Development of advanced fuel injection system requires in-depth investigations of microscopic spray characteristics. Over the years, diesel injector has undergone exponential improvement to be able to deliver fuel at high injection pressure. Phase Doppler interferometry (PDI) was implemented in a constant volume spray chamber to investigated spray characteristics of fuels such as mineral diesel, and Jatropha biodiesel (JB100)/ blends (JB5, JB10, JB20, JB40). A constant volume spray chamber was designed and fabricated, which was fitted with high pressure compressor and high-pressure lines. A leak test was performed upto 20 bar on this chamber. Stand Alone Direct Injection (SADI) system (injection driver) was synchronized with PDI system using NI drivven card and a TTL pulse was given by a customized circuit using function a generator. Alignment of laser beams was performed carefully, since PDI results were sensitive to alignment. Jatropha biodiesel was manufactured in for this study. It was observed that with increase in fuel injection pressure, average axial velocity of spray droplets increased. Spray droplet size decreased with increasing in fuel injection pressure. Microscopic spray characteristics were investigated for determining mean axial velocity distribution of spray droplets and droplet size distribution using PDI. Mean diameters D10, D20, D30 and D32 was found to increase with increasing blending ratio of biodiesel. JB100 showed maximum sauter mean diameter amongst all test fuels at 500 bar FIP.