**Standard Technical Protocols for Collection and Handling of Disease Samples**

**GENERAL REQUIREMENT**

The following general provisions apply to the protocol related tosampling and handling:

 Laboratory tests may involve the use of chemicals or equipment which may present a certain hazard. In all cases, safety procedures should be strictly followed.

 Use of names of chemicals or equipment in this protocols implies no approval of them to the exclusion of others that may also be suitable.

 The procedures presented in the protocols may be adjusted to the need of individual case of collection and handling, provided that they are adequately validated.

**Training of staff**

The staff responsible for survey work and collecting and handling disease sample should have knowledge and skill of plant disease diagnosis. The staff should be trained for the skill and knowledge. NPQP should organize training program for the newly recruited staff before giving task of sample collection and handling. Refresher training should be organized to update new development in the subject regularly.

**SPECIFIC REQUIREMENTS**

**Procedure for Sample Collection, Packagingand Submission**

A suggested list of supplies to include in a field kit used for collecting diseased plant samples is given in Appendix 2. After deciding what to include in the sample, the following procedures for obtaining, packaging, and submitting the sample need to be followed:

**Collecting plant samples**

It is important to collect the best plant samples possible and to record all pertinent information for the diagnostic purpose. Following are general guidelines for collecting disease samples.

**Examine the entire plant for symptoms**

One or more pathogenic microorganisms may infect diseased plants, although they may also have an abiotic disease that does not involve a plant pathogen. Diseased plants often display a range of symptoms. Often, not all symptoms of a particular disease will appear on any one plant within a diseased crop, and more than one plant organ may be affected by a given disease. All of the main plant parts/organs should be for disease symptoms: roots, stems, leaves, and blossoms. Samples from various plant parts/organs should be collected as needed. Plants may suffer from more than one disease simultaneously. Different types of plant parts with different symptoms should be segregated into different samples. 4

**Multiple disease specimens**

A single sample may not be enough to allow a correct diagnosis of the disease problem; several plant samples showing the range of symptoms or not having symptom may be needed. Samples with various stages of disease development should be selected (early and late stages). Samples should be as typical or representative of the overall disease problem as possible. The best plant tissues for diagnosis are the ones showing the symptoms in various stages of disease development, and adequate amounts of them are important, but submitting excessive amounts of leaves or soil should be avoided. Suitable plant material for varietal and or species identification should be included, since occasionally field identifications of the host variety or species may not be known.

**Avoid dead plants or plant parts/organs**

Dead plants or plant parts/organs may not be useful for diagnosis. Often their tissues have been invaded by saprophites and the original pathogens are no longer detectable. Always select plant samples from living tissues and focus your attention on plants or plantparts/organs that are in early stages of disease or are in the process of dying, and not already dead.

**Collect the entire plant whenever possible**

For the correct diagnosis with confidence correct plant parts should be submit; for example, some leaf symptoms of disease (wilting, for example) are the result of damaged or diseased roots that have rotted and are no longer functioning to support the plant; in such cases, a correct diagnosis often depends on having a sample of the roots. Plants should be carefully dug from the ground (not pulled out) so the root systems remain relatively intact. When the entire plant cannot be sampled, shipped, or submitted, collect the largest plant sample possible, or portions of each major plant organ (roots, stems, leaves, flowers). Branch specimens should include the diseased area and part of the healthy area and may be at least 8–12 inches long. Root samples should be taken from the affected plants. Roots of theadjacent weeds should be avoided. If entire plants cannot be sampled, photographs of affected plants where possible should be submitted. For soil and root sampling, roots should be carefully lifted so as not to leave feeder roots or rotted roots behind. About a kilogram of soil fromrhizoshere should be taken for pH, soluble salts, and possibly a nematode identification. Samples should be placed in appropriately sized plastic bags, including a paper towel (better sterilized) or a blotter if sample is very wet. Do not use only plastic bag for wet sample. Duplicate samples are recommended if the sample is succulent or fragile. When whole plant sample is taken, wrap a wire twist-tie around stem at ground line to keep soil off of above-ground plant parts. Samples should be accurately labeled and should be place in a paper bag or an unsealed plastic bag. Samples should be kept cool and should be protect from crushing (Use icebox with sufficient ice to cool the sample in the transit). Sample should not be frizzed. Sample should be delivered promptly to the laboratory.

**Provide backgroundand related information to the maximum extent possible**

Good information contributes to a better understanding of the problem. A complete description of the problem and the crop’s history should accompany the sample. The name or number of the plant 5

sampled should be given. When the problem appeared and when the sample was taken should be indicated. The crop growing site/area should be examined carefully and the prevailing conditions should be noted. The conditions for the site such as elevation, flooding, previous crop history, etc. should be noted. Any observable pattern of disease occurrence should be noted (for example, in random patches, or uniformly throughout the crop). The worksheet information should be reviewedas required. This information is helpful in making a distinction between damage caused by different types of pathogen and damage caused by other factors.(Appendix 1.) In short, the collector should make the following observation critically:

 Spatial and chronological pattern of disease in crop, on individual plants.

 Symptoms- different or consistent, uniform or scattered?

 Spreading disease- across crop or progressing on individual plants

 Frequency and intensity of the disease problem

 Signs of pathogen/causal agent- (fruiting structures, chemical residues, etc.).

 Vector (insects and others) and its different stages

 Any evidence of host recovery

 Symptoms of nearby plants (same or different)

 Internal symptoms/signs of disease (after cut-open)

**Preserving plant samples**

 After collecting the samples, do not expose the samples to direct sunlight.

 Keep them cool and do not allow them to dry out.

 Place samples in plastic bags in the shade or in a cooler until they are ready for delivery to the laboratory. Leaves may be pressed between newspapers in plant-press or between the pages of a book or magazine or wrapped in tissue.

**Isolation of Pathogen in the field**

Some fungal pathogens are too slow growing and get easily overtaken by saprophites in the sample. They need to be isolated in the field and kept viable in the transit. In the field portable isolation chamber (Fig.1.) can be used to reduce contamination by other organism. Sterilized petri plated with 15% water agar is prepared and sealed with parafilm and used to isolate the fungal or bacterial pathogens. The disease sample with the suspected pathogen is surface sterilized with 0.1 % sodium hypochroride, rinsed with sterilized distilled water three times and transferred to the petriplates with agar and resealed with parafilm tape. In the laboratory, the sample is used to isolate the pathogen in suitable selective or non-selective growth medium. The sample can also be used for other diagnostic purposes like DNA-analysis.

**Root/Soil/plant sample for nematode**

**Field Crops**

Nematodes do not necessarily occur uniformly throughout a field, therefore several sub-samples must be taken from across the field, and then combined. Twenty to thirty random sub-samples should be collected from each block of 1-2 hectares. Samples should be taken directly from the root zone. Sub-samples should be mixed thoroughly and place 500 g of soil, with roots, in a plastic bag, for laboratory analysis. Because nematode damage within a crop can be patchy, samples from healthy 6

plants, as well as from plants showing symptoms of decline should be collected. Samples should be kept separately and label them as.

**Trees and Shrubs**

Soil from around the rhizosphere containing feeder rootlets or intact root systems should be collected and place them in a plastic bag. Samples should be collected from the upper 20-30 cm depth around drip rings. Several sub-samples should be taken from each tree or shrub. A minimum of 500 g of soil, and 10 g or a handful of feeder roots, per sample is recommended. Thick woody roots are of little use for nematode analysis. Take Samplesshould be taken separately from healthy trees and from those showing symptoms of disease.

**Nurseries and Greenhouses**

About 500 g of soil including feeder roots should be. Leaf, stem, seed or other aerial plant material should be collected where symptoms are noticed and nematodes suspected.

**Avoid cross contamination:**

There is always chance of cross contamination from one sample to other. The chance of cross contamination should be minimized at the process of handling sample. The equipment like spatula, forceps, pruning knives, etc. used for collecting and handling sample should be disinfected before using to the new place or area. The collector should use disposable hand gloves and clean hand and equipment each time they star handling the sample. It will be safe to keep samples collected from one lot or area in one container like plastic bag or ice- box.

**Labeling Samples**

Labeling should be done properly so that identity of the sample does not get lost on the way. NPQP should develop coding system for the labeling so that uniformity is there and identification of the sample become easier (ex. d/h/t----). Labeling should be done right after collection. Labels should be there both inside and outer surface of the container. The label should be strong enough to withstand the rough handling. Permanent marker is not good for labeling soil and moist sample. Pencil written label on strong paper should be used to label samples. The label should be small and readable. The detail of the label should be noted on the note book or form designed. Blank labels with adhesive are available in the market. Such label should be tested before using for the sample.

**Transportation of disease samples to laboratory**

The sample should reach laboratory in minimum time possible. The sample should be transported in cool conditioned van because sample get contaminated or destroyed at high temperature. If cool van is not available, sample should be transported in icebox containing enough dry ice so that fresh samples reach to the laboratory in time. The sample should be categorized in fragile group and should not be staggered one above the other heavily.

**Handling quarantine samples**

The quarantine samples should be handled carefully so that pathogens do not escape in the transit to containment area. The sample should have warding sign in the sample that the sample may contain quarantine pest, while forwarding to the laboratory for diagnosis. In the laboratory the quarantine samples should be stored or preserved separately. The sample should be properly disposed after the lab work so that it does not escape the laboratory.