Penicillin production, a significant area of research, utilizes various *Penicillium chrysogenum* strains. Improving production efficiency focuses on cheaper substrates and simpler cultivation methods. Agroindustrial wastes like sugarcane bagasse and corn steep liquor offer a cost-effective alternative. This study aimed to isolate *P. chrysogenum* from local sources and assess its penicillin production potential using these wastes in shake-flask cultivations, leveraging Pakistan's abundance of such resources.