

Survey and Monitoring Protocols for Establishment of Pest-Free Areas  
for Brown Rot (*Ralstonia solanacearum*) Of Potato



**Government of India**  
**Ministry of Agriculture**  
**Department of Agriculture & Cooperation**  
**Directorate of Plant Protection, Quarantine & Storage**  
**N.H.IV., Faridabad-121001**

**CONTENTS**

<b>Section</b>	<b>Particulars of contents</b>	<b>Page</b>
	<i>Title</i>	1
	<i>Contents</i>	2
	<i>Endorsement</i>	4
	<i>Review &amp; Amendment</i>	5
	<i>Control &amp; Distribution</i>	5
	<b>INTRODUCTION</b>	6
	<b>Scope</b>	6
	<b>References</b>	6
	<b>Definitions &amp; terms</b>	6
	<b>Outline of requirement</b>	8
<b>1.</b>	<b>GENERAL REQUIREMENTS</b>	9
		9
<b>1.1.</b>	<b>Documentation of Information</b>	9
<b>1.2.</b>	<b>Operation of National Plan</b>	9
<b>1.3.</b>	<b>Management Structure &amp; Responsibilities</b>	9
<b>1.4.</b>	<b>Training requirements</b>	9
<b>1.3.</b>	<b>Funding &amp; Resources</b>	9
<b>2.</b>	<b>SPECIFIC REQUIREMENTS</b>	10
<b>2.1.</b>	<b>Designation of Pest Free Areas (PFAs)</b>	10
<b>2.2.</b>	<b>Survey Plan</b>	10
<b>2.3.</b>	<b>Survey Area</b>	10
<b>2.4.</b>	<b>Period of Survey</b>	11
<b>2.5.</b>	<b>Frequency of Survey</b>	11
<b>2.6.</b>	<b>Pre-Cropping (Soil) Survey</b>	11
<b>2.6.1.</b>	<i>Sampling Intensity (No. of fields to be sampled/Sampled Area)</i>	11
<b>2.6.2.</b>	<i>Sampling sites</i>	11
<b>2.6.3.</b>	<i>Soil sample size</i>	11
<b>2.6.4.</b>	<i>Sampling/analysis protocols</i>	12
<b>2.6.5.</b>	<i>Recording of field data</i>	12
<b>2.7.</b>	<b>Cropping Survey</b>	12
<b>2.7.1.</b>	<i>Sampling intensity (No. of fields to be sampled/Sample Area)</i>	12
<b>2.7.2.</b>	<i>Sampling units</i>	12
<b>2.7.3.</b>	<i>Sample size</i>	12

2.7.4.	<i>Sampling/field diagnostic protocols</i>	12
2.7.5.	<i>Recording of field data</i>	13
<b>2.7.</b>	<b>Post-harvest (tuber) Survey</b>	13
2.7.1.	<i>Sampling intensity (Location/No of lots sampled/lot size)</i>	13
2.7.2.	<i>Sampling units</i>	13
2.7.3.	<i>Sample size</i>	13
2.7.4.	<i>Sampling/detection protocols</i>	13
2.7.5.	<i>Recording field data</i>	13
<b>2.8.</b>	<b>Collection &amp; forwarding of soil/plant/tuber samples</b>	14
<b>2.9.</b>	<b>Laboratory testing</b>	14
<b>2.10.</b>	<b>Reporting of Survey</b>	15
<b>2.11.</b>	<b>Notification of PFAs</b>	15
<b>2.12.</b>	<b>Implementation of Domestic Quarantine measures</b>	15
<b>2.13.</b>	<b>Export inspection &amp; Phytosanitary certification</b>	16
<b>2.13.</b>	<b>Routine monitoring in the designated PFAs</b>	16
<b>2.14.</b>	<b>Notification of detection of brown rot in PFAs</b>	16
<b>2.15.</b>	<b>Suspension and Re-instatement of PFAs</b>	16
<b>2.16.</b>	<b>Auditing &amp; Review</b>	16
<b>Appendices:</b>		
	Appendix-1: Steps involved in establishment of pest free areas	17
	Appendix-2: Field Data Recording Form (Soil Survey)	18
	Appendix-3: Field Data Recording Form (Crop Survey)	19
	Appendix-4: Field Data Recording Form (Tuber Survey)	20
	Appendix-5: Sample Collection/Forwarding Form	21
	Appendix-6: Laboratory Diagnosis Report Form	22
	Appendix-7: Survey Report	23
	Appendix-8: Auditing of Surveillance of Brown Rot of Potato	24

## Endorsement

This standard on '**Survey and Monitoring Protocols for Establishment of Pest-Free Areas for Brown Rot (*Ralstonia solanacearum*) of Potato**' provides necessary guidelines for undertaking survey and monitoring of brown rot and to meet the requirements of establishment, maintenance and verification of pest free areas for brown rot of potato and use as a risk management option for undertaking phytosanitary certification of export of table potato from pest-free areas to European Union or provide scientific justification for phytosanitary measures for protection of endangered pest free area. This standard would enable the recognition of pest free areas in line with provisions of international agreements and thus facilitate the trade.

This standard is approved for adoption on \_\_\_<sup>th</sup> day of February 2005 by:

\_\_\_\_\_  
(P.S. Chandurkar)  
Plant Protection Adviser  
Dte of Plant Protection, Quarantine & Storage,  
NH-IV., **Faridabad-121001.**

## Review and Amendment

This standard would be subject to periodic review and amendment. The next review date for this standard would be February 2007 or any other date as may be considered by Plant Protection Adviser (PPA). The standard would be updated and revised if necessary. The standard holder should ensure that the current edition of this standard is being used.

## Control & Distribution of the standard

The master copy of this standard shall be held with PPA and controlled copies would be distributed by Joint Director (PQ), Directorate of Plant Protection Quarantine & Storage, Faridabad to the National/Regional Plant Quarantine Stations; Director of Agriculture/Horticulture of concerned States; Central Potato Research Institute (CPRI), Shimla/Modipuram; State Agriculture Universities (SAUs) of concerned States; and APEDA as listed below and to any other organization, to whom the distribution has been approved by the PPA. Any clarifications/enquiries regarding this standard would be made to the Joint Director (PQ), Dte of PPQS, Faridabad-121001.

<b>Controlled Copy Holder</b>	<b>Copy No.</b>
Joint Director (PQ), Dte of PPQ&S, N.H.IV., Faridabad	1
Dy Director (PP/Ent), National Plant Quarantine Station, New Delhi	2
Dy Director (Ent/PP.), Regional Plant Quarantine Station, Amritsar	3
Dy Director (Ent./PP), Regional Plant Quarantine Station, Kolkata	4
Dy Director (Ent/PP), Regional Plant Quarantine Station, Chennai	5
Dy Director (PP/Ent.), Regional Plant Quarantine Station, Mumbai	6
Director of Agriculture/Horticulture, U.P. State	7
Director of Agriculture/Horticulture, Punjab State	8
Director of Agriculture/Horticulture, Haryana State	9
Director of Agriculture/Horticulture, Madhya Pradesh State	10
Director of Agriculture/Horticulture, Chattisghar State	11
Chairman, Agriculture Product Export Development Authority, New Delhi	12
Director, Central Potato Research Institute, Shimla/Modipuram	13.
Head, Division of Plant pathology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, U.P. State	14
Head, Division of Plant Pathology, Punjab Agriculture University, Ludhiana, Punjab State.	15
Head, Division of Plant Pathology, Haryana Agriculture University, Hissar, Haryana State.	16
Head, Division of Plant Pathology, Jawaharlal Nehru Agriculture University, Jabalpur, Madhya Pradesh State.	17
Head, Division of Plant Pathology, Chattisgarh Agriculture University, Raipur, Madhya Pradesh State.	18.


## INTRODUCTION

### SCOPE

This standard describes the guidelines for undertaking survey and monitoring of brown rot of potato and requirements for the establishment and use of pest free areas (PFAs) for brown rot of potato as a risk management option for phytosanitary certification of table potato for export to European Union or scientific justification for phytosanitary measures taken for protection of an endangered PFA.

### REFERENCES

- Agreement on the Application of Sanitary and Phytosanitary Measures, 1994. World Trade Organization, Geneva.*  
*Determination of pest status in an area. ISPM No. 8, FAO, Rome*  
*Guidelines for surveillance, 1998. ISPM No. 6, FAO, Rome.*  
*International Plant Protection Convention, 1997. FAO, Rome.*  
*Requirements for the establishment of pest free areas. 1997. ISPM No. 4, FAO, Rome*

### DEFINITIONS AND ABBREVIATIONS

Area:	An officially defined country, part of a country or all or parts of several countries.
Buffer zone:	An area in which a specific pest does not occur or occurs at a low level and is officially controlled, that either encloses or adjacent to an infested area, an infested place of production or a pest free production site, and in which phytosanitary measures are taken to prevent the spread of the pest.
Delimiting survey:	Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest.
Detection survey:	Survey conducted in an area to determine if pests are present.
IPPC:	International Plant Protection Convention as deposited in 1951 with FAO, Rome and as subsequently amended.
Monitoring survey:	Ongoing survey to verify the characteristics of a pest population.
National Plant Protection	

Organization (NPPO):	Official service established by a government to discharge the functions specified by the IPPC.
Official:	Established, authorized or performed by a NPPO
Pest:	Any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products.
Pest free area (PFA):	An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained.
Pest record:	A document providing information concerning the presence or absence of a specific pest at a particular location, at a certain time, with in an area (usually a country) under described circumstances
Pest status (in area):	Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgment on the basis of current and historical pest records and other information.
Phytosanitary certification:	Use of phytosanitary procedures leading to the issue of phytosanitary certificate
Phytosanitary measure:	Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of pests.
Phytosanitary regulation:	Official rule to prevent the introduction and/or spread of quarantine pests, by regulating the production, movement or existence of commodities or other articles, or the normal activity of persons, and by establishing schemes for phytosanitary certification.
Quarantine pest:	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.
Survey:	An official procedure conducted over a definite period to determine the characteristics of a pest population or to determine which species occur in an area.
Surveillance:	An official process, which collects and records data on pest occurrence or absence by survey, monitoring or other procedures.

## **Outline of requirement**

This standard outlines procedures for undertaking survey and monitoring protocols for brown rot of potato (*Ralstonia solanacearum*). The developments of such protocols are critical for establishment of pest free areas for brown rot of potato in line with the established international standards to facilitate export of table potato to European Union. Collection and documentation of information regarding the occurrence and distribution of brown rot pathogen and its races and host species, operation of a national plan with clearly defined management structure and responsibilities is vital for the success of the programme and also trained technical experts and the adequate funding to meet the operational costs.

It is very much essential to organize detection surveys for a period of two years in nominated pest free areas with in the considered agri-export zones in identified States. Further this standard describes the proper surveillance procedures viz., scale of sampling, number of sampling units and sample size for pre cropping (soil) survey, cropping survey and post-harvest tuber survey for establishing the pest free areas in considered agri-export zones and maintenance of designated pest free areas. The standard also describes the requirements of notification of designated PFAs and the domestic quarantine measures required to be implemented by the concerned States to ensure pest-free status of the area is maintained.

## **1. GENERAL REQUIREMENTS**

### **1.1. Documentation of Information**

The information on detailed occurrence and distribution of brown rot (*Ralstonia solanacearum*) in India and its races and host range should be clearly documented based on review of scientific literature and the results of the earlier surveys organized by the Central Potato Research institute, Shimla.

### **1.2. Operation of National Plan**

The Department of Agriculture & Cooperation, Ministry of Agriculture, Government of India should establish a national plan for brown rot surveillance, outlining clear objectives of survey, action plan, management structure and responsibilities, training and resources and budgetary support, in cooperation with the Central Potato Research Institute, Shimla involving concerned State Governments/Agriculture Universities and Agriculture Processed Food Export & Development Authority (APEDA)

### **1.3. Management Structure & Responsibilities**

Directorate of Plant protection, Quarantine & Storage (Dte of PPQS) established under Ministry of Agriculture, being the National Plant Protection Organization has the overall responsibility for implementation of survey and monitoring programmes for determination of pest free areas (PFAs). The Central Potato Research Institute, Shimla will undertake the survey in the nominated agri-export zones in the identified States for identifying the pest free areas. The Director of Agriculture/Horticulture of identified States will be responsible for effective implementation of domestic regulatory measures for restricting the movement of propagating plant material and other solanaceous crops from other areas into designated pest free areas and regulating the exports from the designated pest free areas of concerned States.

### **1.4. Training Requirements**

The technical personnel drawn for the survey should have post-graduate degree in agriculture in the field of plant pathology. They should be adequately trained in brown rot surveillance viz., soil/plant/tuber sampling, field diagnosis of brown rot in growing crop of potato including tuber infections and bacterial wilt in other Solanaceous hosts, collection and forwarding of samples of soil/plant/tubers for detailed laboratory testing and recording of field data. The training programmes should be organized at the Central Potato Research Institute, Shimla and should be of at least 3-4 days duration at the beginning of each season prior to initiation of survey. The trained personal should be audited at the end of training for their capabilities in undertaking proper survey. .

## 1.5. Funding & Resources

The Central Government should provide sufficient budgetary support for organizing the survey for a period of two years and to meet the resources required for the survey.

## 2. SPECIFIC REQUIREMENTS

### 2.1. Designation of Pest Free Areas (PFAs)

The survey should be carried out in the potato growing areas in the agri-export zones of UP, Punjab, MP and Chhatisgarh States to identify the pest free areas (PFAs) for brown rot of potato (*Ralstonia solanacearum*).

Areas to be surveyed will include:

- **Punjab State:** Jalandhar, Amritsar, Hoshiarpur, Ludhiana, Moga and Kapurthala;
- **Haryana State:** Karnal and Kurukshetra;
- **Uttar Pradesh State:** Muradabad, Meerut, Allahabad, Jaunpur, Ghajipur, Faizabad, Barabanki, Agra, Etawa, Farukhabad, Kanpur Rural and Mainpuri;
- **Madhya Pradesh State:** Indore, Dewas, Ujjain, Betul and Chhindwara; and
- **Chhatisgarh State:** Raipur

### 2.2 Survey plan

A suitable survey plan should be drawn by the Central Potato Research Institute and forwarded to the Plant Protection Adviser sufficiently in advance at the beginning of each year for necessary approval.

The survey plan should include:

- Purpose of survey
- Target pest (s) involved
- Target crop(s) involved
- Scope of the survey (geographical area, production system, season)
- Timing of survey (dates, frequency, duration)
- Methodology of survey (statistical sampling/detection protocols etc)
- Mapping (survey area/routes)
- Field recording

The survey should be carried out as per approved plans of the Plant Protection Adviser, Directorate of Plant Protection, Quarantine & Storage, Faridabad.

### 2.3. Survey Area

- Map showing nominated pest free areas with survey routes should be prepared. The nominated pest free areas (PFAs) should be clearly demarked preferably by GPS coordinates and the boundaries
- Information on size of potato growing areas (Blocks/No of fields/Extent of potato growing area (in ha) should be collected for each agri-export zones in which designated pest free areas (PFAs) required to be established and documented prior to initiation of survey
- Information on crop density (plant population/m<sup>2</sup>); cropping intensity (cropping season/No of crops); cropping patterns; agronomic practices being adopted in each agri-export zone and agro-climatic data should be collected and documented
- The fields selected for survey as far as possible should be based on random statistical sampling and should be clearly documented

#### **2.4. Frequency of Survey**

- Monitoring of pathogen in the absence of crop (pre-cropping survey) should be carried out in July-August;
- Monitoring of growing crop of potato (cropping survey) should be carried out during September-October and December-February. Solanaceous crops viz., brinjal, capsicum and tomato will be surveyed during September-October
- Monitoring of tuber infections after crop harvesting (post-harvest tuber survey) should be carried out in February-March to determine symptomatic/latent infections

#### **2.5. Period of Survey**

The detection surveys should be carried out for a minimum period of two years. The detection surveys should also include seed production areas located in the above agri-export zones

#### **2.6. Pre-Cropping (Soil) Survey**

##### *2.6.1. Sampling intensity (No. of fields to be sampled/Sampled Area)*

A minimum of 10% of fields should be selected at random for sampling of soil in each block

##### *2.6.2. Sampling sites*

Soil samples, each of 100-200 g should be collected from at least from ten random sites in each selected field and composited.

### 2.6.3. *Soil sample size*

A composite sample of 1 kg should be collected for laboratory analysis to detect the presence of brown rot pathogen.

### 2.6.4. *Sampling/analysis protocols*

The soil samples should be collected around the rhizosphere zones or hulms of potato/solanaceous crops left over in the field. The soil samples should be collected up to a maximum depth of 10-15 cm.

The soil samples should be forwarded for detailed laboratory testing at CPRI, Shimla.

### 2.6.5. *Recording of field data*

The field data of soil survey should be recorded as per the format prescribed in Appendix-2.

## **2.7. Cropping Survey**

### 2.7.1. *Sampling intensity (No. of fields to be sampled/Sample Area)*

A minimum of 20% fields/cropped area should be selected at random in each block of designated agri-export zone of identified State.

### 2.7.2. *Sampling units*

The plants grown in at least five random spots each of 10 m<sup>2</sup> should be selected in the field representing all the four sides and one at the center of the field.

### 2.7.3. *Sample size*

A total of about 1000 plants per unit should be visually inspected for the symptoms of bacterial wilt.

### 2.7.4. *Sampling/field diagnostic protocols*

The sampling of plants should be carried out with the help of quadrat. All the plants falling within quadrat should be visually examined for the typical symptoms of bacterial wilt. These include wilting of the leaves at the ends of the branches during the heat of the day with recovery at night; eventually, plants fail to recover and die. As the disease develops, a streaky brown discoloration of the stem may be observed on stems up to 2.5 cm or more above the soil line, and the leaves have a bronze tint. The stems of suspected plants will be subjected to ooze test. A white, slimy mass of bacteria exudes from vascular bundles, which are broken or cut. This slime oozes spontaneously from the cut surface of a potato stem in the form of threads, when kept in a

beaker with water. Such threads are not formed by other bacterial pathogens of potato. The suspected plants should be collected and forwarded for detailed laboratory testing at CPRI, Shimla.

#### 2.7.5. *Recording of field data*

The field data of cropping survey should be recorded as per the format prescribed in Appendix-3.

### **2.7. Post-harvest (tuber) Survey**

#### 2.7.1. *Sampling intensity (Location/No of lots sampled/lot size)*

A minimum of 30 % of tuber lots should be sampled in each block of designated agri-export zone of identified State.

#### 2.7.2. *Sampling units*

A minimum of 10 primary samples each of 100 tubers should be drawn at random from each lot of tubers and composited.

#### 2.7.3. *Sample size*

At least a sample of 600 tubers should be drawn from the composited sample for examination.

#### 2.7.4. *Sampling/detection protocols*

All the tubers should be visually examined for typical symptoms of brown rot (*Ralstonia solanacearum*), which is characterized by the emergency of the bacterial ooze that often from the eyes and stem-end attachment of infected tubers. When this bacterial exudate dries, a mass of soil adheres to the tubers at the eyes. The suspected tubers should be cut open and examined for typical browning and necrosis of the vascular ring and immediately surrounding tissues up to 0.5 cm each side of the ring. A creamy fluid exudate usually appears spontaneously on the vascular ring of the cut surface a few minutes after cutting. However, in the case of ring rot (*Clavibacter michiganensis sub sp. sephedonicum*) the tuber has to be squeezed in order to press out a mass of yellowish dissolved vascular tissue and bacterial slime. The suspected tuber samples should be collected and forwarded for detailed laboratory testing at the CPRI, Shimla. In the event of tubers showing no apparent symptoms a sample of 200 tubers shall be collected and forwarded to CPRI, Shimla for laboratory diagnosis of latent infections, if any.

### 2.7.5. Recording field data

The field data of post-harvest tuber survey should be recorded as per the format prescribed in Appendix-4.

## 2.8. Collection & forwarding of soil/plant/tuber samples

The soil samples should be packed in a self-sealing polythene bag and the plant samples should be wrapped between tissue papers and placed in self-sealing type polythene bag. The tuber samples should be packed in cloth bags sufficient to hold 200 tubers. The samples of soil/plant/tubers collected from the field should be labeled giving information about Name of Farm, field number, location, date of collection and the collector and should be forwarded to the Central Potato Research Institute in the prescribed for mat given in Appendix-5

## 2.9. Laboratory testing

The soil samples should be analyzed by dilution plate technique on selective medium (SMSA). The plant/tuber samples should be subject to indirect immunofluorescence antibody staining (IFAS), plating onto a selective medium (SMSA) and a pathogenicity test on tomato to confirm positive results. IFAS or selective plating result, and, in addition, PCR, ELISA or fluorescent in-situ hybridization (FISH) can be performed. Standard samples of 200 tubers per 25 t of potatoes are taken (Janse, 1988; Anon., 1997). Recently a very effective selective medium has been described (Engelbrecht, 1994, and modified by Elphinstone et al., 1996), that can also be applied for detection in environmental samples such as surface water, soil and waste (Janse et al., 1998; Wenneker et al., 1999). ELISA and the polymerase chain reaction (PCR), based on 16S rRNA targeted primers as well as fluorescent in-situ hybridization (FISH), using 16S and 23S rRNA-targeted probes, have also been used successfully. Biochemical tests, fatty acid analysis, RFLP and protein analysis can be used for identification purposes (Seal et al., 1993; Seal and Elphinstone, 1994; Anon., 1997; Stephani and Mazzucchi, 1997; Hartung et al., 1998; Wullings et al., 1998; Seal et al., 1999).

Anon., 1997. Interim testing scheme for the diagnosis, detection and identification of *Pseudomonas solanacearum* (Smith) Smith in potatoes. Publication 97/647/EC, Official Journal European Communities, 273:1-25.

Elphinstone JG, Hennessey JK, Wilson JK, Stead DE, 1996. Sensitivity of different methods for the detection of *Pseudomonas solanacearum* (Smith) Smith in potato tuber extracts. Bulletin OEPP/EPP Bulletin, 26:663-678.

Elphinstone JG, Stanford HM, Stead DE, 1998. Detection of *Ralstonia solanacearum* in potato tubers, *Solanum dulcamara*, and associated irrigation water. In: Prior P, Allen C, Elphinstone J (eds) Bacterial Wilt Disease: Molecular and Ecological Aspects. Berlin, Germany: Springer publishing, 133-139.

Engelbrecht MC, 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. Bacterial Wilt Newsletter, No. 10:3-5.

Hartung F, Werner R, Mülbach HP, Büchner C, 1998. Highly specific PCR-diagnosis to determine *Pseudomonas solanacearum* strains of different geographic origins. Theoretical and Applied Genetics, 96:797-802.

Seal SE, Elphinstone JG, 1994. Advances in identification and detection of *Pseudomonas solanacearum*. In: Hayward AC, Hartman GL, eds. Bacterial wilt: the Disease and its Causative Agent, *Pseudomonas solanacearum*. Wallingford, UK: CAB International, 35-57.

Seal SE, Jackson LA, Young JPW, Daniels MJ, 1993. Differentiation of *Pseudomonas solanacearum*, *Pseudomonas syzygii*, *Pseudomonas pickettii* and blood disease bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. Journal of General Microbiology, 139(7):1587-1594

Seal SE, Taghavi M, Fegan N, Hayward AC, Fegan M, 1999. Determination of *Ralstonia (Pseudomonas) solanacearum* rDNA subgroups by PCR tests. Plant Pathology, 48(1):115-120

Stefani E, Mazzucchi U, 1997. Protein electrophoretograms for the identification of *Ralstonia solanacearum* in potato tubers. Journal of Plant Pathology, 79(3):189-195

Wenneker M, Verdel MSW, Groeneveld RMW, Kempenaar C, Beuningen ARvan, Janse JD, 1999. *Ralstonia (Pseudomonas) solanacearum* race 3 (biovar 2) in surface water and natural weed hosts: First report on stinging nettle (*Urtica dioica*). European Journal of Plant Pathology, 105(3):307-315.

Wullings BA, Beuningen ARvan, Janse JD, Akkermans ADL, 1998. Detection of *Ralstonia solanacearum*, which causes brown rot of potato, by fluorescent in situ hybridization with 23S rRNA-targeted probes. Applied and Environmental Microbiology, 64(11):4546-4554.

The laboratory test results should be reported in the format prescribed in Appendix-6.

## **2.10. Reporting of Survey**

The results of survey should be reported in the format prescribed in Appendix-7. A copy of survey report should be forwarded to the Plant Protection Adviser, Directorate of Plant Protection, Quarantine & Storage, N.H.IV, Faridabad-121001 at the end of each survey.

## **2.11. Notification of PFAs**

Department of Agriculture & Cooperation (MOA) should notify the identified pest free areas and advise the concerned State Governments about required phytosanitary measures should be undertaken for maintaining pest free status of area concerned.

## **2.12. Implementation of Domestic Quarantine measures**

State Governments of Uttar Pradesh, Punjab, Haryana, Madhya Pradesh and Chhatisgarh should implement the regulations restricting the movement of seed material of potato from other areas into the notified pest-free areas established within agri-export zones of the identified states and to undertake specified phytosanitary measures to prevent the introduction of brown rot (*Ralstonia solanacearum*) affected plant material of potato into the designated pest free areas or to destroy any previously undetected infestation.

### **2.13. Export inspection & Phytosanitary certification**

Directorate of Plant Protection, Quarantine & Storage (MOA) will organize inspection of export consignments from the designated pest-free areas established within the agri-export zones of identified states. A phytosanitary certificates will be issued in IPPC format confirming that brown rot (*Ralstonia solanacearum*) is not known to occur in designated pest free area (s) and further that the consignment is free from the brown rot as indicated above.

Any detection of brown rot in consignments offered for export inspection and phytosanitary certification will result the suspension of the exports from that area until the pest free status of that area is reinstated.

### **2.14. Routine monitoring of designated PFAs**

Regular monitoring to confirm on-going freedom of pest-free areas should be undertaken by the Directorate of Plant Protection, Quarantine & Storage in association with the Central Potato Research Institute, Shimla and the results would be communicated to Department of Agriculture & Cooperation (MOA) for further communication to European Union before the commencement of each export season.

### **2.15. Notification of detection of brown rot in PFAs**

Department of Agriculture & Cooperation (MOA) will notify the concerned States and European Union of any detection of brown rot of potato (*Ralstonia solanacearum*) in designated pest free areas (PFAs) established within agri-export zones of identified States during routine monitoring and surveys conducted in the production season.

### **2.16. Suspension and Re-instatement of PFAs**

The concerned State Governments should immediately suspend the exports from the brown rot detected agri-export zones and undertake immediate eradication measures. Pest free area status would be reinstated only after two years of intense monitoring surveys confirming negative results for brown rot infection.

### **2.17. Auditing & Review**

The Directorate of Plant Protection, Quarantine & Storage will conduct auditing of brown rot survey to ensure that the survey and monitoring protocols specified in this Standard are complied with The results of auditing will be reported to the Plant Protection Adviser in

prescribed format given in Appendix-8. The establishment and maintenance of a PFA should be adequately documented and periodically reviewed by the Directorate of Plant Protection, Quarantine & Storage (Ministry of Agriculture). The documentation should include supporting evidence describing official controls including phytosanitary regulations, survey and monitoring protocols and results of survey and phytosanitary measures undertaken.

## Annexure-1

### Steps involved in Establishment of Pest-free areas for brown rot of potato (*Ralstonia solanacearum*)

1. A National Plan for undertaking brown rot surveillance should be prepared by CPRI in consultation with Dte of PPQS/APEDA.
2. Dte of PPQS will prepare a standard on survey and monitoring protocols for establishment of PFAs for brown rot of potato in considered agri-export zones and submit to Department of Agriculture & Cooperation (MOA) for approval
3. CPRI, Shimla will prepare survey plan as per the Standard in the beginning of each year and submit for the approval of Plant Protection Adviser.
4. A team of experts from the CPRI, Shimla/Modipuram will conduct the detection surveys in proposed PFAs and submit the report for consideration of PPA and approval of Department of Agriculture & Cooperation (MOA).
5. Department of Agriculture & Cooperation (MOA) will hold consultation with Dte of PPQS/State Department of Agriculture/Horticulture on the survey results and recommendations and adopts the report.
6. Department of Agriculture & Cooperation (MOA) will notify the designated PFAs and advise the concerned State Governments regarding appropriate phytosanitary measures to be undertaken to maintain PFAs..
7. Department of Agriculture & Cooperation (MOA), if feasible, should issue a domestic regulatory measures for restricting the movement of plant material of mango (*Mangifera indica* and *M. foetida*) including fresh fruits from mango pulp weevil infested States to any other Sstate.
8. Dte of PPQS will regularly audit pest records, review of survey programmes, evaluation of measures undertaken and quality control protocols with CPRI and implementation of domestic regulatory measures with concerned State Governments.
9. Dte of PPQS will undertake the inspection of export consignments at the warehouses of potato located in the designated PFAs established within the agri-export zones and issue phytosanitary certificates confirming that brown rot of potato is not known to occur in designated pest free areas and that the consignment is free from the pests indicated above.
10. Any pest detection in export consignments should be reported to the Dte of PPQS for further communication to European Union and further export of consignments would be suspended from that area until such time the pest free status of that area is reinstated.
11. Dte of PPQS in association with CPRI and concerned State Director of Agriculture/Horticulture will conduct monitoring survey to verify the characteristics of pest population in established PFAs
12. Department of Agriculture & Cooperation (Ministry of Agriculture) will periodically reviews the documentation for establishment of PFAs with CPRI/APEDA/ concerned State Governments













## **Appendix-7: Survey Report**

**Title**

**Purpose of Survey**

**Target pest (s) involved**

**Target crop(s) involved**

**Scope of the survey (geographical area, production system, season)**

**Timing of survey (dates, frequency, duration)**

**Survey Team**

**Survey Methodology**

- Pre-cropping (Soil) Survey
- Cropping Survey
- Post-harvest (tuber) Survey

**Mapping (survey area/routes)**

**Results of Field Survey**

**Results of Laboratory Testing**

**Summary**

## Appendix-8

### Auditing of Surveillance of Brown Rot of Potato

#### 1. Area audited

#### 2. Survey Team involved

#### 3. Audited by

#### 4. Date (s) of Auditing

#### 5. Details of Auditing

5.1. Whether the technical personnel drawn for the survey has undergone training in survey of brown rot

- Yes**
- No**

#### Comments:

5.2. Whether the technical personnel drawn for the survey is fully acquainted with the requirements of the Standard established by the Dte of PPQS

- Fully acquainted with the Standard**
- Partly aware of the Requirements**
- Not aware of the Standard**

#### Comments:

5.3. Whether the technical personnel drawn for the survey is capable of diagnosis symptoms of brown rot in the field

- Fully competent**
- Require further training**
- Not capable**

#### Comments/Action needed:

5.4. Whether the survey carried out as per approved survey plan by the Plant Protection Adviser

- Yes**

- No**

**Comments/Action needed:**

5.5. Whether proper sampling protocols as specified in the Standard are adopted and in drawing the soil samples for laboratory diagnosis and field data is recorded in specified format

- Yes**
- No**

**Comments/Action needed:**

5.6. Whether proper sampling/detection protocols as specified in the Standard are adopted for detecting brown rot in growing crop of potato and other Solanaceous hosts and field data recorded in specified format

- Yes**
- No**

**Comments/Action needed:**

5.7. Whether proper sampling/detection protocols as specified in the Standard are adopted for detecting tuber infections and field data is recorded in specified format

- Yes**
- No**

**Comments/Action needed:**

5.8. Whether the samples of soil/plants/tubers collected from the field are forwarded to the CPRI, Shimla in time for laboratory diagnosis

- Yes**
- No**

**Comments/Action needed:**

5.9. Whether the technical personnel assigned for the laboratory diagnosis are fully familiar with the test techniques and competent to run confirmatory tests (IFAS/ELISA/PCR techniques) for brown rot (*Ralstonia solanacearum*) and its races and the laboratory test results are reported in prescribed format

- Yes**
- No**

**Comments/Action needed:**

5.10. Whether the survey reports are prepared as per the harmonized format specified in the standard and forwarded to Dte of PPQS at the end of each cropping season

- **Yes**
- **No**

**Comments/Action needed:**

5.11. Any other observations:

5.12. Name & Signature of Auditing Officer (s) with date

1. \_\_\_\_\_ 2 \_\_\_\_\_

5.13. Name & Signature of Area Coordinator with date

5.13. Name & Signature of Programme Coordinator with date