Madder (Rubia tinctorum L.) is a perennial plant rich in anthraquinone (AQ) derivatives including alizarin and purpurin in its roots and rhizomes. AQS are important compounds not only in textile and food industries regarding dying properties but also in medicine and pharmaceutical industry because of its pharmacological and biological activities. AQS in the madder can be obtained economically from plants that are at least 3 years old, collected from the nature or cultivated. In this case, the number of plants may decrease and cause destruction. Consistently destruction of plants from in nature can also cause this plant to extinction in the future. Commercially, in the short time to maximum production of AQS can be achieved by applications elicitor both callus and roots obtained in vitro. Thus, from the callus and root obtained through tissue culture, existed two fold more AQS can be obtained compared to the normal plant. Phenolic compounds are another unique metabolite group for cosmeceuticals, foods and pharmaceutical industries. Because of their important properties it is necessary to get AQS and phenolics with high quality and quantity. In recent years, there has been an increased interest in in vitro techniques for secondary metabolite production because of their some advantages including no seasonal constraints and more rapid, efficient, reliable, simple and predictable production. In order to increase the metabolite synthesis in in vitro conditions elicitor applications could have been done effectively. Salinity is an important stress factor influencing growth and secondary metabolite metabolism in plants. This study was carried out to determine the effect of sodium chloride (NaCl) on root growth and secondary metabolite accumulation in madder. For this aim, madder roots obtained from stem explants in in vitro conditions were used as plant materials. Roots were cultured in MS medium containing different concentrations of NaCl (0, 1, 2, 3 and 4 g l-1) for 7 days. Then roots were evaluated in terms of root growth index, total AQ, alizarin, purpurin and total phenolic contents. Based on the results, root growth decreased in line with the elevating level of NaCl while secondary metabolite accumulation significantly increased with NaCl applications compared to the controls. It was determined that NaCl at 3 g l-1 concentration was the most effective application in terms of total AQ, alizarin, purpurin and phenolic accumulation.