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# Optimization of salinity and temperature for the efficient seed germination of *Momocardia charantia*

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## Abstract

The study was aimed to examine the effects of temperature and NaCl concentration on the seed germination and early seedling growth of *Momocardia charantia* (Bitter gourd). Each set of triplicate petridishes were filled with sterile cotton wool moist with water and ten bitter gourd seeds were placed and allowed to germinate in dark environment, at different temperatures for seven days. Parameters measured were, the percentage of germination, germination index, mean time to germination and the length of germ tube. Similar experiment was done using different NaCl concentrations with optimized temperature at 35$^{o}$C. Seed germination and seedling growth of bitter gourd were optimum at 35$^{o}$C of germination temperature and 100mM NaCl concentration under moderate moisture content. Large scale multicentre studies should be done to further confirm this finding in the field condition.

**Keywords:** *Momocardia charantia*, germination index, NaCl, temperature, length of germ tube.

## Introduction

*Momocardia charantia*is (Bitter gourd) a subtropical and tropical vine, classified under Cucurbitaceae family. In Sri Lanka, Bitter gourd is widely used as an ingredient for curry and sambol which are served with rice. For a long time bitter gourd has been used as herbal medicine. The plant parts have diverse medicinal values such are stomachic, laxative, antibilious, emetic, anthelmintic agent, for the treatment of skin diseases, wounds, ulcer, diabetes, and rheumatism. Bitter gourd seeds are easily susceptible to the adverse environmental conditions. During the period of storage the seed content undergo different biochemical reactions and cause seed damage [17]. In the plant life, seed germination is a critical stage. During germination temperature and salinity tolerance are crucial for the establishment of plants that are grown in saline soils. For the successful seed germination, plants require different components at different rates [25]. Seed germination depends on both internal and external factors.

Salinity defined as the accumulation of salt in soil and water. Soil structure, infra-structure, water quality, plant growth and yield can be adversely affected by higher level of NaCl concentration [26]. When the salinity level increased that caused a significant reduction in germination percentage, rate of germination, individual length of shoot and roots and fresh weights of the root [13]. In tropical regions of the world, the crop production was particularly affected by soil salt concentration [1].

Territory plants distribution and seed germination are mainly affected by temperature [10]. Some seeds show optimum rate of germination in cool temperature but other seeds show optimum germination in between warm and cool temperatures. To break the dormancy different seeds require various temperatures. Cellular metabolism of the germinating seeds mainly depends on the amount of water uptake by seeds. In many mesophytic crops moisture availability imposed severe limitations on seed germination (Bonvisutto and Busso, 2007). For germination, plant seeds that have specific germination requirements can establish more successfully than those with few restrictions [13]. When the water potential is low, the germination percentage and germination velocity of a seed also would be low. The lower velocity of coefficients at lower water potential show greater germination times [26].

Bitter gourd is cultivated in a wide range of climatic and geographic locations of the Island of Sri Lanka as a vegetable. There have been no researches conducted to compare the differences in germination potential /index and seedling growth rates of bitter gourd growing in different saline and temperature zones of Sri Lanka. Therefore, this study was focused to examine the effects of temperature, and salinity level on the seed germination and early growth of *Momocardia charantia.*

## Materials and Methods

### *Source of seeds*

The *Momocardia charantia* (bitter gourd) seeds were obtained from Agricultural farm school of Northern province, Thirunelvely, Sri Lanka.

### *Effect of temperature*

A set of three sterile petridishes were taken and they were filled with sterile cotton wool moist with sterile water. In each petridish, ten seeds of *Momocardia charantia* were placed separately. After moisten by 20ml of sterile water, the petridish was kept at a water bath set at 20$^{o}$C, under dark warm condition that is favourable for natural seed germination for 7 days. The same experiment was repeated to 25, 30, 35 and 40$^{o}$C temperatures. To avoid the seeds getting dried, the cotton wools were moisten by 5ml of sterile distilled water every day. The seeds were carefully observed daily and the percentage of germination, germination index, general normal seedling percentage and mean time to germination and the length of germ tube were measured. At the end of the germination period, germination percentage, germination index and mean time to germination were calculated [27].

### *Effect of Salinity*

A set of three sterile petridishes were taken and they were filled with sterile cotton wool moist with 50 mM. In each petridish, ten seeds of *Momocardia charantia* were placed separately. Then the petridishes were moistening by 40ml of 50mM Sodium Chloride solutions. All petridishes were placed in water baths set at 35$^{o}$C, and left in dark for 7 days for natural seed germination. The same experiment was repeated with 100,150, 200, 250 and 300mM concentration of Sodium Chloride solutions. The seeds were carefully observed daily and all the same parameters were recorded as for the temperature experiment.

### *Statistical analysis*

Statistical analyses were performed using R 2.15.3 statistical software at $\alpha = 0.05$ confidence level. The data sets were checked for the parametric assumptions of normality and homogeneity of variances. The data were analyzed using ANOVA followed by Tukey's multiple comparison test to determine significant differences at $p \leq 0.05$ [24].

## Results and Discussion

*Momocardia charantia* has the capacity of growing in a wide range of temperatures. After five days of germination period, the germination percentage was very low at 20$^{o}$C (Figure 1). Germination percentage was increased with the temperature up to 35$^{o}$C and beyond this temperature germination percentage started to decline. The length of germ tube also showed similar trend as the germination percentage (figure 2). The germ tube length was significantly lower at 20$^{o}$C than that of 35$^{o}$C.

After seven days of germination of seeds of *Momocardia charantia*, the germination mean time (GMT) was found to be higher at 35$^{o}$C. GMT obtained at 35$^{o}$C was significantly higher than the other temperatures. When the temperature was increased to 40$^{o}$C, the GMT declined (Figure 3). Under various temperature treatments seeds of bitter gourd showed diversity in responses. The Germination index of the seeds of bitter gourd expressed similar trend as germination mean time (Figure 4).

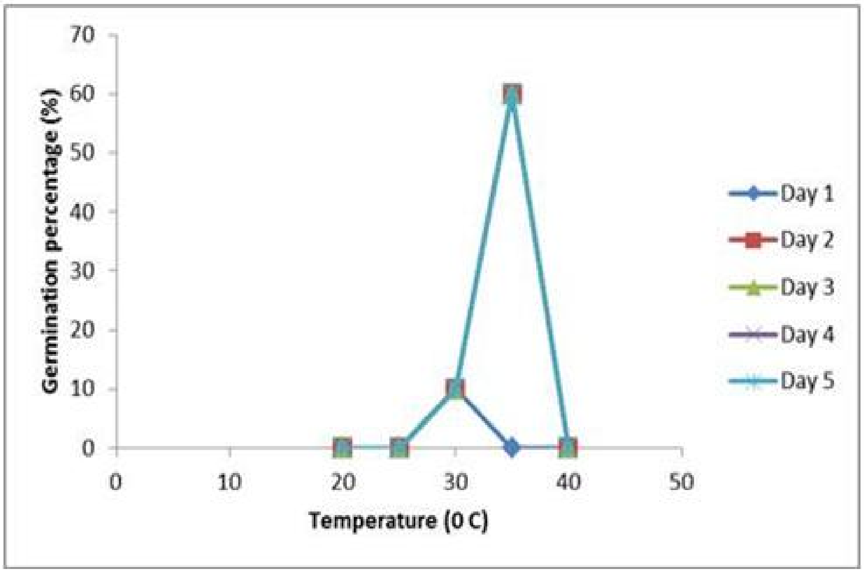


**Figure 1**: Percentage of germination of *Momocardia charantia* seeds at different temperatures.

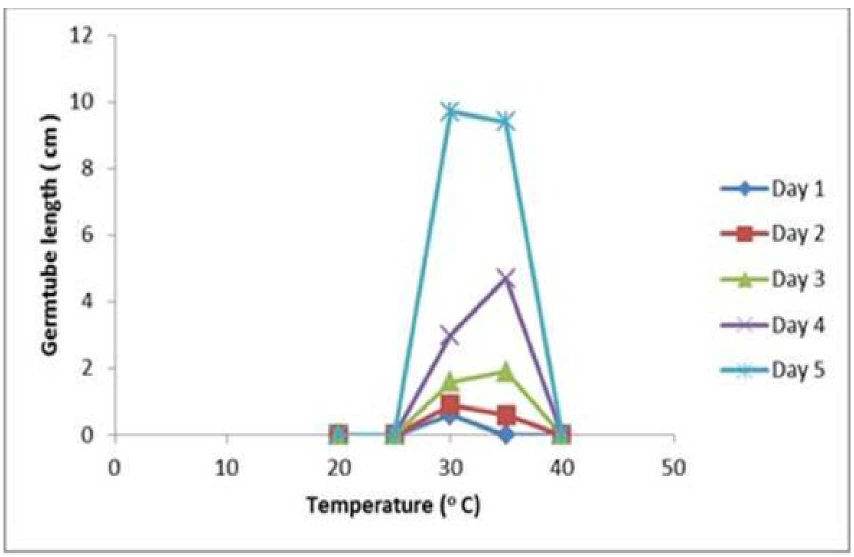


**Figure 2**: Length of germ tube of the seedlings of *Momocardia charantia* at different temperatures.

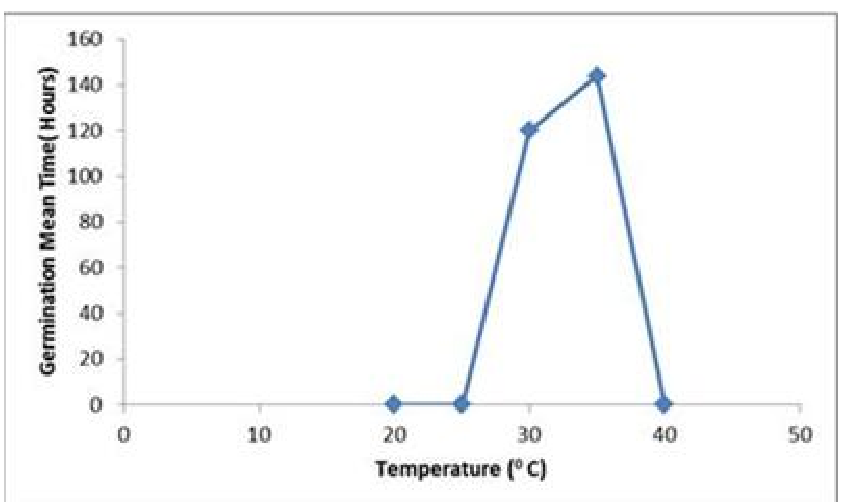


**Figure 3**: Mean time of germination of *Momocardia charantia* seeds at different temperatures.

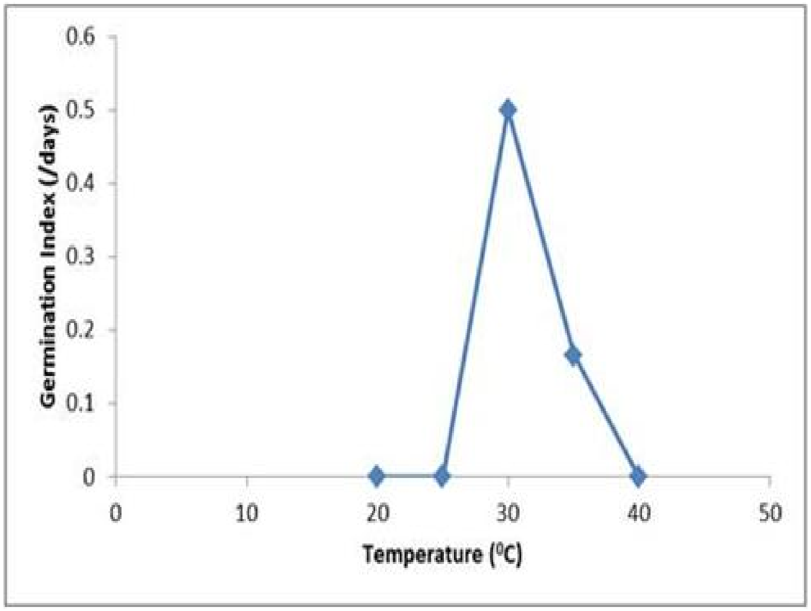


**Figure 4**: Germination index of *Momocardia charantia* seeds at different temperatures.

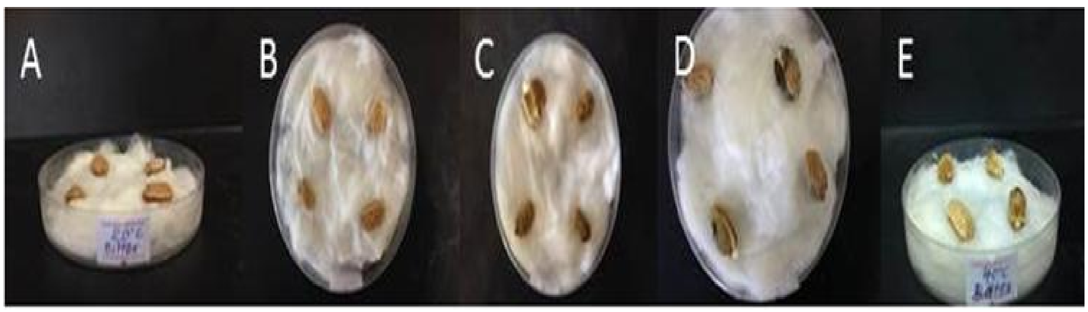


**Figure 5**: Seeds of *Momocardia charantia* showing different responses at different temperatures after 5 days. A- 20$^o$C, B- 25$^o$C, C- 30$^o$C, D- 35$^o$C and E- 40$^o$C.

Aquaporins, the membrane proteins play a key role in plant water homeostasis in response to various environmental stresses [30]. At lower temperatures, pores of aquaporins will get closed and the water conductivity will be highly reduced. When the temperature is high, rate of evapo-transpiration from plants will be higher and this might inhibit seed germination. Root water flux decreases with decreasing temperature [19]. The percentage of seeds of bitter gourds germinated was maximum at the optimal temperature (35$^o$C) for all individuals of same species. As the temperature declines or advances from 35$^o$C, two things happen at the same time. While the percentage of germinating seeds decreases, the number of days to germination increases. That is the basic relationship between seed germination and growing temperature. For every species of seed, there is an optimal soil temperature for germination, and at that temperature, the maximum number of seeds will germinate and in less time than at any other temperature tested [21, 25 and 27]. The enzymes that involved in germination might be more active at the optimum temperature [8].

After five days of seed germination, the germination percentage of bitter gourd seeds at 100mM of NaCl was significantly higher than the other NaCl concentrations (100, 150, 200 and 250mM). The germination percentage of bitter gourd was inhibited in very high salinity concentrations. Beyond 200mM of NaCl concentration, germination was inhibited (Figure 6). Increase in NaCl concentration will inhibit the seed germination. The variation in them germ tube length also showed similar trend as the germination percentage (Figure 7). Bitter gourd has a very limited tolerance to salinity and cannot grow in a wide range of salinity variations.

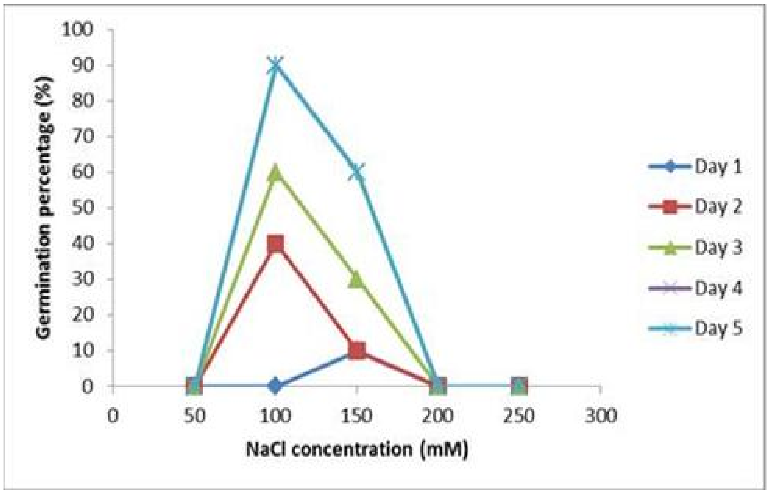


**Figure 6**: Percentage of germination of *Momocardia charantia* at different NaCl concentrations.

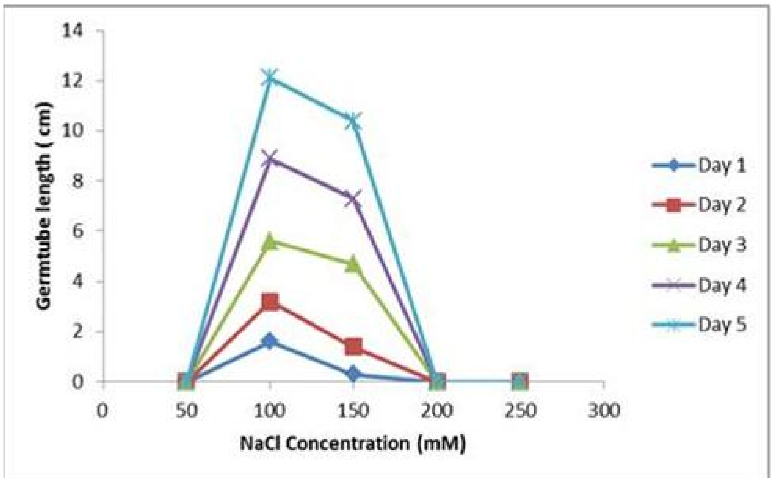


**Figure7**: Length of germtube of *Momocardia charantia* at different NaCl concentrations.

After seven days of germination period of *Momocardia charantia*, Germination Mean Time was stable at lower concentrations of NaCl (50mM, 100mM and 150mM). Beyond 150mM of NaCl, Germination Mean Time of *Momocardia charantia* was significantly lower than all the other tested NaCl concentrations. When the NaCl concentration was further increased to 200mM of NaCl, the GMT declined and no germination was reported (Figure 8). Germination index of *Momocardia charantia* showed similar trend as the germ tube length (Figure 9). Under different salinity treatments *Momocardia charantia* seeds showed various seed germination responses.

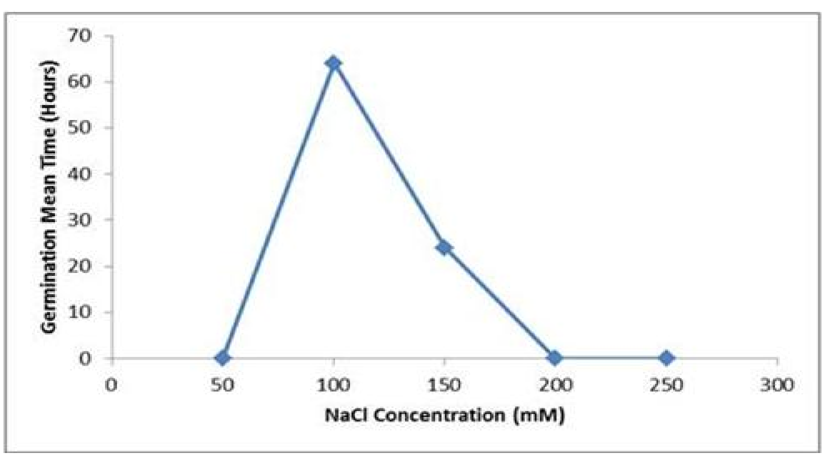


**Figure 8**: Germination Mean Time of *Momocardia charantia* at different NaCl concentrations.

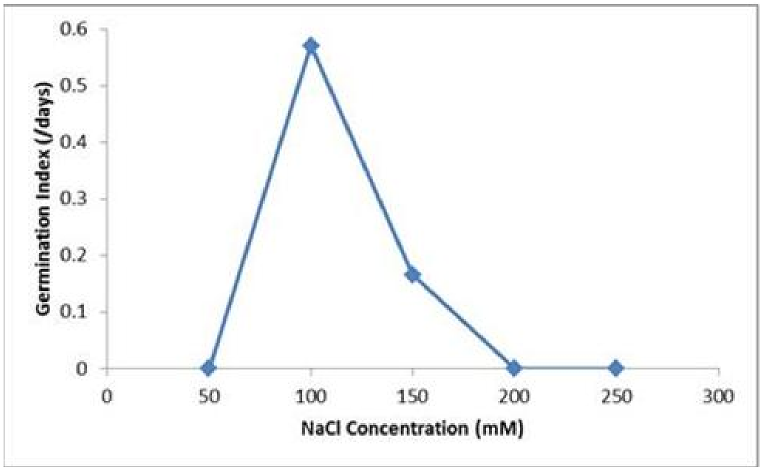


**Figure 9**: Germination Index of *Momocardia charantia* at different NaCl concentrations.

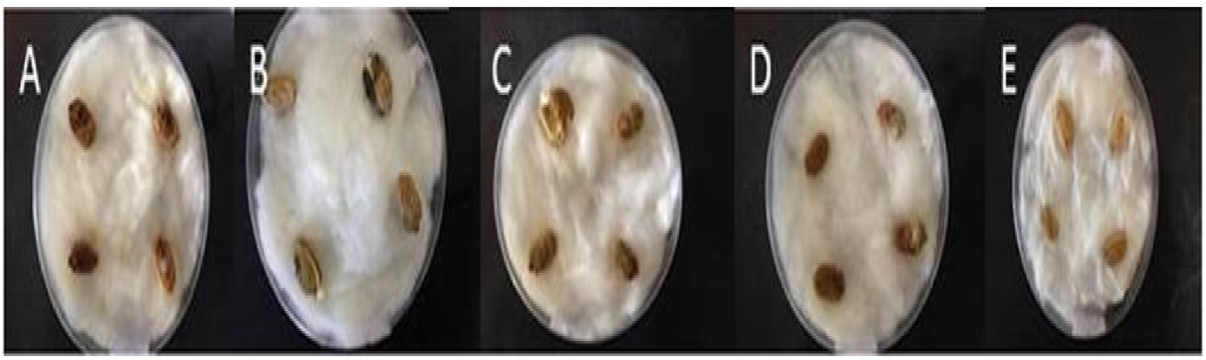


**Figure 10**: Seeds of *Momocardia charantia* showing difference responses at different NaCl concentrations after 5 days. A-50, B-100, C-150, D-200 and E-250 mM NaCl concentration.

Increased sodium chloride concentration levels caused a significant reduction in the seed germination and seedling growth rate. Effect of salts would cause physiological drought in seed and plant growth. Salt reduces the water potential of the substrate solution, which inhibits plant water supply. In salty media plants receive higher amount of salt in root cells that leads to water potential reduction. Therefore there will be a raise in the water absorption under physiological drought condition to a certain extent [2, 12]. There are detrimental effects of NaCl may be linked to long-term effects of accumulated toxic ions. Na+ will inhibit most of the enzymes at a concentration above 100 mM. The concentration at which Cl– becomes toxic beyond certain level and this is not well defined, but is probably in the same range as that for Na+ [21]. The activity of most enzymes inside the seeds and seedlings is negatively affected by high salt concentrations due to perturbation of the hydrophobic–electrostatic balance between the forces maintaining protein structure. However, toxic effects on plant tissues occur even at moderate salt concentrations of about 100 mM, unveiling specific salt toxicity targets [33]. Apoplastic enzymes from most of the halophytes have been shown in vitro to be salt insensitive, coping with NaCl concentrations up to 500mM [34]. The interaction between temperature and salinity plays an important role in the growth of seedlings in natural habitats [28].

## Conclusion

The tolerance of diverse range of environmental factors improves efficient germination of *Momocardia charantia* seeds. Optimum germination responses were expressed by *Momocardia charantia* at 35$^{o}$C temperature and100mM NaCl concentration.

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