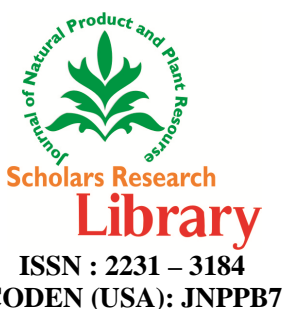




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Factors influencing on germination of some plant seeds by bacterization of *Serratia marcescens*

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ABSTRACT

Plant growth promoting bacteria make differences in plant growth by different mechanisms which can be mediated by direct or indirect methods. Nowadays these bacteria can be used as natural bio fertilizers instead of synthetic fertilizers and dramatically reduce the environmental hazards. Root colonizing bacteria have the ability to increase the seed germination rate, growth of plant and yield of crops. Therefore present study was performed in order to find out the effect of one of the soil borne gram (-) ve , chemoorganotrophic , facultative anaerobic bacteria *Serratia marcescens* on seed germination of some selected healthy plant seeds varieties , *Vigna mungo* (black gram) , *Vigna radiata*(green gram) , *Vigna unculata* (long bean) , *Hibiscus esculentus* (lady's finger),*Cucurbita maxima* (pumpkin), *Capsicum annum*(chilli) and *Solanum melongena* (brinjal), which were collected from Agrarian service center ,Thirunelvely, Jaffna, Srilanka. Twelve surface sterilized seeds were placed into $X \times 10^7$ cells/mL concentration of 75 mL bacterial suspension and soaked for overnight separately then seeds were transferred to sterile petridishes which containing sterile moistened filter paper and kept in dark at room temperature for 3-5 days, where sterile distilled water was used as control. By using above method, this study was also carried out for various soaking time intervals, (0,2,4 and 6 hours) and for different bacterial concentrations($X \times 10^7$ cells/mL - $X \times 10^2$ cells/mL). Finally the mean length of developed germ tube was measured separately and the results were subjected to analysis of variance (ANOVA) and Tukey test at $p=0.05$ using statistical software minitab (version16). The analysis showed that the growth of *C.annum* and *V.radiata* were not affected by *S.marcescens* while the growth of *V.mungo*, *H.esculentus* , *C.maxima* and *S.melongena* were promoted by the bacterium. Unfortunately the germination of *V.unculata* was suppressed by the bacterium for overnight soaking. Moreover, long bean, brinjal, black gram and lady's finger exhibited optimum growth rate at 2 hours soaking period whereas, the development of germ tube for chilli revealed maximum rate at 4 hours soaking time. The concentration of bacteria at $X \times 10^3$ cells/mL and $X \times 10^4$ cells/mL were found as the optimum for the better growth of pumpkin and green gram respectively. Rest of the tested seeds exhibited highest growth activity at $X \times 10^2$ cells/mL concentration. Therefore it was concluded that the bacterial activity on seed germination varied among the seed varieties, soaking time period and inoculum concentration. Hence, this study reveals the primary platform for the practical benefits of employing *S.marcescens* for a sustainable farming system.

Key words: *Serratia marcescens*, plant seeds, soaking time, inoculum concentration

INTRODUCTION

Serratia marcescens is a gram negative, rod shape, and citrate positive bacterium. Colonies on nutrient agar are convex, circular with entire margin .*Serratia marcescens* is a chemoorganotrophic, facultative anaerobic bacteria with low nutritional requirement . Those are classified as a member of the Enterobacteriaceae .It is a widely

distributed saprophytic bacterium and has been found in food especially on starchy food like potatoes, bread and also found in water, air and soil. Some strains produce some characteristic red colour pigment called prodigiosine. Prodigiosine has anticancer, immunosuppressive, antifungal and algicidal effect [1]. Prodigiosine showed inhibitory effect against some plant pathogens [2]. This prodigiosine production is optimum at 28°C at pH 7 and the rate was reduced as the temperature increases [3]. Plant growth promoting rhizobacteria (PGPR) affect growth by direct or indirect mechanisms. PGPR are soil and rhizosphere bacteria that can benefit for plant growth by different mechanisms. They used as natural biofertilizers is advantageous one not only from the economical but also from the ecological point of view. Soil or seed application with PGPR has been used to enhance growth of several crops. Besides promoting plant growth PGPR can also suppress the growth of plant pathogen.

The direct effect of PGPR has been most commonly attributed to the production of plant hormones such as auxins, gibberellins and cytokinins or by supplying biologically fixed nitrogen. These PGPR also affect growth by indirect mechanisms such as suppression of bacterial, fungal and nematode pathogens by production of siderophores, HCN, ammonia, chitinase, antibiotics and volatile metabolites etc. [4]. Plant growth promoting bacteria bind to the surface of seed and in response to tryptophan and other amino acids exuded from the germinating seed, synthesize and secrete IAA (indole acetic acid). This IAA may be taken up by the seeds and together with endogenous IAA stimulates plant cell proliferation, cell elongation or induces the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase. The enzyme catalyses the conversion of S-adenosyl-L-methionine (SAM) to ACC, the immediate precursor of ethylene [5]. Based on the background present study was conducted in order to find out the effect of soil borne bacteria, *Serratia marcescens* on some plant seed germination.

MATERIALS AND METHODS

Collection of plant seeds.

Seven varieties of healthy plant seeds, *Vigna mungo* (blackgram), *Vigna radiata* (green gram), *Vigna unculata* (long bean), *Hibiscus esculentus* (lady's finger), *Cucurbita maxima* (pumpkin), *Capsicum annum* (chilli) and *Solanum melongena* (brinjal), were collected from Agrarian service centre, Thirunelvely, Jaffna, Sri Lanka.

Sub culturing of bacterial culture

The soil born bacteria, *Serratia marcescens* was obtained from laboratory culture collection of the Department of Botany, University of Jaffna, Jaffna, Sri Lanka.

The bacterial culture was sub cultured by streaking on nutrient agar medium in petridish with the help of a sterile inoculating loop under aseptic condition and incubated at 37°C for 24-48 hours. Purity of the bacterial culture was checked by staining methods and stored at 4°C in slant culture for further studies.

Determination of effect of soaking time on seed germination.

Seven varieties of the selected healthy plant seeds were surface sterilized with 0.4% of sodium hypochlorite (NaOCl) for 2 minutes and they were washed thoroughly with sterile distilled water for three times. Twelve surface sterilized seeds from each variety were soaked into 75 mL of bacterial suspension at a particular concentration ($X \times 10^7$ cells/cm³) separately and they were allowed for overnight. The seeds were soaked in 75 mL sterile distilled water was used as control.

Likewise, fifty surface sterilized tested healthy seeds were chosen and transferred into 75 mL of the same concentration ($X \times 10^7$ cells/cm³) of bacterial suspension separately. Then twelve seeds were transferred at different time intervals, 0, 2, 4 and 6 hours interval into sterile petridishes which had sterilized moistened filter paper. Controls were also maintained. Finally, the petridishes were kept in dark at room temperature for 3-5 days. The development of germ tube was observed and mean length was measured for each variety of seeds. In addition to that percentages of germinated seeds were calculated.

Determination of effect of inoculum concentration on seed germination.

The bacterial suspension was prepared ($X \times 10^7$ cells/cm³) and serial dilution was carried out from 10⁻¹ to 10⁻⁵ using sterile distilled water in order to obtain different concentrations of suspensions. Then twelve surface sterilized seeds from each varieties were soaked into the different concentrations of bacterial suspensions separately and it was allowed for overnight. Then the seeds were transferred into sterile petridishes which had sterile moisture filter paper separately. Whereas sterile distilled water was used as control instead of bacterial suspension. Petridishes were kept in dark at room temperature for 1-3 days and the mean length of germ tube was measured as well as percentage of germination was calculated upto three days.

Statistical analysis

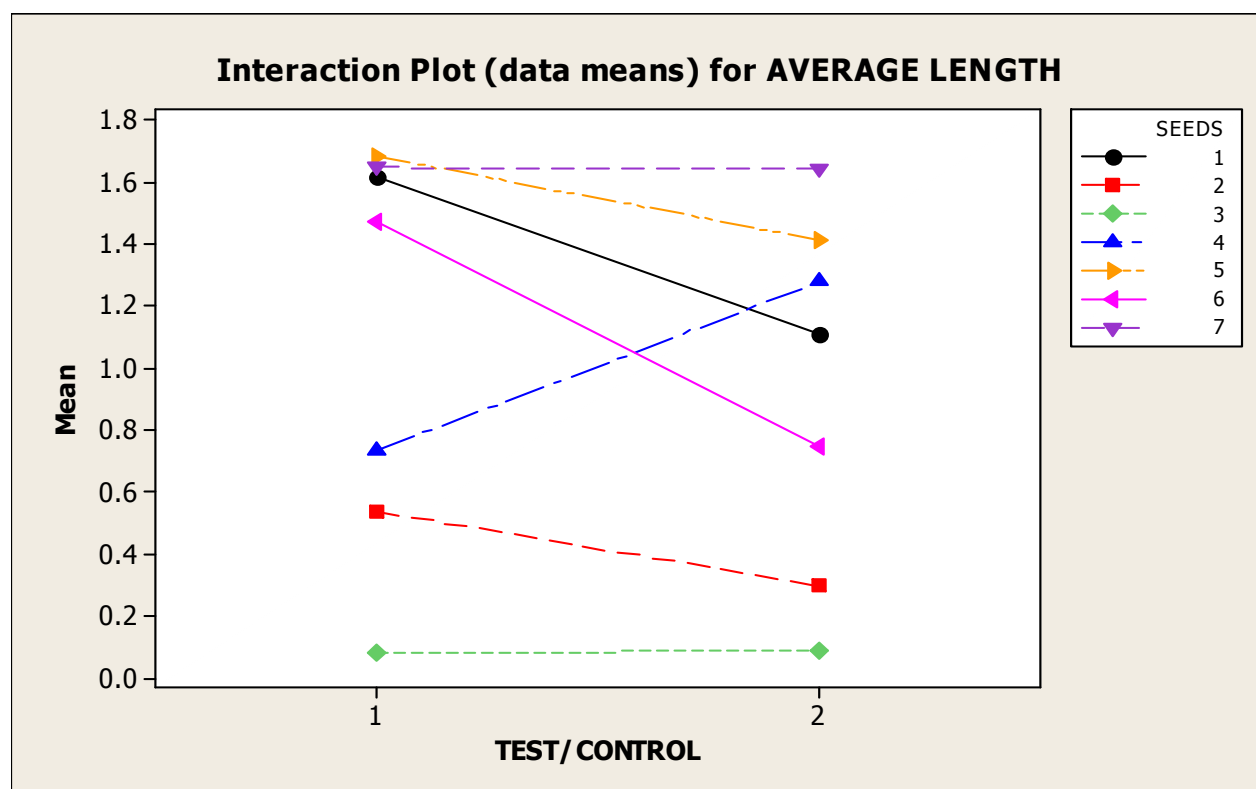
The results were subjected to analysis of variance (ANOVA) and Tukey test at p=0.05 using statistical software, minitab (version16).

RESULTS AND DISCUSSION

Effect of bacteria on seed germination depend on type of bacteria [6], type of seeds, concentration of bacterial inoculum, soaking time ,competitive microorganisms [7] and environmental factors[8]. Our study revealed that the growth of some plant seeds were promoted by tested bacteria, *Serratia marcescens*. At the mean while germination of the some seeds were inhibited by the organism.

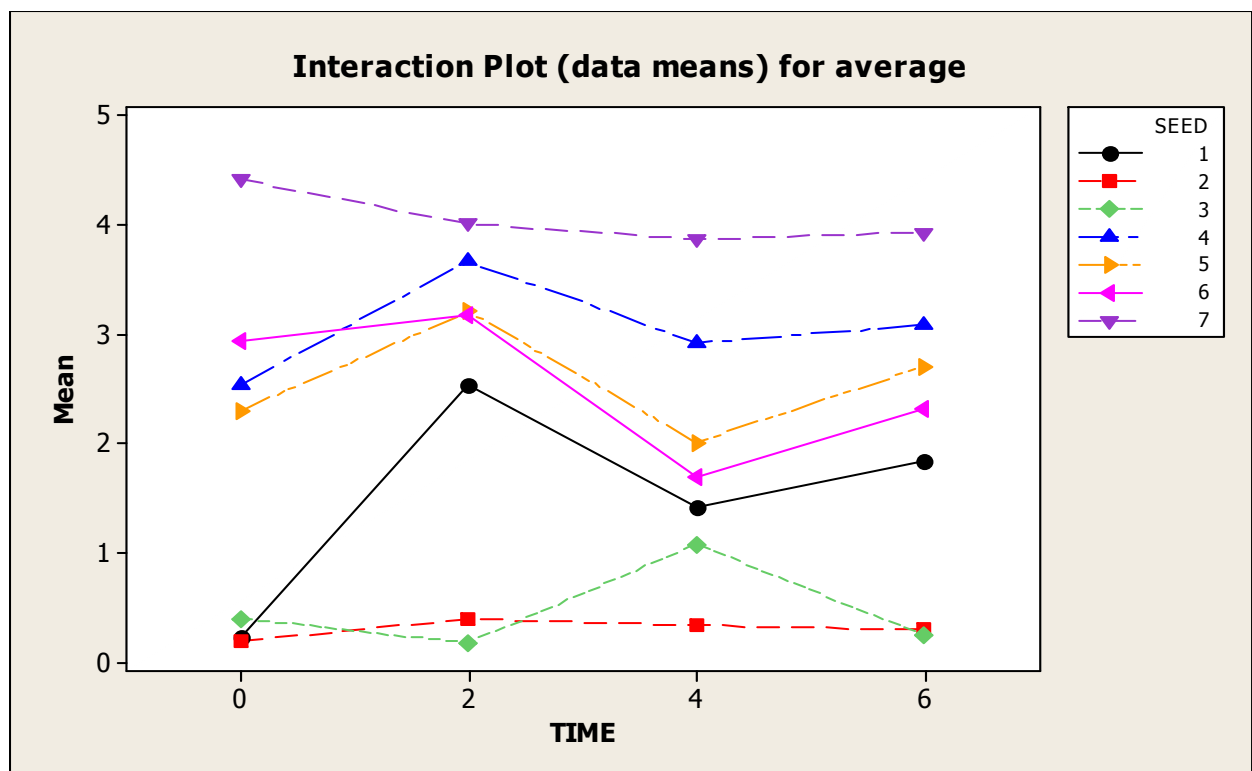
Table 1: Tested plant seeds

SEEDS	CODE
<i>Hibiscus esculentus</i>	S ₁
<i>Cucurbita maxima</i>	S ₂
<i>Capsicum annum</i>	S ₃
<i>Vigna uniculata</i>	S ₄
<i>Vigna mungo</i>	S ₅
<i>Solanum melongena</i>	S ₆
<i>Vigna radiata</i>	S ₇



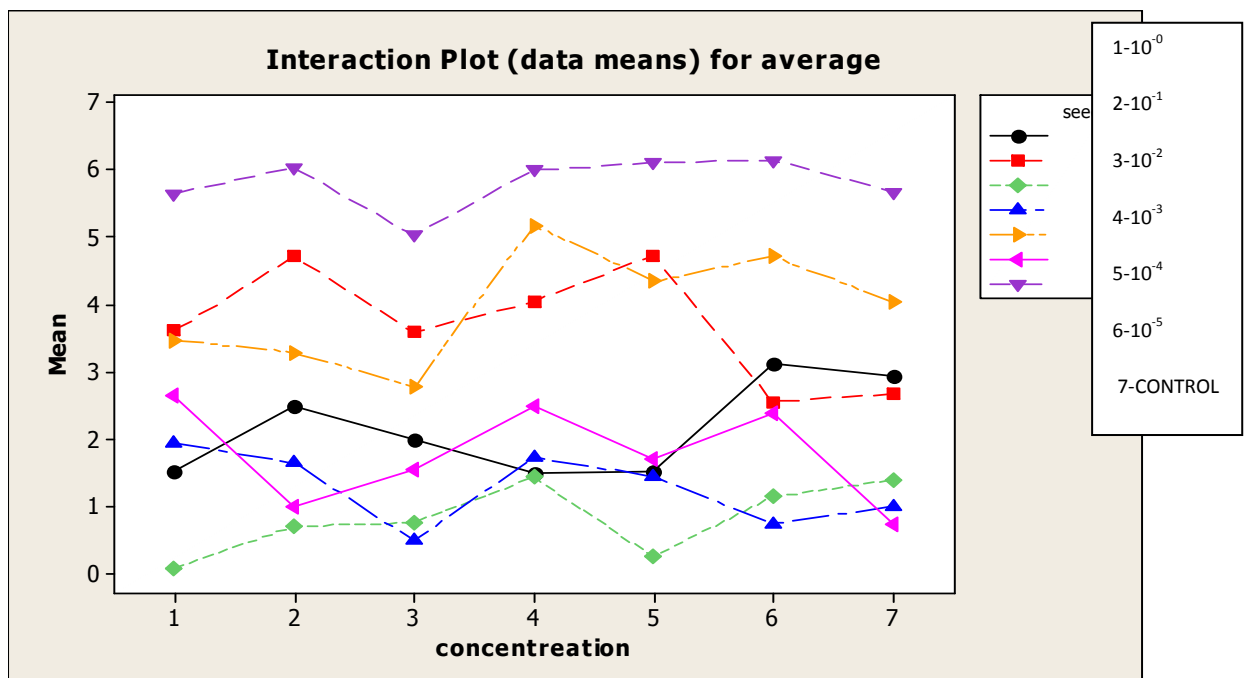
Graph1: The mean length of tested seeds after the overnight soaking
Test-1, Control-2

Among these seven tested seeds, the *S.marcescens* induced the germination of four seeds varieties, lady’s finger, blackgram, brinjal ,and pumpkin and there was no effect against chilli and green gram when compared with control. However, unfortunately the growth of long bean seeds was only inhibited by the tested soil borne bacteria. This study indicated that bacteria not only promote the germination of seeds but also inhibit the germination of some seeds and also concluded that the bacterial activity on seed germination varied among the seed varieties (Graph 1).



Graph 2: The mean length of tested seeds after the different soaking time period

Hence, analysis showed that the germination rate of seeds also depend on the soaking time period which varied within the seeds . Long bean, brinjal, black gram and lady’s finger exhibited optimum growth at two hours soaking period. Furthermore, at four hours soaking period, chilli produced better growth rate (Graph 2).



Graph 3: The mean length of tested seeds in different concentration of bacterial suspension

The seed germination promoted as optimum in a particular concentration. This concentration was varied among the seeds. In our experiment, initial concentration of bacterial inoculum was taken as $X \times 10^7$ cells/ml. After the dilution, at 10^{-3} dilution the maximum seed germination was observed in chilli , long bean, black gram and brinjal.

However the seeds of pumpkin and lady's finger showed highest growth germination at 10^{-4} and 10^{-5} dilution respectively.

In most of the cases the seeds germination in the highest dilution of the bacterial suspension exhibited very less significant difference compared with control. This effect was mainly influenced by the diluents rather than bacterial concentration. But, in lowest dilution effect on seed germination was mainly determined due to the bacterial cell concentration. Overall, bacterial colonization depend on the concentration of bacteria and physiological properties of the tested seeds. In this study rate of colonization varied among different concentrations may be due to cell interactions. Therefore the net effect on seed germination varied among different tested seeds.

CONCLUSION

The study showed that the growth of *C.annum* and *V.radiata* were not affected by *S.marcescens* while the growth of *V.mungo*, *H.esculentus*, *C.maxima* and *S.melongena* were promoted by the bacterium. Unfortunately the germination of *V.uniculata* was suppressed by the bacterium for overnight soaking. Moreover, long bean, brinjal, black gram and lady's finger exhibited optimum growth rate at 2 hours soaking period whereas, the development of germ tube for chilli revealed maximum rate at 4 hours soaking time. The concentration of bacteria at $X \times 10^3$ cells/mL and $X \times 10^4$ cells/mL were found as the optimum for the better growth of pumpkin and green gram respectively. Rest of the tested seeds exhibited highest growth activity at $X \times 10^2$ cells/mL concentration. Therefore this study enriched the knowledge on agro ecological friendly farming methods to improve the plants growth.

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