*., 2018). Moreover, essential oil content and yield are modified by the rate of applied nitrogen. Zinc application increased the fresh and dry matter production and essential oil concentration of japenese mint (Misra & Sharma, 1991). Similary, the foliar spray with Zinc (100 ppm) in blue sage (*Salvia farinacea* L.) enhanced the length of peduncle and length of main inflorescences (Nahed Abd El-Aziz & Balbaa, 2007). Micronutrients such as iron, manganese and Zinc have important roles in plant growth and yield of aromatic and medicinal plants (AbdEl-Wahab, 2008).

Genotypes, climatic condition, edaphic factors relative humidity, growing season, agronomic practices (such us fertilization, irrigation, harvesting) etc. affect the growth and production of roses (Boskabady *et al.*, 2011).

Present attempt was to evaluate the effects of SA, Zinc Sulphate and rhizobacterial inoculation on the growth and flowering of rose and rose water quality in 2 different seasons.

Material and Methods

Plant material and growing conditions: *Rosa gruss an teplitz* cuttings were taken from 3-4 years old plant cultivated in Floriculture research area, institute of horticulture, University of Agriculture, Faisalabad. Prior to sowing the cuttings were and soaked for 3-4 h in broth culture of *Pseudomonas putida* and *Bacillus cereus* having 10^8cells/ml. Aqueous solution of salicylic acid (10^{-5} M) was foliarly sprayed 30 days after sowing at bud initiation stage and 1% Zn $(SO4)_2$ was added to the rhizosphere before planting. The experiment was conducted under natural condition both in field and in pots. In pots the experiment was conducted under semi sterilized condition. The soil was autoclaved, and the pots were sterilized. In the field, the row to row distance was 30 inches, and the plant to plant distance was 25 inches. The following treatments were made (Table 1).

Physiological analysis and growth parameters of plants

Determination of growth parameters: Data on plant growth was collected after the first leaf emergence. The stem and leaf girth were measured by vernier caliper (Miranda *et al*., 2011), plant spread, root length and plant height was measured according to Paul *et al*., (2010).

Distillation of rose water: Steam/hydro-distillation apparatus was implicated for extracting rose water from fresh petal (Koksal *et al*., 2015).

Organoleptic evaluation of rose water: Rose water of *Rosa gruss an teplitz* was analyzed for Aroma, Color, Texture, Taste/ flavor. Viscosity by using Hedonic scale technique (Peryam & Pilgrim, 1957). Viscosity was measured by viscometer.

Determination of soil physio-chemical analysis: After 90 d of sowing soil samples were collected from rhizosphere of all treated plants for macro and micronutrients determinations. The organic matter (OM), P content and K content was measured according to Walkley & Black (1934) Olsen *et al*., (1954) and Beckett (1964) respectively. Micro nutrients viz., Fe, magnesium, zine Copper and Boron were determined by using atomic absorption spectrophotometer (Sims *et al*., 1989).

Results

Result presented in (Fig. 1) revealed that in winter the T1 (*Pseudomonas putida*) exhibited significant stem and leaf girth while both were maximum in T7 (*Pseudomonas putida* + salicylic acid) followed by T8 (*Bacillus cereus* + salicylic acid). In potted plants, the maximum (500%) stem girth was exhibited by T11 (*Bacillus cereus*), Leaf girth was not significantly affected over control. In summer grown plants, the stem girth showed significant decrease in the control plant though leaf girth was not significantly affected as compared to that of winter grown plants. Except T1 and T2 (which showed nonsignificant increase over control) stem girth was markedly higher in the treatment, maximum increase was recorded in the PGPR+SA The % increase was higher in leaf girth.

During winter, in potted plants no significant difference was recorded between treated and control plants for stem and leaf girth but in summer both the PGPR treatments showed significant increase over control. The T11 was more effective.

Plants spread (Fig. 2) was also increased in summer compared to winter grown plants in untreated control plants. All the treatments increased the height and spread of plant, the % increase was greater in summer and the treatment effect was higher on plant height. Under semi sterilized condition in pots, *Bacillus cereus* appeared more effective.

(Fig. 3) revealed higher number of flowers produced in winter but more buds were recorded in summer. All the treatments increased both flower number and buds Maximum increase was due to PGPR the effect of which was further augmented in presence of SA. In potted plants significant increase in flower opened and the buds produced was recorded T11 being most effective.

The root length was higher in summer over winter (Fig. 4). All the treatments significantly increased the length of root over control. Both the PGPR treatments performed better and the synergistic effects of PGPR with SA was more pronounced particularly for summer grown plants showing maximum increase (144%) as a result of T7 (*Pseudomonas putida* and salicylic acid), In the potted plants the PGPR effect was significantly higher over control. *Pseudomonas putida* and *Bacillus cereus* species treatments were at par to each other in both the season.

(Fig. 5) demonstrated that root weight was higher in summer. Except zinc sulphate treatment all the treatments significantly increased the root weight and the % increase was higher in summer. PGPR was more effective and further augmented the promotive effects of SA > zinc sulphate. For the combined treatment of T7, the root weight was 44 % greater than that of control. In potted grown plants, PGPR treatments differ non significantly over control but during second year, in summer there was significant increase in root weight due to T11.

Table 1. Treatments.

Labelling	Description
C	Control (untreated seeds soaked in distilled water
T_1	Inoculation with *Pseudomonas putida*
T_2	Inoculation with *Bacillus cereus* prior to sowing
T_3	Soil supplemented with 1% Zinc Sulphate
T_4	Inoculation with *Pseudomonas putida* and treatment of rhizosphere soil with Zinc Sulphate (1%)
T_5	Inoculation with *Bacillus cereus* and treatment of rhizosphere soil with Zinc Sulphate
T_6	Plants sprayed with foliar Salicylic acid at leaf bud initiation stage
T_7	Inoculation with *Pseudomonas putida* and foliar spray with SA.
T_8	Inoculation with *Bacillus cereus* prior to sowing and foliar spray with SA.
	Pot studies
T_9	Control (untreated seeds soaked in distilled water
T_{10}	Inoculation with *Pseudomonas putida* before sowing
T_{11}	Inoculation with *Bacillus cereus* prior to sowing

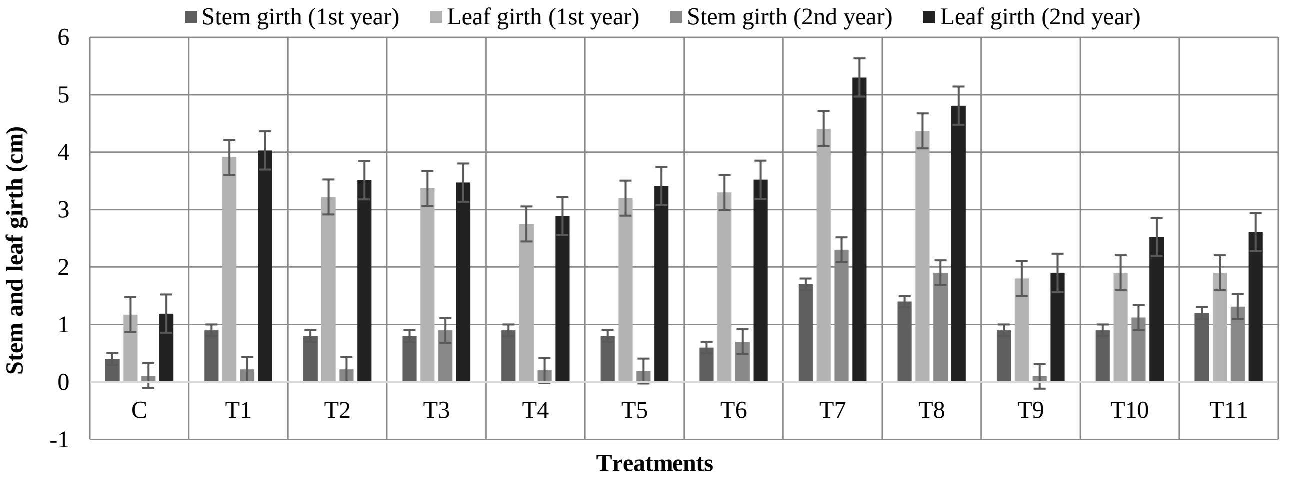

Fig. 1. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on stem and leaf girth of *Rosa gruss-an-teplitz*. C= Control (untreated cuttings soaked in distilled water, T1=Soaking of rose cutting with *Pseudomonas putida*, T2=Soaking of rose cutting with *Bacillus cereus*, T3=Cutting of rose sown in soil supplemented with 1% Zn (SO_4), T4= Soaking the cutting of both cultivars with *Pseudomonas putida* + 1% Zn (SO_4), T5= Soaking the cutting of both cultivars with *Bacillus cereus*+ 1% Zn(SO4), T6= Cutting treated with foliar spray of SA, T7= Soaking the cutting with *Pseudomonas putida* and spray with salicylic acid, T8= Soaking the cutting of the cultivars with Bacillus cereus and spray with SA, T9= soaked in distilled water for 2-3 hours prior to planting in pots, T10= Soaking with inocula of *Pseudomonas putida*, T11=Soaking with inocula of *Bacillus cereus* in pots.

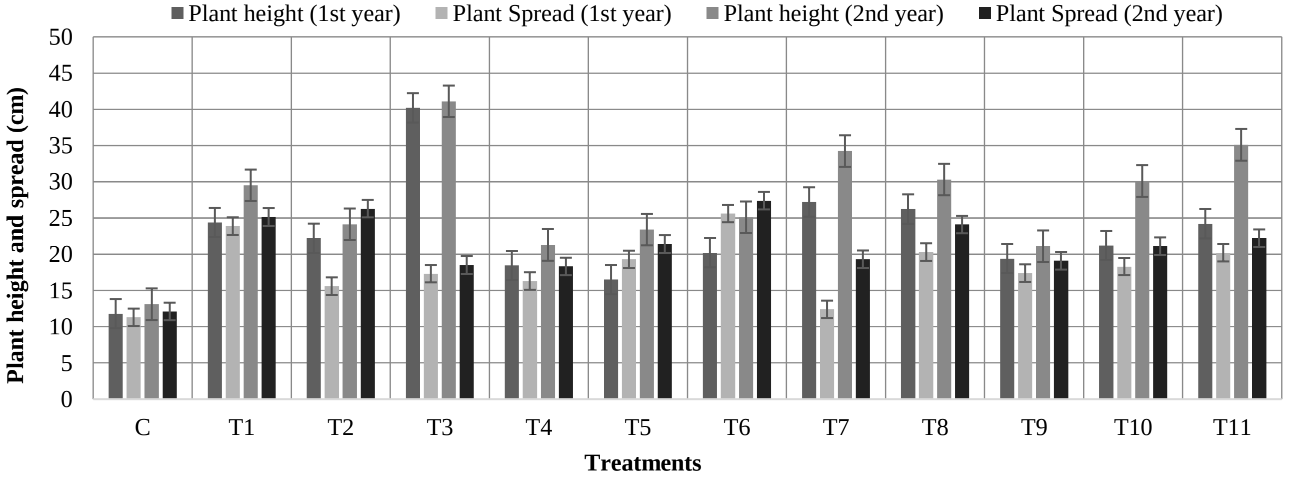

Fig. 2. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on plant height and plant spread of *Rosa gruss-an-teplitz*. Treatment details as in Fig.1 The bar on graph represent std error value. Mean of 5 replicates per treatments.

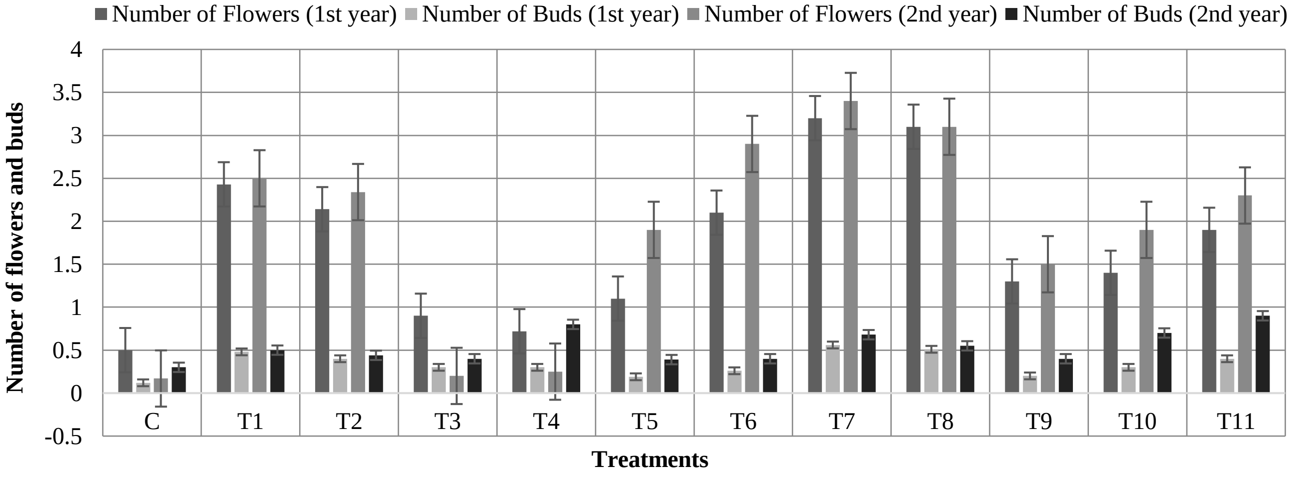

Fig. 3. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on number of flower and buds of *Rosa gruss-an-teplitz*. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

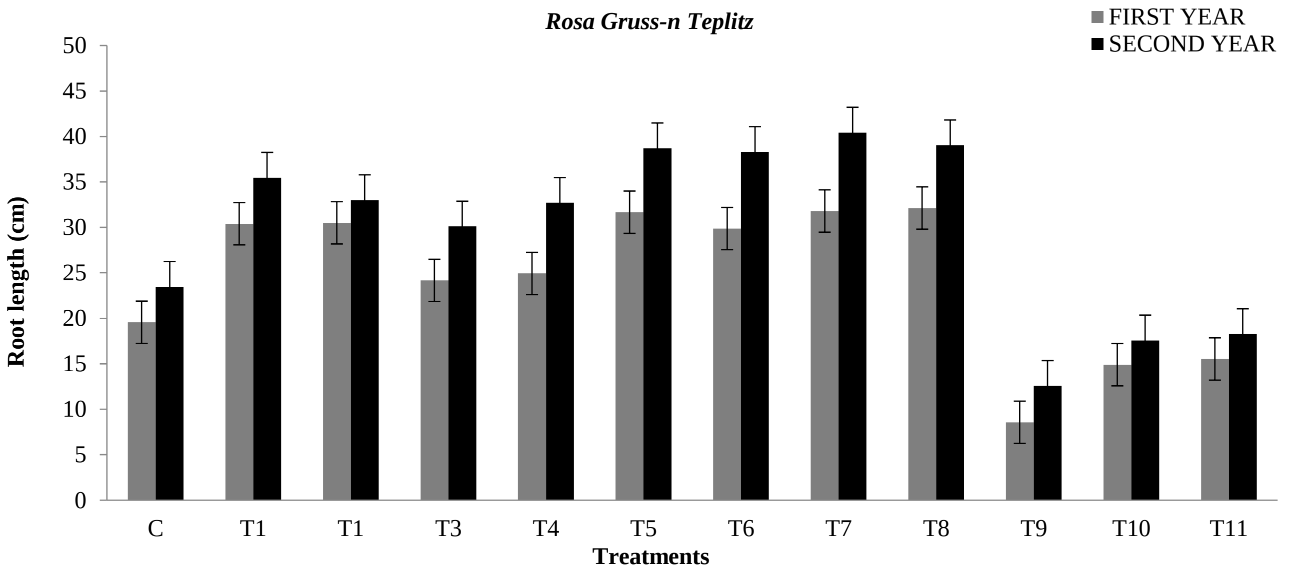

Fig. 4. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on Root length of *Rosa gruss-an-teplitz*. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

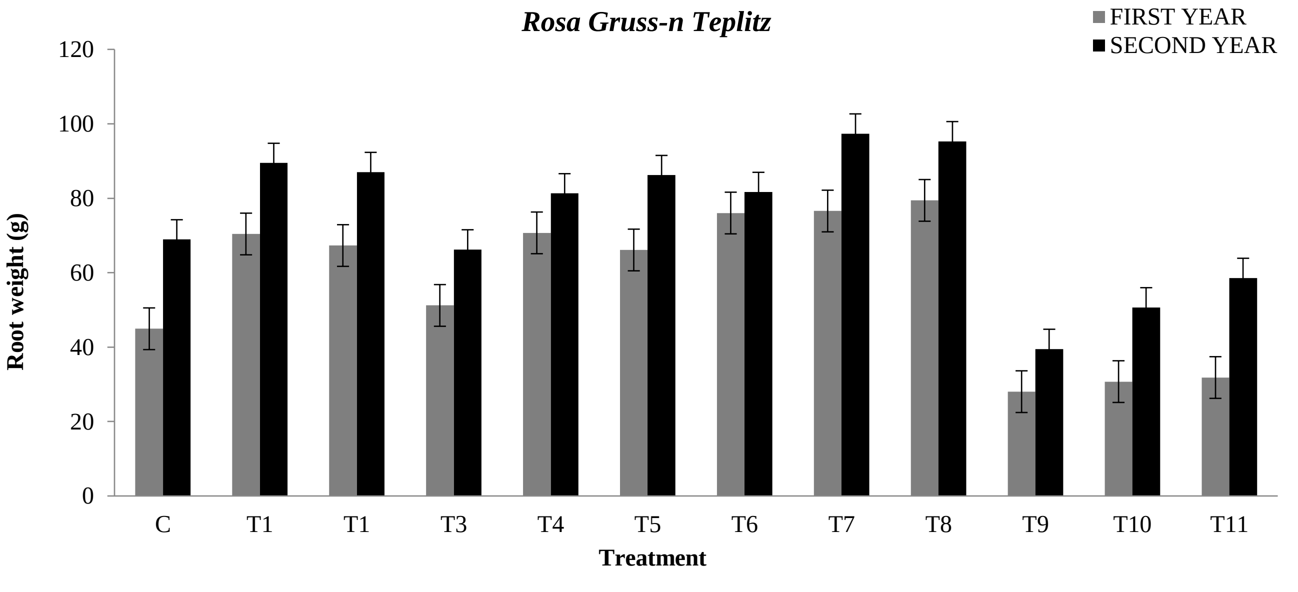

Fig. 5. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on Root weight of *Rosa gruss-an-teplitz*. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

The organic matter was greater in winter than that in summer (Fig. 6). T1 treatment (*Pseudomonas putida*) did not show any marked difference with the control but *Bacillus cereus* showed significantly higher organic matter content in summer. Nevertheless, all other treatments showed tremendous increases over control and the extent of increase was higher in summer. Potted plants followed the similar pattern and showed significant increase in both years over control, summer grown plants were more responsive.

(Figs. 7 and 8) demonstrated that in rhizosphere of winter grown plant K>Ca>Mg>P was recorded in untreated control. All the treatments increased Ca and K content but P and Mg content was increased in PGPR and combined treatment of *B. cereus* in Zinc sulphate and SA + PGPR. Potted plants have greater response to PGPR and showed similar pattern of response to the nutrient. Similar pattern of response to treatments was observed in the rhizosphere of summer grown plants All the treatments showed higher k content but Ca, Mg and P content was significantly higher in PGPR treatments and combined treatment of PGPR + SA and *P. putida* +Zinc sulphate.

(Fig. 9) revealed that in the rhizosphere of winter grown plants, rhizosphere Fe and Mn were higher in PGPR and PGPR +SA treatment only. In potted plants both the PGPR were stimulatory to Fe and Mn, the T11 (*B. cereus*) was more effective.

(Fig. 10) demonstrated enhanced Fe content in PGPR treatments and Fe and Mn in the PGPR+SA contents during summer. Potted plants rhizosphere showed stimulatory effects of both PGPR on Fe and Mn contents of rhizosphere soil over untreated control plants rhizosphere.

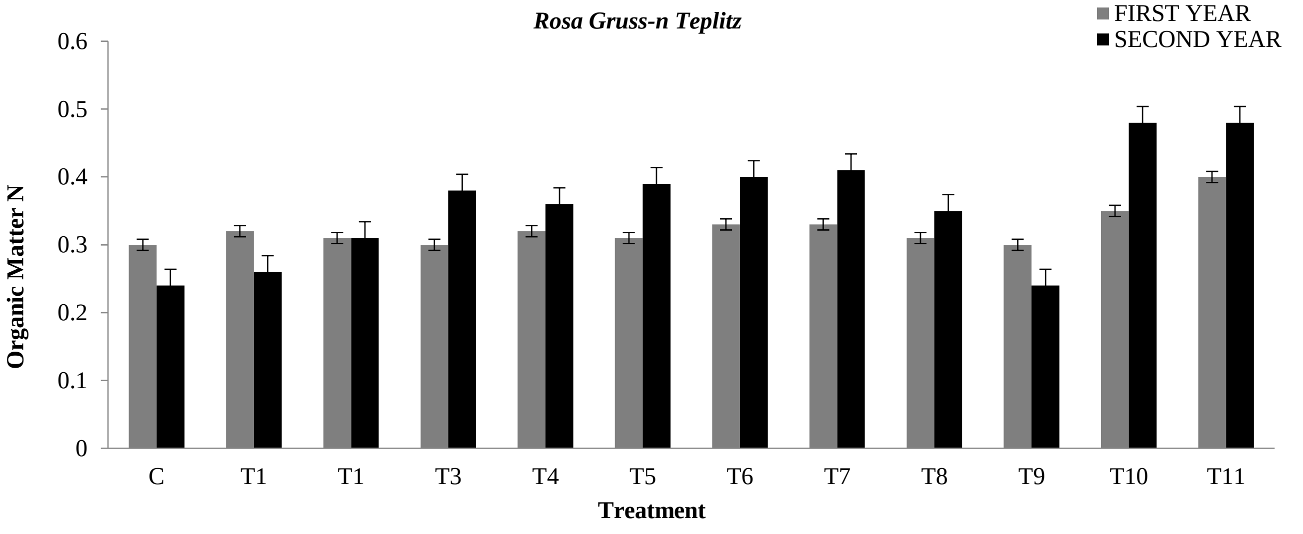

Fig. 6. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on organic matter of *Rosa Gruss-an-teplitz*. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

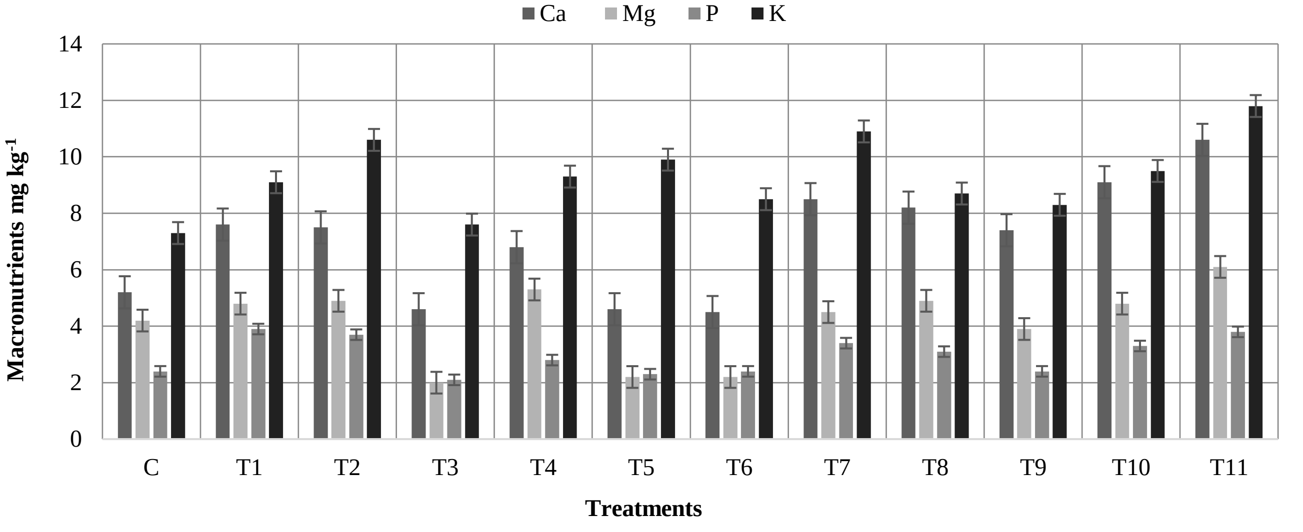

Fig. 7. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on macronutrient of *Rosa gruss-an-teplitz* 1st year. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

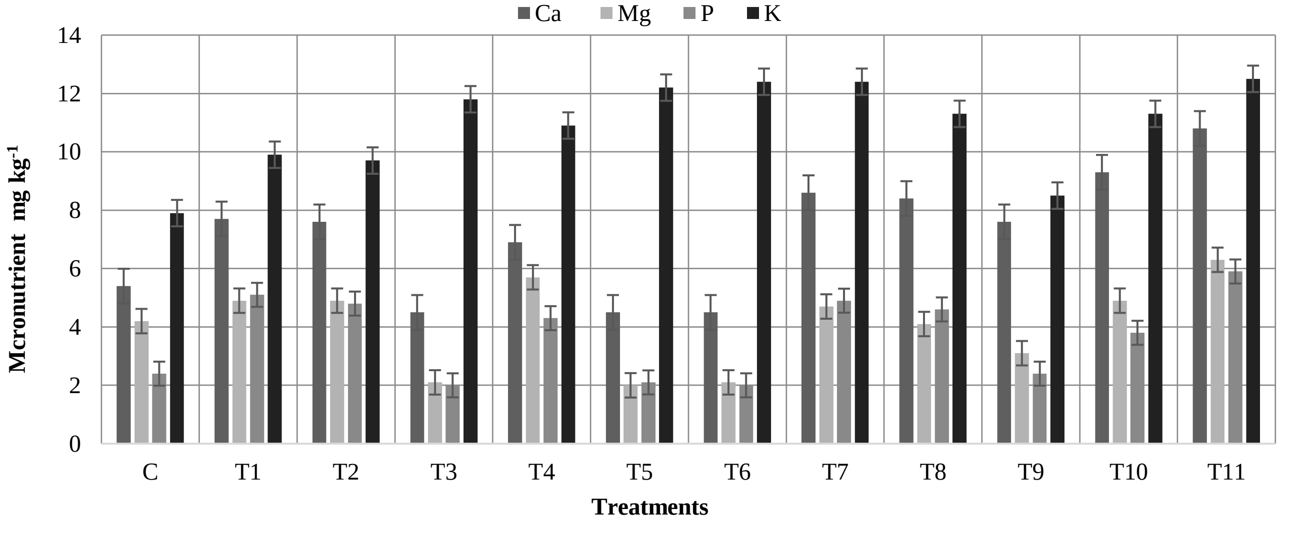

Fig. 8. Effects of Rhizobacteria, Zinc Sulphate and Salicylic acid on macronutrient of *Rosa gruss-an-teplitz* 2nd year. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

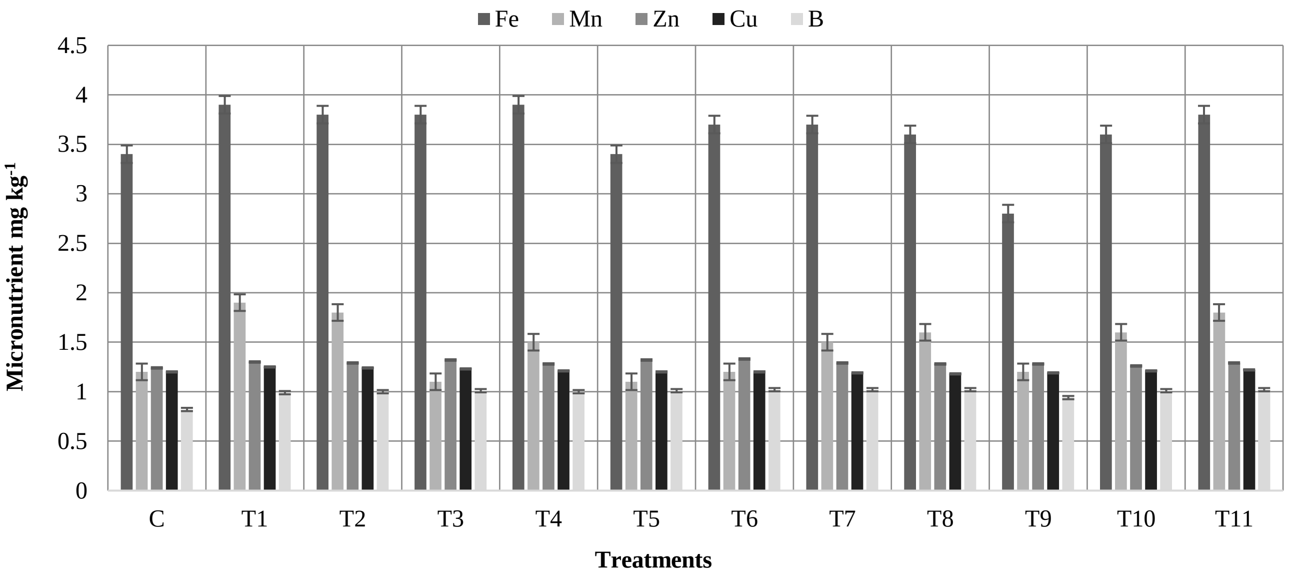

Fig. 9. Effect of Rhizobacteria, Zinc sulphate and Salicylic acid on micronutrients of *Rosa Gruss-an-Teplitz* 1st year. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

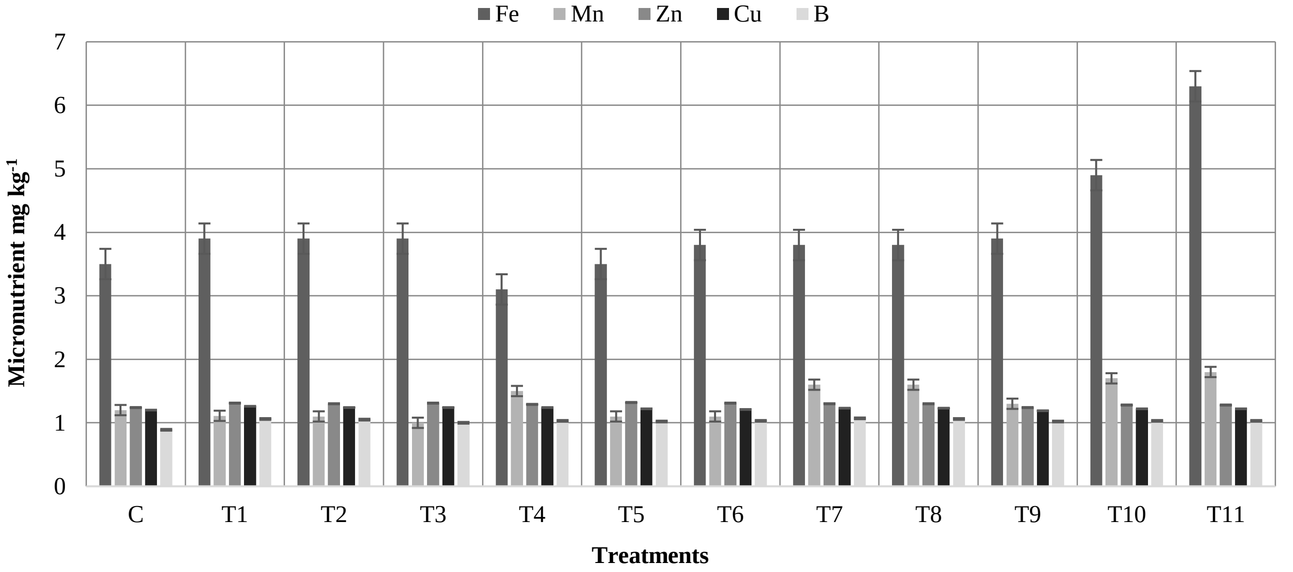

Fig. 10. Effect of Rhizobacteria, Zinc sulphate and Salicylic acid on micronutrients of *Rosa Gruss-an-Teplitz* 2nd year. Treatment details as in Fig. 1. The bar on graph represent std error value. Mean of 5 replicates per treatments.

Organoleptic analyses of rose water revealed variations in flavor and aroma (Table 2). The color, texture, and viscosity of the rose water under the various treatments were all the same. All plants that had been treated had stronger aromas. The T2 (*Bacillus cereus*) treatment produced the most aroma, followed by the combined T7 (Salicylic Acid + *Pseudomonas putida*) treatment in field-grown plants. Rose petal water from potted plants revealed higher aroma in winter as compared to summer. Similar trend was noted in case of field grown plants.

Discussion

Present result corroborates earlier finding of Nehra *et al*., (2014) who demonstrated PGPR induced stimulation in growth and its further augmentation with Salicylic acid.

The effect of PGPR was greater than salicylic acid or zinc sulphate applied alone. The rhizobacteria appeared to assist Salicylic acid to augment growth parameters. Sharaf (2019) demonstrated stimulation in root and shoot growth and metabolic activities following the combined effect of rhizobacteria and Zinc.in *Pisum sativum.* Inoculation of PGPR in combination with Salicylic acid, Zinc and Indole acetic acid increased Nitrogen content and plants dry weight significantly (Abdelaziz *et al*., 2018). Maximum growth was recorded with 0.5 M and 0.25 M ZnSo4 (Iqbal *et al*., 2021).

In field grown plants, photo assimilates appeared to be accumulated in stem during winter leading to increased stem growth which are utilized for increased leaf growth as evidenced by the greater leaf girth and reduced growth of stem in summer. The stimulatory effects on stem girth were evident in field grown plants only in PGPR treatment demonstrating the PGPR mediated phytohormone production (Kashyap *et al*., 2019).

Table 2. Organoleptic analysis of Rose water (*Rosa gruss-an-teplitz*).

Treatments	Aroma (ouE/m^3)		Color		Texture		Viscosity		Taste/Flavor	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Control	6.5	6.5	Color-less	Colorless	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Sweet	Sweet
Pseudomonas putida	8	7.5	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
Bacillus cereus	8.5	7.3	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
1% Zn $(SO_4)_2$	7.3	7	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
1% Zn $(SO_4)_2$ +*Pseudomonas putida*	8	7.5	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
1% Zn $(SO_4)_2$+*Bacillus cereus*	7.5	8	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
Salicylic Acid (SA)	7.5	8	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
Salicylic Acid + *Pseudomonas putida*	8.3	8.5	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
Salicylic Acid+ *Bacillus cereus*	8	8	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Aromatic and Sweet
Potted plants untreated control	7	6	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic	Aromatic
Plants inoculated with *Pseudomonas putida*	7.5	7.5	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Sweet
Plants inoculated with *Bacillus cereus*	8.3	7.5	Color-less	Color-less	Non-Sticky	Non-Sticky	Non-Viscous	Non-Viscous	Aromatic and Sweet	Sweet

This may be attributed to the biomass accumulation in stem to protect the plant from low temperature stress. In summer, enhanced production of growth promoting hormones viz. IAA and GA were recorded. However, in potted plants both the stem and leaf girth were increased in PGPR treatments. This difference may indicate the competitiveness of the PGPR applied with the native microorganism in field as well as other environmental factors. Similar pattern was reported for PGPR induced increase in plant height and plant spread.

The PGPR, *Bacillus subtilis* and *B. safensis* in *Zea mays* mitigated environmental stress by reducing ethylene level in host plant and improved growth (Misra *et al*., 2020). The rhizobacteria enhanced the dry weight, plant height, root length, root average diameter, root surface area, root volume and chlorophyll content of plants (Li *et al*., 2020).

The field grown plants showed more flowers opened; noteworthy the PGPR produced buds much greater in number than that of control. PGPR exhibited synergistic response with SA to produce higher number of flowers. The potted plants showed PGPR induced significant increase in flower and buds in summer grown plants. *B. cereus* effect was more pronounced as it is endospore forming bacteria and can tolerate stress more effectively (Chu & Chung, 2020). The PGPR induced increase in root length and root weight may be attributed to the phytohormone production (Khan *et al*., 2020).

PGPR and salicylic acid both had significant influence on shoot and root growth of wheat (*Triticum aestivum* L.) and in woody plants (Nawaz *et al*., 2020). The PGPR along with other physiological functions, also promote bud formation and flowering of a plant. *Pseudomonas putida* and *Bacillus subtilis* significantly increased bulb diameter, length and weight of *Hyacinthus orientalis* L. cv. Azolos. (Karagöz *et al*., 2019).

Higher flower number per square meter in marigold (*Calendula officinalis* L.), was observed following inoculation of *Enterobacter* and *Pseudomonas* species. Leaf number, stem branches, receptacle diameter, and capital diameter were observed (Hormozinejad *et al*., 2018).

Another very important function of salicylic acid is that it lengthens the vase life of rose flower. SA in 0.5 to 1.5mM concentration can prevent oxidation and increases pre and post harvested rose flowers (Kazemi *et al*., 2018). The results revealed that SA in postharvest flowers of *Nicotiana plumbaginifolia* inhibit metabolic process and activate antioxidant enzymes which retard senescence (Nisar *et al*., 2021).

The lower organic matter detected in the rhizosphere of summer grown plants may indicate the utilization of organic matter by plants grown in summer to boost up the growth of plants after the winter dormancy. The Ca, Mg, P and K were higher in PGPR treatment and synergistic effects were observed with SA in winter, but in summer *Pseudomonas putida* was more effective in field grown plants. Similar pattern was followed in summer with much greater % increase in Ca and K particularly with Salicylic acid.

In the rhizosphere of potted grown plants both the PGPR significantly increased the concentration of K>Ca,>Mg>,P in both the season. This may be attributed that under sterile condition PGPR inoculant applied has its sole effect and do not have to compete with indigenous microbes as in field grown plants.

Significant increase was observed in rhizosphere soil during winter regarding the concentration of Fe, >Mn >B following inoculation of PGPR and PGPR + salicylic acid. Similar effects were observed in maize plant (Alaey *et al*., 2011). Khan *et al*., (2021) showed the positive effects of PGPR and Salicylic acid on IAA (73%) and GA (70%) contents but decreased (55%) the ABA content of shoot (Khan *et al*., 2021). Increase in the content of Cu and Manganese was observed in alfalfa with PGPR (Miri *et al*., 2016). The observed lower % increase in Fe and Mn in the rhizosphere of PGPR or PGPR +SA treatments in their rhizosphere soil may be attributed to the accelerated growth of the plant after winter dormancy break.

The higher organic matter content was recorded in the rhizosphere soil of potted grown plants treated with PGPR. *Bacillus* being more effective. Ullah *et al*., (2019) reported significant stimulation of organic matter in maize treated with PGPR. The OM participated for increase in moisture content, micro and macronutrients etc (Villa *et al*., 2021).

The aroma of rose water was higher in plants inoculated with *Bacillus* sp. All the treatments enhanced the aroma, taste and flavor of rose water. The PGPR and PGPR+SA being more effective. Winter grown plants responded to treatment more effectively than that in summer. The aroma and flavor imparted to rose water due to treatments may be attributed to the production of aromatic compounds e.g. mono and diterpenes (unpublished data). Cappellari *et al*., (2020) demonstrated the induction of monoterpenes and phenolics following SA or methyl jasmonate combined with PGPR in Mentha x piperita which might may be responsible for enhanced aroma and taste of rose water.

The sweetness was also increased in potted plants. Banchio evaluated the effect of Plant growth promoting rhizobacteria such as *Pseudomonas fluorescens, Bacillus subtilis, Sino rhizobium meliloti,* and *Bradyrhizobium* sp. in *O. majorana* and stated that *P. fluorescens and Bradyrhizobium* sp., help the plant not only in growth of shoot length, weight, leaves and nodes proliferation but also increased the production of essential oil when compared with control (Banchio *et al*., 2008).

Conclusion

PGPR effectively promoted growth and flower production as well as imparted aroma and sweetness to rose water. PGPR acted synergistically with SA in field grown plants. Zinc sulphate was least effective compared to PGPR and SA but it is inferred that zinc sulphate effect can be augmented when used in association with PGPR. The plant was more responsive to treatments in summer. The organic matter and macro and micronutrient contents of rhizosphere soil can also be significantly enhanced by PGPR treatments which can be beneficial for successive crop. *Pseudomonas* sp., was more effective under field condition, whereas, *Bacillus* sp., was more effective in plants grown in pots under semi sterilized condition. PGPR and SA treatments enhanced aroma and sweetness in rose water. PGPR in combination with SA is recommended for enhanced growth, yield and better quality of rose water.

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