Aflatoxins are the most potent mycotoxins produced by *Aspergillus flavus* infection and pose a serious health hazard to the consumers of peanuts and peanut products. *A. flavus* contamination occurs during pre- or post- harvest stage, at this stage there is no active genetic resistance from the live cells. However, peanut seed coat serves as the outer layer in physically protecting the endosperm from pathogen infection. We aimed to exploit the physical and biochemical resistance mediated by seed coat against *A. flavus*. In vitro Seed Colonization Assay (IVSC) showed that line 55-437 is resistant to *A. flavus* infection compared to TMV2. To assess, if the resistance is due to the seed coat biochemicals, the seed coat extracts from 55-437 and TMV-2 and was used for radial growth bioassay using Aflatoxin producing *A. flavus* strain. 55-437 showed a greater level of inhibition than TMV-2 in radial growth bioassays in three independent replicates. Thirteen lines were obtained from USDA using seed coat color as a criteria and two lines (PI 561680 with pinkish tan color and PI 544346 with tan color) showed higher resistance to *A. flavus* compared to 55-437 during in vitro seed colonization. The result observed indicates that seed coat biochemical plays a critical role in *A. flavus* resistance observed in peanut germplasm.