Thymol, is one of an active compound in black seed (Nigella sativa L.) extract and showed beneficial immunomodulatory properties as augmenting the T cells and immune responses mediated by natural killer cells. Also, the important secondary metabolite thymol played important roles in inhibition of cancer cells, antibacterial activity against bacteria, antioxidant activity via scavenging free radicals, anti-inflammatory activities via inhibiting recruitment of cytokines and chemokines, antitumor, antiseptic, analgesic and fungicidal activities, carcinogenicity and can attach with the mutagenic substances. Plant tissue culture techniques like biotic elicitation is applied for enhancing the production of valuable secondary metabolites in medicinal plants. This study indicates the effect of chitosan, a biotic elicitor, on thymol production in callus culture of black seed. Leaves were used as explants and cultured on MS medium supplemented with 2.0 mg/l benzylaminopurine (BAP), 1.0 mg/l naphthaleneacetic acid (NAA) and different concentrations of chitosan (0, 5.0, 15.0, 10.0 and 20.0 mg/l). Calli formed after 5-6 weeks and subcultured two times each of 2-3 week duration. Thymol content was measured by the high performance liquid chromatography (HPLC). Among the tested elicitor concentrations, 10 mg/l chitosan was the best enhancing concentration with the accumulation value of 6.5 µg/g of thymol in N. sativa. It was seen that 5.0 and 15.0 mg/l chitosan also increased the thymol content compared to the control group. On the other hand, chitosan concentration of 20 mg/l decreased the amount of thymol in calli. As a result of this study, it has been determined that the amount of thymol varies according to different concentrations of chitosan and the most effective dose, 10 mg/l chitosan, can be used successfully in black seed callus cultures for enhancing the production of thymol. 

**Keywords:** Black seed, Callus culture, Chitosan, Nigella sativa L., Thymol