Plant cell culture is an adorable alternative source to whole plants for the production of beneficial secondary metabolites. The accumulation of secondary metabolites in plant tissue cultures can be stimulated by the elicitors. In the present study, the effects of different concentrations of exogenous melatonin (as an elicitor) on the callus induction and secondary metabolite production of basil (Ocimum basilicum L.) were tested. Donor plants were grown in the plant growth chamber at 18–20°C with a 16/8-hour (day/night) photoperiod, 12 klux light intensity, 51–54% humidity. Leaf explant of basil were cultured on MS medium supplemented with melatonin (0, 100.0, 200.0 µM), NAA (2 mg/l) and BAP (1 mg/l). Cultures were incubated at 28°C in the dark. Callus induction was observed within four to five weeks after 2-3 times subcultures. The results showed that the supplement of melatonin at 100.0 µM and 200.0 µM in the MS medium decreased the frequency of callus induction compared with the control (MS medium without melatonin). Lower concentration (100.0 µM) of melatonin added in the medium could increase the differentiation frequency of adventitious buds from callus, however higher level of melatonin (200.0 µM) inhibited the bud differentiation. Also, secondary metabolite levels of callus were determined in control, 100.0 and 200.0 µM melatonin treatments. Results showed that the addition of melatonin affected either phenolics (like caffeic acid, rosmarinic acid, cinnamic acid, p-coumaric acid and vanilin) or aromatics (like 1,8-Cineole, dl-Limonene, Methyleugenol, 3-Methylbutanal, 2-Methylbutanal, Hexanal, 2-Furancarboxaldehyde, Benzaldehyde, Bergamotene) accumulation in various degrees. **Key Words:** Basil, Callus, Melatonin, Ocimum basilicum L., Secondary Metabolites