Biological Forum – An International Journal **16(8): 129-139(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of *Rhodopseudomonas* Strains Biomass on Morphological Parameters of Seed Germination of *Capsicum annuum* L

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(Received: 22 May 2024; Revised: 13 June 2024; Accepted: 12 July 2024; Published: 14 August 2024)
(Published by Research Trend)

ABSTRACT: Sustainable agriculture and horticulture are currently an ongoing research effort for reaching communities current food demand without understanding the future requirement and development. Quality farming is needed for the maintenance of soil fertility; use of the wide application of manmade agrochemicals (e.g chemical fertilizer) has been a notable contributor to environmental pollution. This research article aims to analyze the potential of *Rhodopseudomonas* strains a purple non-sulphur bacteria, which promotes plant growth. When used as a commercialized bio-fertilizer *Rhodopseudomonas* strains is examined based on two main features of sustainability, including the effects on plant growth, environmental impact, and easy production. The productiveness is dependent on the improvement of plant growth through the secretion of extracellular metabolites, resistance to abiotic stresses, bioremediation of heavy metals, and mitigation of greenhouse gas emissions. This article suggests the vitally important roles of *Rhodopseudomonas* strains as an effective bio-fertilizer in Agriculture and Horticulture. However, the amount of production and application earned more attention. The potential substrates ranging from various waste streams and formulation methods for *Rhodopseudomonas* strains production are summarized to discuss environmental and economic stability.

Keywords: *Rhodopseudomonas strains*, Bacterial biomass, Bio-fertilizer, Seed germination, Agriculture and Horticulture.

INTRODUCTION

Modern farming techniques are very costly and helpful in increasing food and agriculture demand, mostly to the natural environment, such as loss of soil fertility and environmental pollution. Problems like high mineral fertilization can be decreased by cost effective use of greener biofertilizers. The plant growth promoting rhizobacteria (bacteria) (PGPR/PGPB) is widely used as biofertilizers have been reported due to their important effects to promote plant growth and yield (Basu *et al.,* 2021). Plant growth is promoted by free living microorganisms. To increase worldwide crop production there is usage of soil microbes; thus, producing a new bacterial genus as a novel bio-fertilizer to increase plant growth and yield is highly desirable. *Rhodopseudomonas palustris (R. palustris)* is a free-living bacterium species under the genus *Rhodopseudomonas*, phylum Proteobacteria. For the biosynthesis of energy this bacteria uses various biotic and abiotic factors. *Rhodopseudomonas palustris* is one of the purple non-sulphur bacteria (PNSB). The four modes of Metabolism are chemoautotrophic, photoautotrophic, chemoheterotrophic, and photoheterotrophic, increasing versatility and flexibility, this is widely used in biotechnological applications because of its multifaceted modes, including a promising bio-fertilizer to be utilized in agriculture. Some beneficial functions of *R. palustris* include (i) nitrogen (N) fixation (Wong *et al.,* 2014), (ii) heavy metal remediation (Batool *et al.,* 2017) and (iii) methane (CH_4) emission mitigation in saline paddy fields (Kantha *et al.,* 2015). Indole-3-acetic acid (IAA) (Wong *et al.,* 2014) and 5-aminolevulinic acid (ALA) are the plant growth promoters produced by the *R. palustris.* They also absorb sodium ions with the help of exopolymeric substances. Which help in stimulation of plant growth and improved hostility to environmental stresses. Another beneficial feature of *R. palustris* is that it can be prepared in a low-cost effective culture medium facilitating high production (Lo *et al.,* 2020). These features make *R. palustris* acceptable as a biofertilizer and biocontrol agent for numerous agricultural applications, although an overview on *R. palustris* multi-functional traits is still lacking. Research has demonstrated that inoculating plants with PGPR as bio-fertilizers can be an effective strategy to increase crop growth. (Backer *et al.,* 2018) have highly reviewed and created the roadmap to commercialize PGPR as biofertilizers. Basu *et al.* (2021) also have

discussed about different aspects of PGPR as biofertilizers, including the beneficial applications, mechanisms of actions and commercialization pathways. Specific PGPR strains have also been studied and covered in different aspects, including *Bacillus* spp. and their role in plant growth development and stress reduction (Radhakrishnan *et al.,* 2017), the impact of bacteria (*Azospirillum* species), in agricultural and environmental applications (Cassán *et al.,* 2020), and the different possible operational mechanisms of rhizobia (Jaiswal *et al.,* 2021) were identified. Most of the highlighted PGPR are from various microbial taxa, with a lack of significance on PNSB to showcase and exploit as biofertilizers. The inclusion of PNSB like *R. palustris* as beneficial bio-fertilizers is often failed to see, probably due to the no proper commercialization or limited application for growth promotion of crops, compared with other bacteria. Recent work by Sakarika *et al.* (2020) leads to a pathway to purple non-sulphur bacteria, providing a detailed explanation on the use for plant growth, environmental aspects, and preliminary cost-effectiveness analysis. The way of transitioning from research to application has also been discussed focused on the shelf-life issues and application methods. The discussion by Sakarika *et al.* (2020) is from a relatively high perspective, covering all the phototrophic microorganisms. Our study serves as an updated work that shows the benefits of a specific strain, mainly *R. palustris*, as promising bio-fertilizers based on three main points of sustainability, including the effects on plant growth, environmental impact, and easy production. The value of *R. palustris* in agriculture is reviewed and discussed, from their benefits on plant growth to synthesize as low-cost commercial agricultural inputs to promote stable agriculture. The commercialization and application of PGPR as a biofertilizer, such as *Pseudomonas* spp. and *Bacillus* spp. among many rhizobacteria, has become a main component of sustainable agriculture practices in many countries (Mustafa *et al.,* 2019). EmFarma Plus commercialized photosynthetic bacteria by a manufacturer called Probiotics Polska (Mącik *et al.,* 2020) in Europe. Nun Kaew *et al.* (2014) have demonstrated the potential of *R. palustris* as a plant growth-stimulating bacterium at a lower cost than commercial ALA. The successful development of an *R. palustris* formulation is mainly dependent on using sustainable materials and application methods, such as utilizing horticultural oil as a potential additive for the liquid-based production of *R. palustris* (Lee *et al.,* 2016). Lo *et al.* (2020) have developed a low-cost medium to facilitate large-scale production of *R. palustris*. These findings suggest that *R. palustris* has the ability to be develop a safe, cost-effective and easy-to-process microbial formulation that would facilitate its practical use in the field at a wide scale. Despite previous studies they have successfully determined the stable *R. palustris* formulations using either liquids or solids as carrier materials or efforts to achieve low-cost production, the commercialization of *R. palustris* inoculant is relatively new and still has a long way to go. Timmusk *et al.* (2017) presented the challenges and procedures for the commercial synthesis of PGPR/PGPB products. Backer *et al.* (2018) have proposed ten steps to develop and commercialize PGPR-based inoculants, while Basu *et al.* (2021) have discussed the industrial production pathways and constraints in developing PGPR bio-fertilizers. Recent work by Sakarika *et al.* (2019) has explained the roadmap for research and valorization of PNSB products used for plant production. Although these reviews have extensively discussed the plan of action or space to commercialize PGPR- or PNSB-based biofertilizers, the naming and application features are not widely discussed. The commercialization strategy stops as there is no correct quality check available for bio-fertilizers at the moment (Basu *et al.,* 2021). For the application of biofertilizers on crops farmers requires correctly labeled information. The quality of bio fertilizers is hard to understand as there synthesizing process is different from chemical fertilizers (Malusà *et al.,* 2016). The Potential of Rhodopseudomonas Palustris as a Bio-Fertiliser for Sustainable Agriculture Sabki *et al*. (2021). Establishing quality control guidelines for biofertilizers is important to avoid failure in the fields. A highly produced quality standard can help to inform the inoculation efficiency of bio-fertilizers and gain confidence among consumers that leads to creating a more demand in the fertilizers market.

MATERIAL AND METHOD

A. In Vivo – plastic tray experiments

(i) Sample preparation of *Capsicum annuum*.L. *Capsicum annuum* (Green Chilli) crop is one of the important crop in Telangana State of India belong to Nightshade family. It is consumed in the form of spices in different curries and pickles used in homemade items. The present research was conducted with short duration of *Capsicum annuum* (green chilli) crop. The main objective is to assess the impact of *Capsicum annuum* on morphology, growth and yield parameters with soil amendment of selected strains of RM01, RM02 and RM03 bacterium at the time of sowing. *Capsicum annuum* (Green Chilli) seeds were shown in measurement units plastic pots with 16 cm height and 12 cm radius at the top filled with two kg of soil. The sowing was done during the first week of March 2022 in a Green House at Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana, India. The maximum and minimum temperatures recorded during the experimental period rang from 20.5°C to 32.8°C. The crop was maintained stress free conditions by irrigating at regular intervals.

Preparation of *Rhodopseudomonas* sp. biomass: *Rhodopseudomonas* sp. RM01, RM02 and RM03 were grown in modified Biebl and Pfennig's (1981) medium. Three bacterium were grown in a ten litter capacity cultured bottle and incubated under 3,000 lux illumination intensity with 28±2^{o}C temperature in anaerobic conditions. Indigenous *Rhodopseudomonas* sp. self-flocculated and on the fifth day the culture were taken for preparation of experimental biofertilizer. The fine powder of soil was thoroughly mixed with ratio of 1: 3 (Biomass: Soil).

(ii) Treatment of *Capsicum annuum*.L with PNSB culture. Fifty milliliters of logarithmically grown cultures of all three strains, such as *Rps. palustris* RM01, *Rps. rhenobacensis* RM02, *Rps. palustris* RM03 centrifuged at 6000 rpm and pellet were washed with distilled water and again centrifuged to get pellet. 50 ml of distilled water were added to this pellet and resultant suspension was added to soil of each pots at time of sowing chilli plants (Arunasri, 2004). Biofertilizer studies were carried out using horticulture crop *i.e., Capsicum annuum* (green chillies) for the current study. The efficiency of three strains were tested by observing the morphological and biomass parameters like plant height (cm),number of leaves, leaf area (cm^2/pl), root length (cm), root volume (ml/pl), dry weight(g/pl) of leaf, stem, root and total biomass were tested with all the three strains of PNSB. The observation were recorded at 18th day, the experiment were conducted with three bacterial strains (*Rhodopseudomonas palustris* RM01, *Rhodopseudomonas rhenobacensis* RM02, *Rhodopseudomonas palustris* RM03). Four Plastic trays were used for seeding each tray contains fifty cavities of depth 3.8 cm, size 54 × 26 cm × 4.5(L×W×H) pack of one tray. Garden soil was taken into the plastic trays. For the seed germination water sprinkled were twice day. The sprouts were observed from tray. The sprouted grains are separated carefully. Out of four plastic trays, one tray used for *Rhodopseudomonas palustris* RM01 biomass (1g/kg soil) second tray used for *Rhodopseudomonas rhenobacensis* RM02 biomass (1g/kg soil) 3rd tray used for *Rhodopseudomonas palastris* RM03 biomass (1g/kg soil) and last one for control each of which contains fifty seed capacity trays. The seed germination were recorded at regular intervals and plants from all the three treatments were harvested on zero day to sixth month and parameters such as plant root length (cm), root volume (ml), shoot length (cm), leaf length (cm), leaf width (cm), Total height of the plant, number of leaves, number of branches, number of clusters, number of pods, plant dry biomass (g/pl), seed weight (g/pl) and husk (seed coat) weight were recorded.

The germinated seeds were removed carefully at seedling stage and washed out. The shoot and root measurements of the plants were calculated. For studies of biofertilizer efficacy of phototsynthesic bacteria, the soil with photosynthetic bacteria dry biomass powder were taken separately at the dose of 3grams (RM01, RM02 and RM03) bacterial biomass per kg of soil separately. The shoot length, leaf length & leaf width, root length and the number of leaves were measured at 8th day,10th day, 12th day,16th day and 18th day and height of chilies plants were measured at the 30th day , 60th day, 90th day, 120th day, 150th day and 180th day. The growth parameters like plant height, leaves of the plant, shoot and root length; fresh and dry weight of shoot and root were measured at 180day. Studies of yield number of fruits, average size of fruits, weight of seed in each fruits and total number of seeds/fruits were also measured at 180days.

Table 1: Isolation of Bacteria and production of large scale biomass (biofertilizer).

Isolation of PNSBs from Water and Soil samples (RM01, RM02 and RM03)
↓
In vivo cultivation of PNSBs (RM01, RM02 and RM03)
↓
Microbial cell harvesting (Growth suitable media/selected media for production of large scale biomass)
↓
Centrifugation at 6000 rpm of media
↓
Collect bacterial pellet(Biomass)
↓
Mixed with seed + bacterial biomass (Biomass used as biofertilizer) + garden soil
↓
Crop cultivation in a plastic tray

Table 2: Field screening of most effective bacterial biomass and production of crop yield.

Isolation of Bacteria from soils and water
↓
Laboratory screening of microbes for seed germination and plant growth
↓
Screening of seed germination & germination percentage (%) of chilli plants treated with microbial biomass along with the control in green house
↓
Field screening of effective of bacterial biomass in chilli crop as a biofertilizer.
↓
Refinement of inoculums(Zero day to180 days)
↓
Environmental impact test and substantiation of microbes biomass
↓
Production of crop yield (Plant leaf length, width, root, shoot length, root volume, fruit length, Fruit size, seed size and weight of (*Capsicum annuum*) fruit (µg/gram fruit) & some other parameters)

RESULT

Among the various biotechnological applications of Purple Non-Sulfur Bacteria, its usage as biofertilizer is gaining importance in recent times. The results obtained from field experiments with soil amendment of PNSB biomass to a crop plant were presented. Pot experiments were carried out to quantify the effect of three PNSB strains as soil amendment on the seed germination of *Capsicum annuum* L and plant growth under experimental conditions. In horticulture and agriculture used two types of chillies and from that one is green chilli which are used in daily cooking as a vegetable and second one is normal chillies used for a commercial use in pickles and industrial purpose Current study were conducted on green chilli crop (*Capsicum annuum*). In the market so many bacterial based biofertilizer are available but phototrophic bacteria as pigment based biofertilizer, has shaded therefore we have selected for study as biofertilizer. We were selected green chillies used for study to check the effect of crop yield by using PNSB as a biofertilizer.

A. Preparation of bacterial biomass for biofertilizer studies

The present investigation were carried out to study the effect of bacterial biomass on chilli crop, using as biofertilizer compared with control plants (without biomass biofertilizer). The biomass of three PNSB strains (RM01, RM02 and RM03) were taken individually, 3 grams of biomass were added to 100 grams of normal garden soil and mixed well and chilli seeds were sowed in tissue culture poly thin tray, which were filled with soil with bacterial biomass.

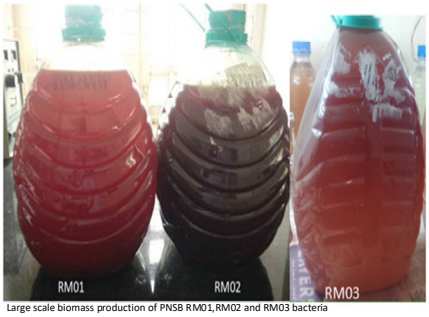


Fig. 1. Bacterial culture of three PNSB strains.

(i) Seed germination of *Capsicum annuum* L. Seed germination and plant growth conditions of green chillis of selected model green chilli seeds, were widely studied in the literature, to investigate the individual and the combined effects of control RM01, RM02 and RM03 biomass biofertilizer treatment on the rate of seed germination and plant growth.

Fifty chilli seeds were sowed in three plastic trays separately, each plastic tray containing fifty seeds single treatment were used for all the experiments carried out under the same conditions to confirm the reproducibility. Seed samples were divided into four groups as shown as follow:

Sample-1: Plastic tray containing fifty seeds used as control (without any treatment biomass).

Sample-2: Plastic tray containing fifty seeds used as test (treatment RM01 biomass).

Sample-3: Plastic tray containing fifty seeds used as test (treatment RM02 biomass).

Sample-4: Plastic tray containing fifty seeds used as test (treatment RM03 biomass).

All the trays were watered with 10 ml of tap water every day at 24hr interval and placed in green house chamber at room temperature and relative humidity which were located in Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana state, India. The number of seed germination were observed and recorded every day. The germination rate were calculated at 0^{th} , 8^{th}, 10th, 12th and 18^{th} days depending on the nature of the seeds. For the growth studies of plants, control (untreated) and treated seeds were planted in green house nursery pots.

(ii) Rate of germination of *Capsicum annuum* L. The number of seeds sprouted divided by the number of total seeds germinated, then multiplied by hundred. The equation is to calculate the germination percentage is:

Formula : % of Seed Germination = No. of Germinated Seeds/Total Seeds × 100

The rate of germination provides a measure of the time course of seed germination by hundred.

B. Effect of RM01, RM02 and RM03 biomass on seed germination of (Capsicum annuum)

In this section the effect of seed germination were evaluated & compared with control. The germination of chilli seeds have been examined every 0^{th} Day, 8^{th} Day, 10^{th} Day, 12^{th} Day and 18^{th} days along with control. Control seeds, RM01, RM03 at 12^{th} day and RM02 at 9^{th} day examined the seed germination (Fig. 2 and Table 3).

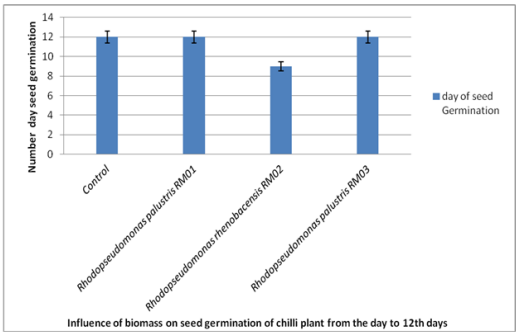


Fig. 2. Influence of biomass on seed germination of plants.

As compared to control plants, *Rhodopseudomonas palustris* RM01 and *Rhodopseudomonas rhenobacensis* (RM02) and *Rhodopseudomonas palustris* RM03 biomass has enhanced the seeds germination rate and the seedling growth. As seen in (Table 3). After 18th day the percentage (%) of seed germination were observed as 82% (control), 88 (%) RM01, 92(%) RM02 & 84 (%) RM03 respectively. In addition, the RM01 and RM03 biofertilizer showed increased rate of seed germination and also improved the seedling growth than control.

Table 3: Effect of three bacterial biomass of RM01, RM02 and RM03 on seed germination of *Capsicum annuum*.L (green chillies).

Sr. No.	Name of the bacterial biomass used as biofertilizer	Number of seed out of fifty (50 Seeds)	Seed germination (%)	Germination starting day
1.	Control	41	82	12th day
2.	RM01 biomass	44	88	12th day
3.	RM02 biomass	46	92	9th day
4.	RM03 biomass	42	84	12th day

(i) Analysis of physical parameters of *Capsicum Annuum* L upto 18th day of growth on period. The positive effects were observed in treated plants in short term germination and seedling growth. Therefore, in order to understand the long term combined effect of treated chilli seeds, the stem and root lengths of chilli were measured after 0th , 8th , 10th , 12th and 18th day of sowing. The chillies were planted in soil substrate and the seedling growth were monitored at regular intervals respectively. In all cases, the plant stem length increased gradually with increasing the cultivation duration. For the treated chilli seeds, data showed that the stem length is high as compared to control sample (without biomass). On the 0th day, 8th, 10th, 12th and 18th day of cultivation. the average root lengths were 2.1 cm, 4.1cm, 1.0cm and 1.8 cm and 2.1cm for RM01, RM02, RM03 and control respectively (Fig. 3-6), the average shoot lengths were 5.0 cm, 3.5cm, 3.2cm and 2.8 cm for RM01, RM02, RM03 and control respectively. The average root volume were 0.25 cm, 0.2 cm, 0.2cm and 1cm for RM01, RM02, RM03 and control respectively. The average plant lengths are 6.0 cm, 7.6cm, 4.2cm and 6.6cm for RM01, RM02, RM03 and control respectively.

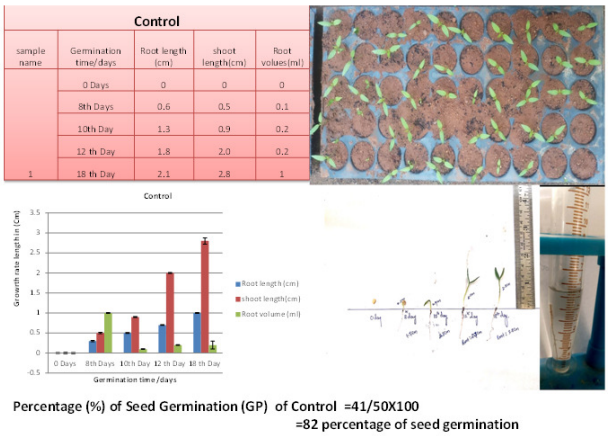


Fig. 3. The growth of root length, shoot length in (cm) and Root volume in (ml) growth from 0th -18th day seedling in control.

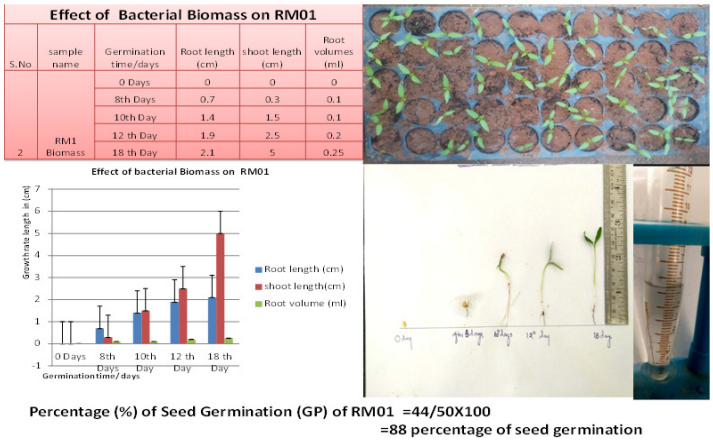


Fig. 4. The growth of root length (cm), shoot length in (cm) and root volume in (ml) chillies 0th -18th day of seedling in soil treated with RM01 biomass.

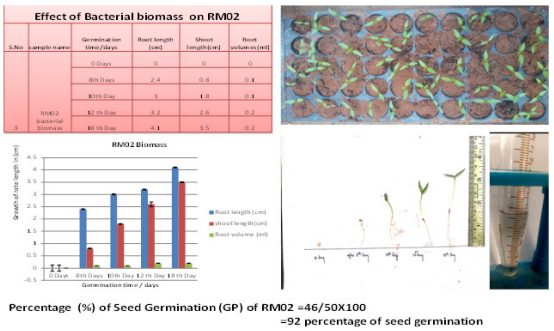
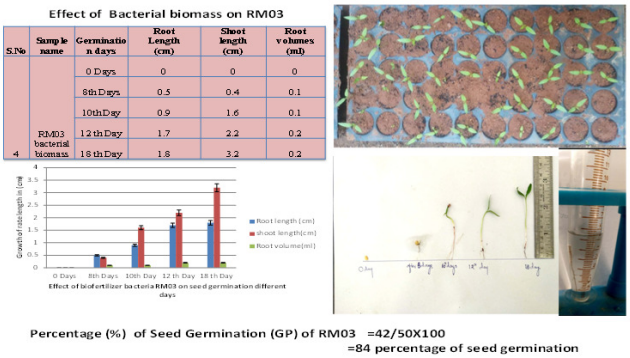


Fig. 5. The growth of root length (cm), shoot length in (cm) and root volume in (ml) chillies 0^{th} -18^{th} day of seedling in RM02 treatment with PNSB biomass



S.No	Sample name	Germination days	Root Length (cm)	Shoot length (cm)	Root volumes (ml)
4	RM03 bacterial biomass	0 Days	0	0	0
		8th Days	0.5	0.4	0.1
		10th Day	0.9	1.6	0.1
		12 th Day	1.7	2.2	0.2
		18 th Day	1.8	3.2	0.2

Fig. 6. The growth of root length (cm), shoot length in (cm) and root value in (ml) chillies 0^{th} -18^{th} day of seedling in soil treated with RM03 biomass.

After cultivation of chilli plants, the percentage of seed germination (%) observed in which the growth were more in RM01, RM02 and RM03 when compared to Control. Therefore, in order to understand the long term combined effect of treated chilli, % of seed germination of chilli plants were calculated at 0^{th} . 8^{th} , 10^{th} , 12^{th} and 18^{th} day .The seed germination of RM01, RM03 biomass treated plants showed less seed germination when compared with RM02 biomass The maximum seed germination percentage was observed on 18^{th} day of sowing the seeds. In our observation the percentage of seed germination inhibition in all three PNSBs were reported as 12% in *Rps. palustris* RM01, 8% in *Rps. rhenobacensis* RM02, 16% *Rps. Palustris* RM03, 18% in control without biofertilizer respectively. The maximum seed germination were observed on 9^{th} day in case of RM02 where as the RM01, RM03 and control showed on 12^{th} day.

C. Effect of bacterial biomass on various parameters of Capsicum annuum L

The present investigation were carried out to study the effect of bacterial biomass on chilli crop. The bacterial biomass of RM01, RM02 and RM03 inoculated in plants showed increase in the growth of chilli plants when compared with control plants. All the parameters like morphological and bio-chemical parameters were increased in soil which is inoculated with bacteria biomass. The present study were well correlated with the previous reports by Gaur and Agarwadi (1989). Where they studied the combined and dual inoculations of *A. brasilense* and Pseudomonas strain in sorghum plant which increased in root length, nitrogenase activity and dry matter, seed yield as compared to single inoculation of both organisms and control plants (Fig. 7- 26, Table 4).

(i) Effect of Bacterial Biomass on the Morphological Parameters of Chilli Leaves.

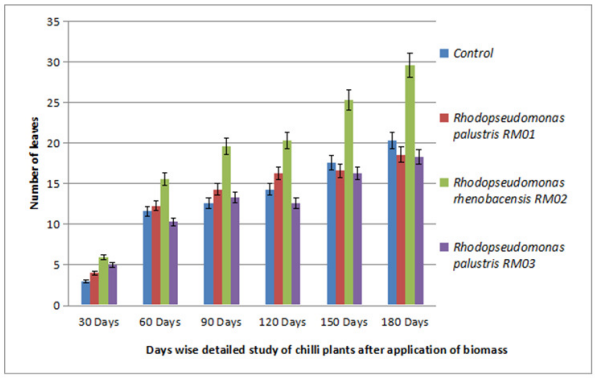


Fig. 7. Effect of bacterial biomass RM01, RM02 and RM03 on the growth of number of leaves.

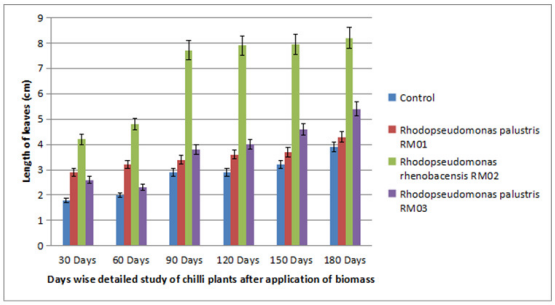


Fig. 8. Effect of bacterial biomass RM01, RM02 and RM03 on the length of leaves.

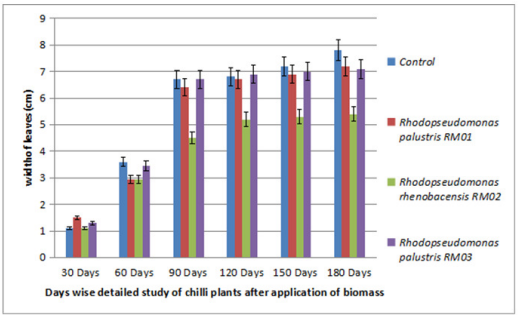


Fig. 9. Effect of bacterial biomass RM01, RM02 and RM03 on the leaf width.

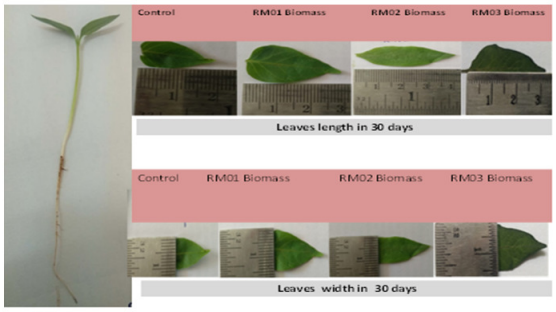


Fig. 10. Measurement of Leaves length (cm) and width (cm) in 30th days.

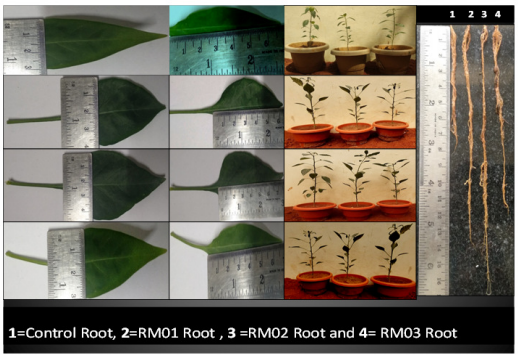


Fig. 11. Effect of three bacterial biomass on shoot length, leaves length, root length, root volume and total height of the plants at 60th days.

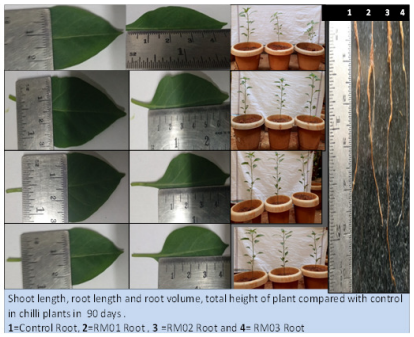


Fig. 12. Effect of three Bacterial biomass on shoot length, root length and root volume, total height of plant compared with control in chillies in 90th days.

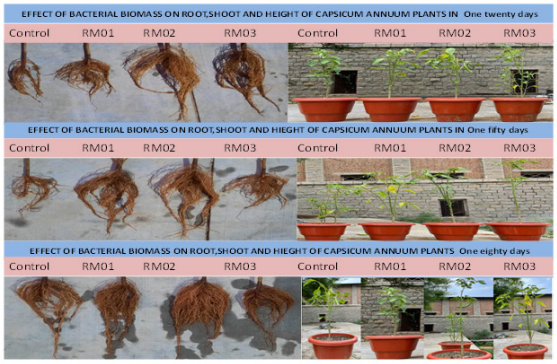


Fig. 13. Effect of three bacterial biomass on shoot length, root length and total height of chillies plants compared with control in on 120 days, 150th day and 180th day.

(ii) Effect of Bacterial Biomass on the Morphological Parameters on Shoot Length, Root Length and Root Volume of Chilli Plant

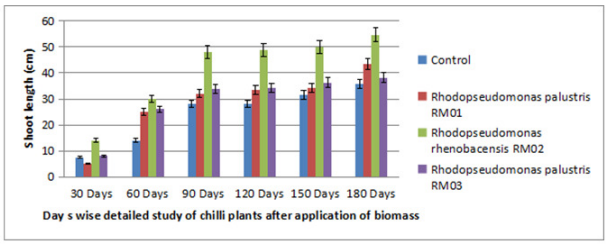


Fig. 14. Effect of RM01, RM02 and RM03 bacterial biomass on the shoot length.

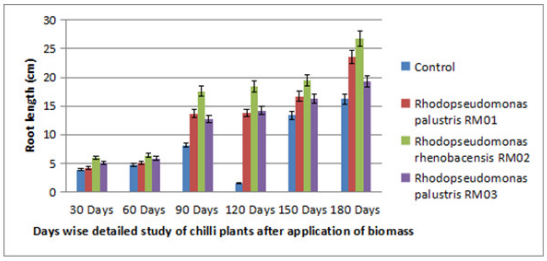


Fig. 15. Effect of bacterial biomass RM01, RM02 and RM03 on root length.

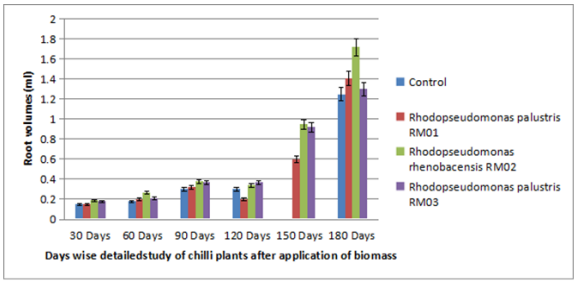


Fig. 16. Effect of bacterial biomass RM01, RM02 and RM03 on root volume.

(iii) Effect of Bacterial Biomass on the Morphological Parameters on Number of Flowers and Total Length of Chilli Plant

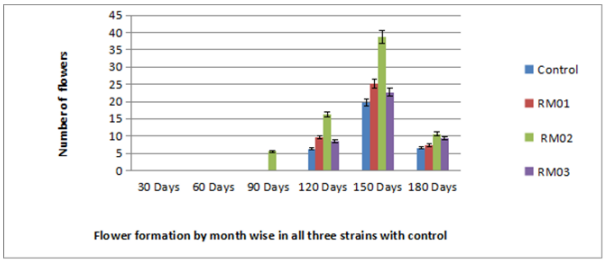


Fig. 17. Effect of bacterial biomass RM01, RM02 and RM03 on Number of flowers.

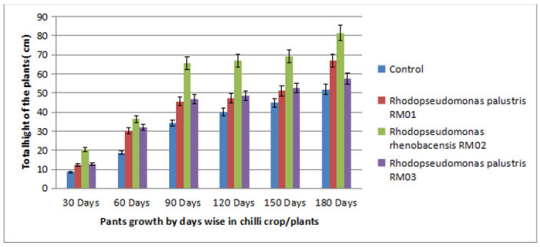


Fig. 18. Effect of bacterial biomass RM01, RM02 and RM03 Total length of shoot, root, chillies)

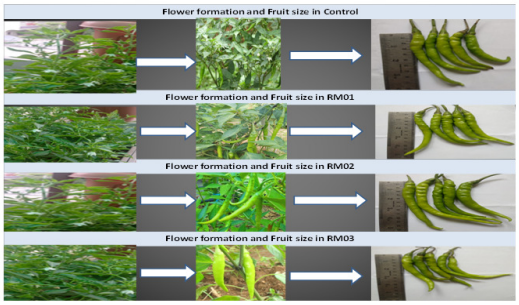


Fig. 19. The yield of *Capsicum annuum* L collected at 120th days.

Fig. 20. Effect of bacterial biomass (biofertilizer) RM01, RM02 and RM03 on growth of fruit and seeds in chilli plants.

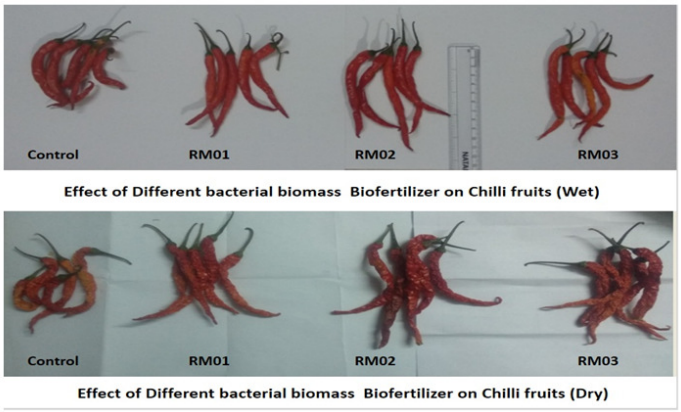


Fig. 21. Effect of bacterial biomass (biofertilizer) RM01, RM02 and RM03 on pigment (Fruit colour) and growth of chilli.

Table 4: Comparative morphological analysis of control & soil treated with bacterial biomass of RM01, RM02 and RM03 effect on growth of *Capsicum annuum* L.

Sr. No.	Morphological studies of green chilli plant effected by biomass of all three PNSB strains	Control plants	RM01 Biomass	RM02 Biomass	RM03 Biomass
1.	Total no. of plants taken for experiment	3	3	3	3
2.	Average no. of branches/plant	4	6	8	5
3.	Average no. of flowers/plants	18	22	28	23
4.	Average no. of fruits/plants	15	19	26	21
5.	Average fruit size/plant	7.94	11.14	13.01	10.84
6.	Average fruit weight (grams)	2.46	3.57	4.66	3.39

7.	Fruit length & diameter (cm × cm)	7.94×1.12	11.14×1.26	13.01×1.58	10.84×1.14
8.	Average number of seeds weight in fruits	31.8	41.2	49.8	37.2
9.	Average number of seeds weight (g)	(15 seed = 0.12g)	(15 seed = 0.1336g)	(15 seed = 0.1583g)	(15 seed = 0.1296g)
10.	Average number of seed weight (mg)	(one seed 0.008mg)	(Average of one seed 0.0089mg)	(Average of one seed 0.01055mg)	(Average of one seed 0.00864mg)
11.	Average fresh weight of plant root (g)	1.982	2.067	2.971	3.258
12.	Average of total dry weight of plant root (g)	1.126	1.532	2.364	2.892
13.	Average fresh weight of plant (g)	9grams	12 grams	13.5 grams	11.2 grams
14.	Average total dry weight of plant (g)	8.65grams	8.94 grams	9.87 grams	8.79 grams

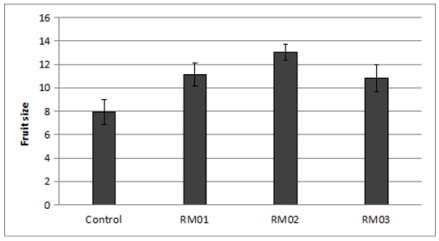


Fig. 22. Effect of bacterial biomass on the fruit size of *Capsicum annuum* L.

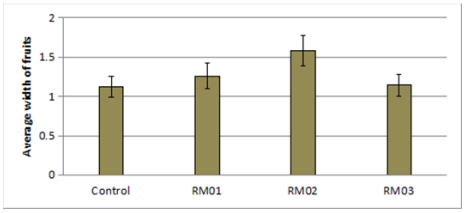


Fig. 23. Effect of bacterial biomass on the fruit width of *Capsicum annuum* L.

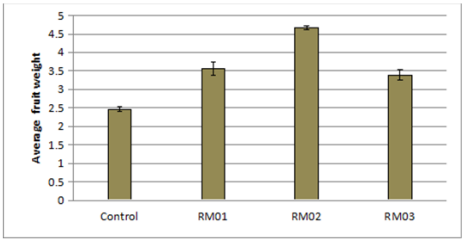


Fig. 24. Effect of bacterial biomass on the fruit weight of *Capsicum annuum* L.

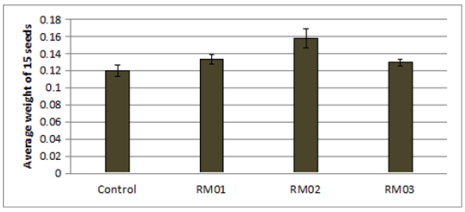


Fig. 25. Effect of bacterial biomass on the seed weight of *Capsicum annuum* L.

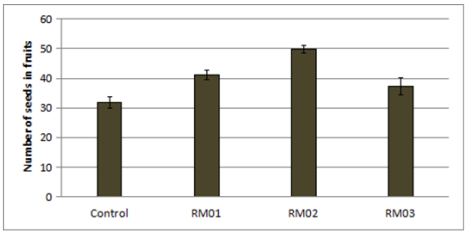


Fig. 26. Effect of bacterial biomass on the number of seeds in fruit in *Capsicum annuum* L.

CONCLUSIONS

In our experiment, we found high capsaicin content in of plant treated with RM02 biomass, The effect of PNSB biomass order of *Capsicum annuum* content were RM02<RM01<RM03<control observed respectively. In addition, determination of capsaicin content in *Capsicum* fruits enables us to recommend a daily dose as a food additive, which would be a very cheap and available preventive nutrient in gastrointestinal disorders. Perucka and Oleszek (2000) were reported extraction and determination of capsaicinoids in fruit of hot pepper *Capsicum annuum* L. by spectrophotometry and high-performance liquid chromatography, Saria *et al.* (1981), Chemistry and quality control of *Capsicum* and *Capsicum* products. Due to the property of enhancing the fertilizer of soil which were helping in the germination of the seed & enhancing the morphological & biochemical parameters the bacterial biomass can be used as a biofertilizer in the field of agriculture and horticulture.

Acknowledgement. I am grateful to thanks the Head Department of Botany for providing experiments for this study. Thanks for the principal University College of Science and Supervisor for laboratory facilities.

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How to cite this article: M. Manjula, K. Rama and B. Rajani (2024). Effect of *Rhodopseudomonas* Strains Biomass on Morphological Parameters of Seed Germination of *Capsicum annuum* L. *Biological Forum – An International Journal, 16*(8): 129-139.