**How to collect disease samples and further processing in lab conditions (A Case study)**

**3.1 Sample Collection**

Diseased samples were collected from different regions of Islamabad, Rawalpindi, Sargodha and Kahta a city in district khushab, which is famous in producing tomatoes in large quantity. After collecting, samples were placed in polythene zip lock plastic bags and carried to lab of Plant Pathology, University college of Agriculture, Sargodha. The samples were stored in refrigerator at 4oC for further use.

**3.2 Isolation and Identification of the Pathogen**

**3.2.1 Isolation on Blotter Paper**

Diseased Samples were cut into small pieces along with growing margins of disease and place on three layer water soaked filter paper .After inoculation PDA plates were placed in incubation chamber at 26 ±1°C for seven days. After 7 days of incubation, fungus colony was examined under stereoscopic microscope.

**3.2.2 Isolation on PDA Medium**

The pathogen was isolated from infected tomato leaves samples and then cut into small pieces along with growing margins of about 1.5-2cm, surface sterilize them with (Bleach) for approximately 2-minutes then washed three times with distilled water and placed on prepared PDA containing Petri plates. These Petri plates were incubated at 26 ±1°C for one week to check the sporulation for further studies.

**3.2.3 Preparation of Pure Culture**

Colony of pathogen from infected samples was observed after five to seven days. Pure culture obtained with the help of single spore technique (Hansen, 1926) or hyphal tip technique (Brown, 1924) by incubating at about 28°C for seven days and observed it daily to get rid of contamination from bacteria and other pathogens.

**3.2.4 Identification of Pathogen**

The purified fungi *Alternaria solani* was identified according to their morphological characters using the description of (Ozcelik, & Ozcelik, 1997; Perez & Martinez, 1997; Patterson, 1991).

**3.2.5 Pathogrnicity Test**

Pathogenicity test was carried out also confirmed pathogen *Alternaria solani* by producing concentric rings on thirty days old plants when sparyed mycelial suspension from seven-day-old culture. Reisolations were made from infected plants and the cultures thus obtained were compared with original cultures to confirm the identity and pathogenicity of pathogen.