Government of the People's Republic of Bangladesh
Ministry of Agriculture
Department of Agricultural Extension
Plant Quarantine Wing
Strengthening Phytosanitary Capacity in Bangladesh Project

Pest Risk Analysis (PRA) of *Globodera* spp. in Bangladesh

June 2017

Report on

Pest Risk Analysis (PRA) of *Globodera* spp.

in Bangladesh

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ACKNOWLEDGEMENTS

We are delighted to extend our appreciation to Strengthening Phytosanitary Capacity in Bangladesh (SPCB) Project under Plant Quarantine Wing (PQW) of Department of Agricultural Extension for keeping reliance on Association of Human Development Program (AHDP) to carry out the "**Conducting Pest Risk Analysis (PRA) for *Globodera* spp. in Bangladesh**". This report has been prepared based on the laboratory activities and extensive field survey of 21 potato growing areas of Bangladesh for the last four months (January 2017 to May 2017) as well as reviewing the secondary information. We have had a pleasant time with SPCB-PQW for getting although support during course of working on setting the indicators, revising the questionnaires, data collection on sampling and field survey, monitoring, supervision, data analysis and report writing. Professor Dr. Md. Atiqur Rahman Khokon, Team Leader, PRA team is the chief author with efforts from Professor Dr. Md. Aminuzzaman of the PRA team.

The authors whole-heartedly acknowledge to all persons involved in the entire PRA study of *Globodera* spp. Our sincere gratitude to Kbd Md. Golam Maruf, Director General, DAE, Bangladesh for providing support and opportunity to meet the Director of Plant Quarantine Wing (PQW) of DAE. We Specially thanks Dr. Mohammad Ali, Project Director, Strengthening Phytosanitary Capacity in Bangladesh (SPCB) Project; Mr. Md. Ahsan Ullah, Consultant (PRA); Mrs. Marina Jebunehar, Senior Monitoring and Evaluation Officer, SPCB for their valuable cooperation, guidance and suggestions to the study team. We are thankful to Kbd Md. Mohsin, Director, PQW of DAE for his kind cooperation and suggestions during the study period. We would like to extend our gratitude to Krishibid Dulal Chandra Das, Executive Director of AHDP and PRA study Coordinator for cordial support during field survey, data collection, monitoring activities and analyzing data.

Professor Dr. Md. Atiqur Rahman Khokon
Team Leader

ACRONYMS

AEZ : AGRO-ECOLOGICAL ZONE
AHDP: ASSOCIATION OF HUMAN DEVELOPMENT PROGRAM
BADC : BANGLADESH AGRICULTURE DEVELOPMENT CORPORATION
BARI : BANGLADESH AGRICULTURAL RESEARCH INSTITUTE
BAU : BANGLADESH AGRICULTURAL UNIVERSITY
BBS : BANGLADESH BUREAU OF STATISTICS
CABI : CENTER FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL
DAE : DEPARTMENT OF AGRICULTURAL EXTENSION
DG : DIRECTOR GENERAL
DR. : DOCTOR
e.g. : FOR EXAMPLE
EPPO : EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
et al. : AND ASSOCIATES
EU : EUROPEAN UNION
FAO : FOOD AND AGRICULTURE ORGANIZATION
FAOSTAT : FOOD AND AGRICULTURE ORGANIZATION STATISTICS
FGD : FOCUS GROUP DISCUSSION
GOB : GOVERNMENT OF BANGLADESH
IPPC : INTERNATIONAL PLANT PROTECTION CONVENTION
IPM : INTEGRATED PEST MANAGEMENT
ISPM : INTERNATIONAL STANDARD FOR PHYTOSANITARY MEASURES
J. : JOURNAL
KII : KEY INFORMANT INTERVIEW
LTD : LIMITED
MD : MANAGING DIRECTOR
NGO : NON-GOVERNMENT ORGANIZATION
NO. : NUMBER
NPPO: NATIONAL PLANT PROTECTION ORGANIZATION
^{O}C: DEGREE CELSIUS
PD: PROJECT DIRECTOR
PFA: PEST FREE AREA
PPW: PLANT PROTECTION WING
PQW: PLANT QUARANTINE WING
PRA: PEST RISK ANALYSIS
PROF.: PROFESSOR
PVT. : PRIVATE
RH: RELATIVE HUMIDITY
SCA: SEED CERTIFICATION AGENCY
SID: STATISTICS AND INFORMATICS DIVISION
SPCB : STRENGTHENING PHYTOSANITARY CAPACITY PROJECT IN BANGLADESH
UK : UNITED KINGDOM
USA : UNITED STATES OF AMERICA
USDA : UNITED STATES DEPARTMENT OF AGRICULTURE
% : PERCNTAGE

EXECUTIVE SUMMARY

Bangladesh is the 7th top potato producing country in the world. Around 10 million tons potato are Produced in the country every year. For cultivation of potato we had to import a large amountof seed potato from European countries following the stringent phytosanitary measures. Currently, Govt. and private sector are producing seed potato for fulfilling the huge demand of seeds. But still to day a substantial amount of seed is being imported from the EU countries. So, there is a chance of introducing the pest *Globodera* spp. in Bangladesh.

The study 'Pest Risk Analysis (PRA)' for potato in Bangladesh has been endeavoured to document the presence of potato cyst nematode (PCN) comprises of *Globodera rostochiensis, G. pallida*, *G. tabacum*, *G. ellingtonae* and *G. mexicana* in Bangladesh and analyze the risk associated with import pathway and prepare necessary recommendations to combat the problem if any.

This study was undertaken following IPPC Rules and Regulations. A project entitled "Strengthening Phytosanitary Capacity in Bangladesh" of the Plant Quarantine Wing of Department of Agricultural Extension under the Ministry of Agriculture is performing to analyse the pest risk of different commodities and the existence of certain pest/pathogens in Bangladesh. The Plant Quarantine Wing is executing this task with the assistance of Private Organization who has qualified personnel. Association of Human Development Program (AHDP) has been awarded to conduct the PRA of *Globodera* spp. in Bangladesh. The PRA process is being conducted with the technical assistance of the Consultant-PRA, Md. Ahsan Ullah, Strengthening Phytosanitary Capacity in Bangladesh Project.

There is no report of occurrence of *Globodera* spp. in Bangladesh. Therefore, one of the basic objectives of this study is to develop baseline information. In order to do it, relevant information were collected from different secondary sources like published papers, books, journals, internet as well as interview with different stakeholders such as farmers, personnel from DAE, experts and professionals. Primary data were collected through field survey and laboratory analysis of plant and soil samples. For this purpose potato growing 70 upazillas under 21 distracts were selected and visited. In each upazila 100 farmers (10 blocks and 10 farmers from each block) were selected for data collection.

A field guide book having the description and the pictures of typical symptoms of PCN affected field collected from different website were provided to the interviewers and data enumerators. Responses indicated that causal organism is not known to the farmers. The farmers have never seen the symptoms like field note book. Neither have they ever complained any production obstacles by PCN. On the other hand, they have experience with potato diseases like late blight, soft rot, dry rot, scab etc. and insect pests like potato cut worm, etc. In some cases they can identify root-knot disease of potato causes by *Meloidogyne* spp. Survey further indicated that

farmers are aware of weeds and insects infestation in the potato field and they usually take some measures during potato land preparation. Farmers used to apply the chemicals of carbamate group in some cases as a routine cultivation practice.

Interviewing with Focus Group Discussion (FGD) and KII (Key Informant Interview) also provided important information and suggestions regarding PCN in Bangladesh. The respondents of FGDs are not aware of the specific sign and symptoms of PCN, although some of them know about diseases of potato, association of weeds with potato, possible precautionary measures to be taken. Key Informant Interview mostly comprises the eminent professors of the universities, scientists of the research institutes and high officials of the DAE, Bangladesh. The responses of KII indicate that the absence of PCN in Bangladesh. The KII respondents have stressed upon the continuous monitoring of the PCN and innovating the quick diagnosis kit. They further suggested for regular and routine application of carbamate pesticides and management of weeds of the solanaceae family.

The laboratory analysis of the plant samples of potato and associated solanaceous weeds, soils, potato tubers following standard isolation technique of nematode indicate the absence of *Globodera rostochiensis, G. pallida*, *G. tabacum*, *G. Ellingtonae* and *G. mexicana*in the surveyed areas. During the course of isolation modified method of Baermann Funnel Technique and modified Flegg's Sieving and Sedimentation Technique was followed. Soil and root samples of potato collected from 21 districts and 70 upazill as comprising farmers' field, potato tubers from quarantine station, BADC, private companies reveal the complete absence of *Globodera* spp. An additional endeavour was considered to record the plant parasitic nematodes in potato field from the surveyed locations. A number of plant parasitic nematodes were isolated from various samples of potato mostly comprise of *Meloidogyne incognita* and *Helicotylenchus dihystera* were isolated from soil and plant samples of potato, brinjal, and tomato.

A number of weed hosts consisting of tit-begun (black nightshade - *Solanum nigrum*) and foska begun (clammy ground cherry - *Physalis heterophylla*) were also taken into consideration for investigation for the association of *Globodera* spp. No visible symptoms were recognized with the weed hosts, neither laboratory analysis showed the presence of any cyst nematode. Rather some common root infesting plant parasitic nematodes were associated as like as potato, tomato and brinjal.

Based on the risk assessment it is revealed that the existence of *Globodera* spp. in potato as well as in brinjal, tomato and relevant weed hosts is absent in Bangladesh. Conversely, Bangladesh is at high risk condition due to import of seed potato tuber every year from those counties where *Globodera* spp. are available. Therefore, several management options can be adopted in a systematic way to restrict the invasion of *Globodera* spp. in Bangladesh and further spreading upon invasion-

Policy level:

1. Imposition of phytosanitary certificate for importing seed potato tuber at public and private sector.
2. Routine geographic positioning of survey and digital archive of data every year.
3. Tagging phytosanitary certificate on the potato bag.
4. Training for the administrative officers.

Capacity building:

1. Molecular-based quick diagnostic protocol and developing skilled manpower at all entry port of Bangladesh.
2. Training at different levels like high officials (AD, DD, Agriculture officer, AEO), private entrepreneurs, technicians, farmers, businessman etc.

Field management:

1. Certified tuber should be used for seed production.
2. Prophylactic measures including chemical nematicides application in the intensively potato growing areas.
3. Weed management especially tit begun and foska begun.
4. Cleaning and burning of potato stubbles.
5. Filed inundation after harvest.
6. Safe disposal of discarded tuber.
7. Disinfecting agricultural tools and equipment.
8. Pre-harvest inspection.
9. Crop rotation.

Chapter 1

Initiation of the process for analyzing the risk assessment of *Globodera* spp. in Bangladesh

1.1 Back ground of the PRA study and justification:

Production of quality potato tuber is the latest thrust sector of agriculture in Bangladesh for its native consumption and export. Bangladesh has promising market for exporting table potato tubers in Russia, Malaysia, Singapore, Indonesia, Sri Lanka, Thailand, Hong Kong, Vietnam, Maldives, Turkey, Azerbaijan, Ethiopia and Nigeria. According to agriculture ministry of Bangladesh, in the 2013-14 fiscal, Bangladesh exported around $34m worth of potato to different countries, including $9m of export to Russia alone. Malaysia is the largest importer of potato from Bangladesh, buying around $13m of potato from Bangladesh during the last fiscal year (DAE, 2014). The production of potato in Bangladesh is surplus at present against its consumption which is approximately 26 lac metric tons (FAOSTAT, 2015). This emerging sector could not be flourished to its expected level due to lack of intensive findings of quarantine pest of potato like *Globodera* spp. Simultaneously, the seed potatoes used for cultivation in Bangladesh mostly imported from the Netherlands as well as from other countries such as Belgium, Germany, Denmark and USA. In the fiscal year 2014-15, Bangladesh imported 5,238.8 MT seed potatoes through Chittagong Seaport, of which 4748.80 MT from the Netherlands, 350.00 MT from Belgium, 140.00 MT from Germany (DAE, 2015). It is observed that import of seed potao is reducing day by day.Therefore, a potential risk of introduction of *Globodera* spp. associated with seed potatoes imported from the country of exports into Bangladesh.

The potato cyst nematodes (PCNs) *Globodera rostochiensis* (Wollenweber) Skarbilovich anc *G. pallida* (Stone) Behrens are the most economically important and well-studied species within the genus *Globodera*. There are some species under the genus *Golbodera* which are not reported to be associated with potato, but can be found in other solanaceous crops. The species under the genus *Globodera* are mainly *Globodera rostochiensis*, *G. pallida*, *G. tabacum (G. tabacum tabacum, G. tabacum solanacearum, G. tabacum virginiae)*, *G. ellingtonae*, *G. mexicana* which can be found in different solanaceous plants.

All G*lobodera* species are primarily root parasites, the activities of which cause reductions in both the yield and quality of the potato crop (*Solanum tuberosum* L.). The PCNs are reported to cause yield losses ranging from 10 to 12% worldwide and 9% in Europe. Annual losses in the UK production system are estimaed at £26 million. Both PCN species are well estabilished with established within the main potato growing areas of the United Kingdom, but *G pallidaincidence* has increased in England and Wales, where 67% of infestations were purely of this species. These nematodes have already been declared as quarantine pests in many countries of the world.

Pest Risk Analysis (PRA) always satisfies the quarantine requirements of the importing countries, provides scientific evidence to understand the existence of quarantine pest, assess the magnitude of potential economic loss and at the same time provides the rationale of developing strategies for phytosanitary measures of a country. PRA study also provides scientific evidences the presence and absence of a quarantine pest in particular area or country. Therefore, SPCB, PQW, DAE has taken this crucial study to obtain intensive observation on *Globodera* spp. that had already been declared as a quarantine pest in many countries of the world. The PRA on *Globodera* spp. will facilitate to identify the existence of the pest in Bangladesh, to quantify the magnitude of potential consequences in a defined area, to identify the possible pathways, and to design the strategies for pest risk management.

The overall objectives of PRA of *Globodera* spp. is to check the existence of this quarantine nematode and/or identify the concern pathways and evaluate the associated risk and develop appropriate management strategies.

1.2 Setting up the scheme of entire PRA study

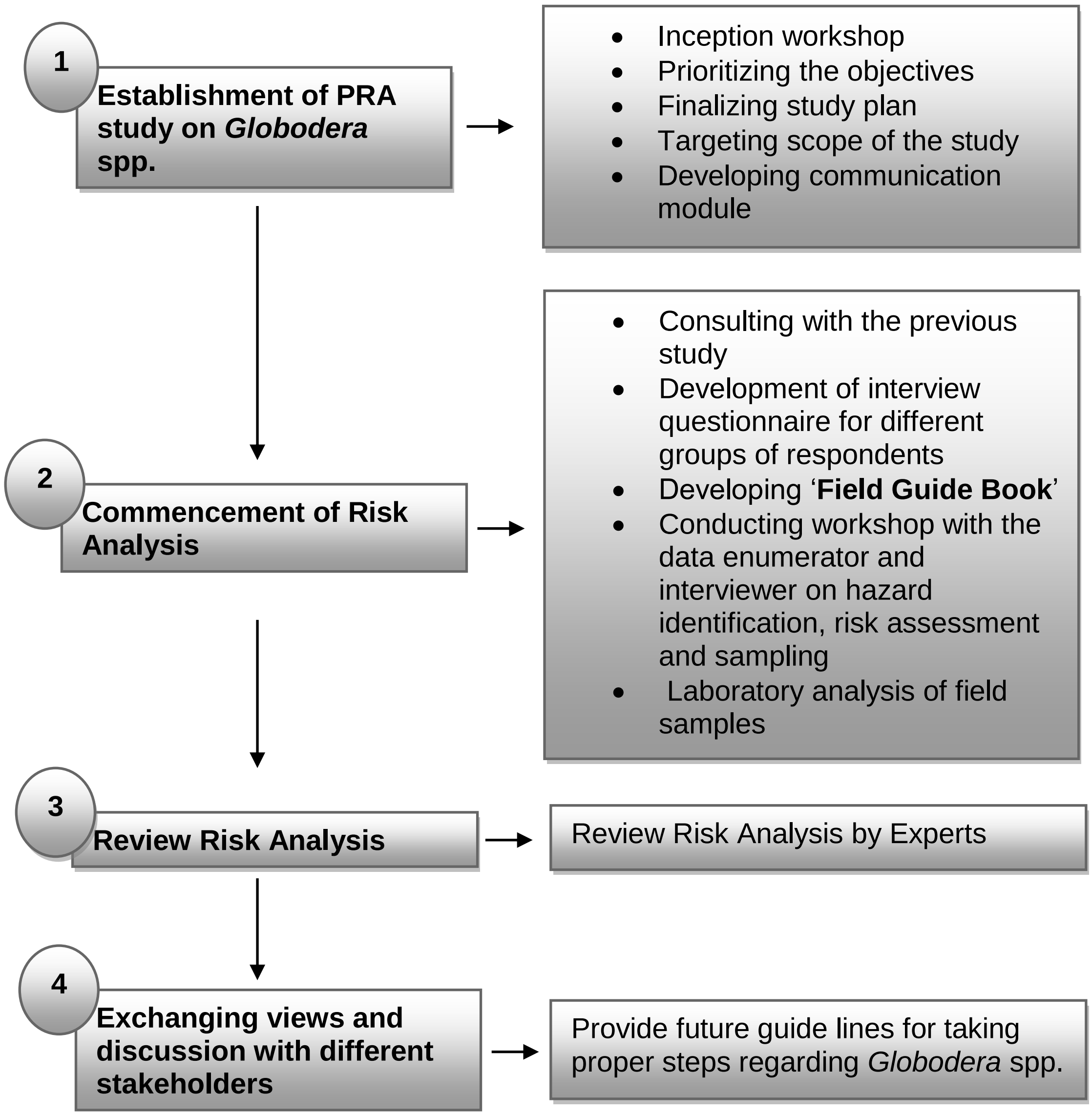


Figure 1: Linear schematic presentation of the activities that will be carried out during PRA study of *Globodera* spp.

1.3 Previous risk assessment in Bangladesh

In 2015, Strengthening Phytosanitary Capacity Bangladesh (SPCB) project under the Department of Agricultural Extension conducted a Pest Risk Analysis (PRA) study on potato as a commodity where various pests of potato had been assessed and possible pathway had been identified. The nematode pests associated with seed potato tuber and potential risk assessed in 2015 are presented in Table no. 1& 2.

Table 1: Pests associated with seed potatoes (*Solanum tuberosum*) in Bangladesh, the Netherlands, Germany, Denmark, USA and other EU countries (Extracted from the report on PRA of potato in Bangladesh, DAE, SPCB, PQW, 2015)

Pest	Common name	Geographic Distribution	Infestation/ Infective Phase	Plant Part Affected	Presence in Bangladesh	Quarantine Pest (Yes/No)	Follow Pathway (Yes/No)	Reference
Order: Tylenchida Family: Hteterodoridae								
Globodera rostochiensis (Wollenweder)	Golden Cyst Nematode	France, Germany, Scotland, Netherlands, India, USA	Juvenile-2	Root, stem, tuber	No	Yes	Yes	CABI 2006, EPPO 1978
Globodera pallida (Stone, 1973) Behrens 1975	Pale Cyst Nematode	EPPO region, Scotland, India, Netherlands, Scotland	Juvenile-2	Root, stem, tuber	No	Yes	Yes	CABI/EPPO, 1999; EPPO, 2006
Order: Tylenchida Family: Anguinidae								
Ditylenchus destructor Thime, 1975	Potato tuber nematode	EPPO region, Mediterranean region, Scotland, Canada, USA	Juvenile-2	Root, leaves, vegetative organs, tuber	Yes	No	Yes	CABI/EPPO, 2001; EPPO 2006
Ditylenchus dipsaci (Kuehn, 1857) Filipjev, 1936	Potato tuber nematode	EPPO region, Mediterranean region, Scotland, Canada, USA	Juvenile-2	Root, leaves, vegetative organs, tubers	No	Yes	Yes	CABI/EPPO, 1999; EPPO, 2006

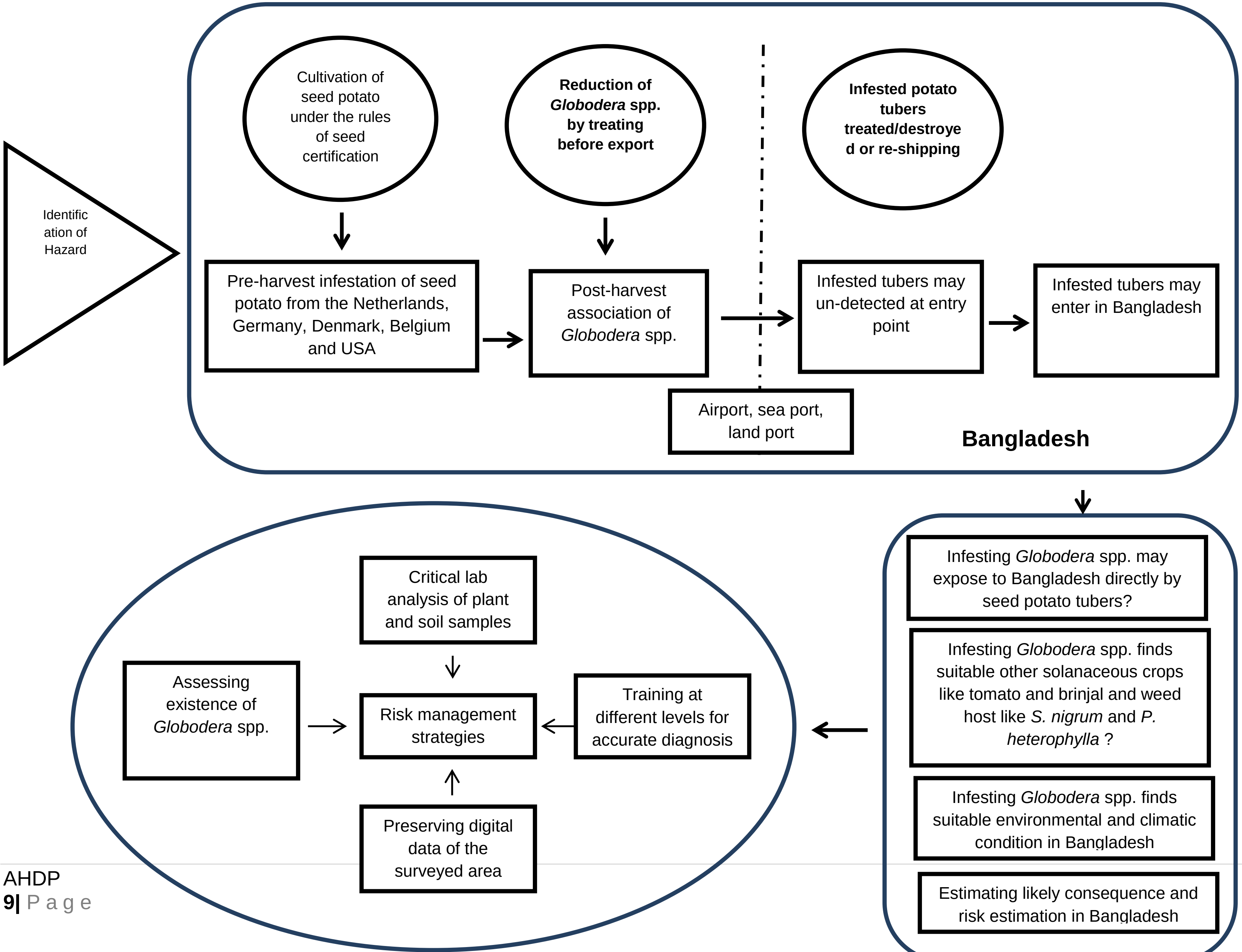
Table 2: The overall Pest Risk Potential rating

(Extracted from the report on PRA of potato in Bangladesh, DAE, SPCB, PQW, 2015)

Pest	Consequences of Introduction	Potential of Introduction	Pest Risk Potential
Globodera rostochiensis (Wollenweder) Order: Tylenchida Family: Heterodoridae	High	High	High
Globodera pallida (Stone) Behrens Order: Tylenchida Family: Heterodoridae	High	High	High
Ditylenchus dipsaci (Kuehn) Filipjev Order: Tylenchida Family: Anguinidae	High	High	High

1.4 Proposed import pathway analysis for *Globodera* spp. in Bangladesh

The seed potatoes are mostly imported from the EU countries like the Netherlands, Belgium, Germany, Denmark and USA. To understand the import risks, it is very important to know the pathway of *Globodera* spp. Moreover, the associated factors in the pathways are very important to take into consideration for taking appropriate measures. The pathways of *Globodera* spp. initiate from the country of import or export. Therefore, sverel steps including pre-harvest inspection of the seed potato field is very important whether the cultivation of seed potato follow the stringent phytosanitary rules of the respective country. Before importing/exporting it is very urgent to know whether any chemical treatment is applied or not which can be mentioned in the phytosanitary certificate or tag. In the port of entry, the associated cyst of *Globodera* spp. may have a chance to be un-detected and can be spread directly in the farmers' field. Potato is cultivated extensively in Bangladesh as well as some other solanacesous crops and weeds *viz*. egg plant/brinjal, tomato, tit begun (black nightshade), foska begun (clammy ground cherry) can be the host of *Globodera* spp. Therefore, to identify the exixtance of *Globodera* spp. in Bangladesh it is imperative to critically investigate potato, tomato, brinjal, black nightshade and clammy ground cherry. After identifying the pthways of *Globodera* spp. the appropriate management can be designed and followed.



Identification of Hazard
Cultivation of seed potato under the rules of seed certification
Reduction of *Globodera* spp. by treating before export
Infested potato tubers treated/destroyed or re-shipping
Pre-harvest infestation of seed potato from the Netherlands, Germany, Denmark, Belgium and USA
Post-harvest association of *Globodera* spp.
Infested tubers may un-detected at entry point
Infested tubers may enter in Bangladesh
Airport, sea port, land port
Bangladesh
Infesting *Globodera* spp. may expose to Bangladesh directly by seed potato tubers?
Infesting *Globodera* spp. finds suitable other solanaceous crops like tomato and brinjal and weed host like *S. nigrum* and *P. heterophylla* ?
Infesting *Globodera* spp. finds suitable environmental and climatic condition in Bangladesh
Estimating likely consequence and risk estimation in Bangladesh
Critical lab analysis of plant and soil samples
Assessing existence of *Globodera* spp.
Risk management strategies
Training at different levels for accurate diagnosis
Preserving digital data of the surveyed area

1.4.1 Environmental condition of Bangladesh

Bangladesh has a subtropical monsoon climate characterized by wide seasonal variations in rainfall, high temperatures and humidity. There are three distinct seasons in Bangladesh: a hot, humid summer from March to June; a cool, rainy monsoon season from June to October; and a cool, dry winter from October to March. In general, maximum summer temperatures range between 30°C and 40°C. April is the warmest month in most parts of the country. January is the coldest month, when the average temperature for most of the country is about 10°C.

Heavy rainfall is characteristic of Bangladesh. With the exception of the relatively dry western region of Rajshahi, where the annual rainfall is about 1600 mm, most parts of the country receive at least 2000 mm of rainfall per year. Because of its location just south of the foothills of the Himalayas, where monsoon winds turn west and northwest, the regions in north-eastern Bangladesh receives the greatest average precipitation, sometimes over 4000 mm per year. About 80 percent of Bangladesh's rain falls during the monsoon season.

The Typhoon season begins in August and lasts to end November. Warnings are not always very effective in Bangladesh, due to the bad Infrastructure of this country. Be careful when you travel around in the coastal areas of Bangladesh in this period, and try to follow the weather forecasts carefully. Typhoons have often catastrophic effects, and can cause flooding that can sometimes last for several weeks. The environmental condition for survival of *Globodera* spp. in Bangladesh is favourable as the potato exporting countries like Denmark, The Netherlands, Germany, USA.

1.4.2 Environmental condition of potato exporting countries

Denmark: Denmark is situated in the zone between three European climatic zones (Borea influence in the north, Atlantic influence the west and Continental influence in the east); the climate throughout Denmark is a mixture of these influences. Generally the western parts of the country has Atlantic climate and the eastern parts a more continental influenced climate.

The yearly precipitation is over 900 mm in some parts of Jutland and below 500 mm over The Great Belt between Jutland and Seeland. The rain is more or less even distributed through the year, but as the evaporation is less in the cool months October to march, the winter is the most humid time of the year. Over the year the temperature is naturally highest, over 8.5 degrees C, in the southern parts of the country and below 7,5 in the northern parts of Jutland. July is the warmest month with a mean temperature over 17,5 degrees C in southeast and just below 16 in the northwest of Jutland. January is the coldest period in Denmark, but the mean temperature of c. 0 degrees C is more even throughout the country because of the warming effect from the surrounding sea.

The climate in Denmark is pleasant in the summertime (May - August). Typical daytime temperatures in the midsummer are a little more than 20 degrees C. May is especially charming month, as the spring is at its height. September can often be very rainy.

The Winter in Denmark can be quite cold. Temperature falls sometimes until 15 - 30°C below zero. Then the country is ruled by snow, ice and icy winds. Even in April it is still possible to have a snowstorm. In the wintertime the sun rises only little above the horizon and for months (roughly October - March) the days are dark and short.

The Netherlands: The Netherlands have a temperate maritime climate influenced by the North Sea and Atlantic Ocean, with cool summers and moderate winters. Daytime temperatures varies from 2-6 °C in the winter and 17-20°C in the summer.

Since the country is small there is little variation in climate from region to region, although the marine influences are less inland. Rainfall is distributed throughout the year with a dryer period from April to September. Especially in fall and winter strong Atlantic low-pressure systems can bring gales and uncomfortable weather. Sometimes easterly winds can cause a more continental type of weather, warm and dry in the summer, but cold and clear in the winter with temperatures sometimes far below zero. The Netherlands is a flat country and has often breezy conditions, although more in the winter than in the summer, and more among the coastal areas than inland.

Germany: Germany's climate is moderate and has generally no longer periods of cold or hot weather. North-western and coastal Germany has a maritime influenced climate which is characterized by warm summers and mild cloudy winters. Most areas on the country's North Sea coast have midwinter temperatures about 1.5°C or even higher. Farther inland, the climate is continental, marked by greater seasonal variations in temperature, with warmer summers and colder winters. Temperature extremes between night and day and summer and winter are considerably less in the north than in the south. During January, the coldest month, the average temperature is about 1.5°C in the north and about -2°C in the south. In July, the warmest month, it is cooler in the north than in the south. The northern coastal region has July temperatures averaging between 16°C and 18°C; at some locations in the south, the average is almost 20°C or even slightly higher.

In addition to the maritime and continental climates that predominate over most of the country, the Alpine regions in the extreme south and, to a lesser degree, some areas of the Central German Uplands have a so-called mountain. This climate is characterized by lower temperatures because of higher altitudes and greater precipitation caused by air becoming moisture-laden as it lifts over higher terrain. An occasional condition in the Alps is called "föhn", or warm wind. In this atmospheric condition, warm tropical air is drawn across the Alps and loses moisture on the southern slopes of the mountains. Sometimes this winds can be as strong as gales, and can cause damage, especially in late autumn and winter. Especially in fall and winter strong Atlantic low-pressure system can bring gales and uncomfortable weather with showers, thunderstorms and heavy rain, especially in the western coastal parts and the mountainous regions of Germany; in summer times weaker low pressure systems can cause showery weather, and sometimes even (severe) thunderstorm. Winters in Germany are generally mild, but can sometimes be harsh with heavy snowfall and temperatures far below zero, especially in the eastern, southern and mountainous regions.

USA: Weather varies widely across the continental USA, as well as in Alaska and Hawaii. In general terms, summers are hot and humid in the plains and southern states, while the southwest is very hot and quite dry. Endless summers are southern California and Florida trademarks, while in the Pacific Northwest and New England states, summers are warm with cool mornings and pleasant evening conditions.

Winters in the southern states are mild, while in the north, northeast, plains states, and in the western mountains, conditions are often quite cold with heavy snow and sub-freezing temperatures. Severe weather in the form of thunderstorms is a normal spring and summer occurrence; devastating tornadoes are also common in the spring and summer months, especially in the central part of the U.S.A. Hurricanes occasionally strike the eastern coastline and Gulf of Mexico states from June through October.

1.5 Methodology

The methodology for the present PRA study used system-wide approach, which involved wide-ranging and sequenced discussion with relevant stakeholders aiming to identify the existence of *Globodera* spp., their potential hazards, quarantine concern of the pests, their risk and management options. The study involved the use of (i) field survey through structured questionnaire, (ii) semi-structured interviews by means of Focus Group Discussions (FGD), (iii) formal and non-formal interviews through Key Informant Interview (KII); (iv) collection of primary and secondary information, reviewing the available reports and (v) physical field visits to the sampled area.

1.5.1 Major activities for data collection

a. Field survey

The study area for field survey includes the entire country.The focal survey areas were selectedbased on the discussion with SPCB, PQW, DAE; considering the major potato growing areas; entry points for importing and exporting potato; agro-ecological zones of Bangladesh. The study survey was conducted with the direct interview of potato growers in 21 major growing districts of Bangladesh for quantitative data aiming primarily to identify the existence of *Globodera* spp. as well as other associated nematodes of potato, tomato, brinjal, and solanaceous weed hosts of potato. Due to very subtle abnormalities can be conspicuous in the affected field or there might be no visible symptoms or confused symptoms with other diseases, a structured questionnaire was followed to get the desired information describing field symptoms, relevant production activities, existing management, possible pathway of introduction, distribution etc. The qualitative data were also collected through Focus Group Discussions (FGD) with progressive potato farmers and through key Informant Iinterviews (KII) with extension personnelat field and headquarter level, Plant Quarantine Centres at Sea and land port, officials of Ministry of Agriculture, Plant Pathologist of Bangladesh Agricultural Research Institute (BARI), and Agricultural Universities.

b. Secondary data collection and review of literatures

The existing PRA related secondary data were collected and gathered from secondary sources such as journals, books, proceedings, CD-ROM (CABI) search, internet browsing especially through website of CAB International, EPPO Bulletin and others. The documents were then critically reviewed, synthesized in relation to identify the quarantine pests of potato available in the potato exporting counties such as India, Japan, and Thailand as well as PRA related activities performed there. Ultimately, the activity scheme was designed based on the formulated and synthesized information to study the present PRA of *Globodera* spp.

c. Listing of nematode pests of potato

Soil and plant samples from each PRA study area were collected and preserved accordingly for isolation, and identification. Survey of field, focus group discussion and key informant interviews were used as the base of laboratory analysis. Previously published reports/articles were also used as key identification tools. At the end of the study both *Globodera* spp. and other plant parasitic nematodes of potato, tomato, brinjal and weed hosts were listed.

d. PRA location and study sampling

The survey area comprised of the whole country. But, based on the discussion with DAE personnel, the focal survey study was conducted in the 21 major potato growing districts and seed entry point, Chittagong sea port of Bangladesh as selected by the client-Project Director, Strengthening Phytosanitary Capacity in Bangladesh (SPCB) under Plant Quarantine Wing (PQW), DAE, Bangladesh. Survey team lead by PRA team leader, team members, SPCB, PQW representative, respective data collector, data enumerator, and respective field officers visited the places to observe the field condition and collecting samples for laboratory analyses. A total 70 upazillas (sub district) were selected under the 21 sampled districts, where 10 agricultural blocks were covered under each upazilla and 10 potato growers/farmers were interviewed in each block through pre-structured questionnaire. Thus, a total of 7000 growers/farmers were interviewed from all of 21 sampled districts. The Focus Group Discussion (FGD) meeting was also conducted for each of 21 sampled districts with the participation of at least 10 potato growers/farmers aiming to gather qualitative data. Besides, one officer designated as Additional Deputy Director (Plant Protection) for each district had also been interviewed through semi-structured Key Iinformant Interview (KII) checklist. The district and upazila-wise distribution of respondents is given below:

Table 3: Distribution of the respondents in major potato growing districts of Bangladesh

SN	District	Upazilla	No. of blocks	No. of farmers	No. of FDGs	KII
1	Rajshahi	Paba	10	10	1	1
		Mohanpur	10	10		
		Putia	10	10		
		Bagmara	10	10		
		Durgapur	10	10		
2	Chittagong	Patia	10	10	1	1
		Chandanaish	10	10		
3	Comilla	Barura	10	10	1	1
		Daudkandi	10	10		
		Titas	10	10		
		Chandina	10	10		
		Meghna	10	10		
4	Chadpur	Hazigang	10	10	1	1
		Motlab (North)	10	10		
		Matlab (South)	10	10		
5	Laxmipur	Raipur	10	10	1	1
		Ramganj	10	10		
6	Jessore	Jessore sadar	10	10		
		Chougacha	10	10		
		Bagarpara	10	10		

7	Jhenaidha	Court Chandpur	10	10	1	1
		Harinakunda	10	10		
8	Gaibandha	Palashbari	10	10	1	1
		Gobindaganj	10	10		
		Sadullahpur	10	10		
9	Manikganj	Singair	10	10	1	1
		Satuaria	10	10		
		Gheor	10	10		
10	Narayanganj	Narayanganj sadar	10	10	1	1
		Sonargaon	10	10		
		Bandar	10	10		
11	Munshiganj	Sreenagar	10	10	1	1
		Munshiganj sadar	10	10		
		Tongibari	10	10		
		Lohaganj	10	10		
		Sirajdikhan	10	10		
12	Dinajpur	Dinajpur Sadar	10	10	1	1
		Fulbari	10	10		
		Birampur	10	10		
		Birganj	10	10		
		Birol	10	10		
13	Thakurgaon	Thakurgaon Sadar	10	10	1	1
		Ranishankail	10	10		
14	Panchagor	Pirgonj	10	10	1	1
		Debiganj	10	10		
15	Noagaon	Atrai	10	10	1	1
		Raninagar	10	10		
		Badalgachi	10	10		
16	Joypurhat	Joypurhat sadar	10	10	1	1
		Panchbibi	10	10		
		Khetlal	10	10		
17	Lalmonirhat	Hatibandha	10	10	1	1
		Lalmonirhat sadar	10	10		
18	Kurigram	Kurigram sadar	10	10	1	1
		Ulipur	10	10		
19	Bogra	Sherpur	10	10	1	1
		Shibganj	10	10		
		Kahalu	10	10		
		Alamdighi	10	10		
		Sonatala	10	10		
20	Rangpur	Rangpur Sadar	10	10	1	1
		Pirgacha	10	10		
		Badarganj	10	10		
		Mithapukur	10	10		
		Taraganj	10	10		
21	Nilphamary	Nilphamary Sadar	10	10	1	1
		Dimla	10	10		
		Jaldhaka	10	10		
		Kishoregonj	10	10		
Total		70		7000	21	21

e. Development of indicators for field survey:

Considering the specific objectives of the study, the major indicators for the field survey were identified in consultation with the Plant Quarantine Wing (PQW) officials under Department of Agriculture Extension (DAE), Bangladesh. The indicators were typical symptoms observation in the field of BADC, private seed importers, research stations, farmers of potato and solanaceous weeds, general farm activities that may be related to possible establishment and distribution of *Globodera* spp., laboratory isolation and identification of *Globodera* spp. from plants and soil samples of potato, tomato, brinjal and associated solanaceous weeds, entry and pathways of quarantine pests; effective measures in controlling these pests; options in preventing the entry and spread of quarantine pests, their risk and management options and phytosanitary measures.

f. Development of data collection tools:

Field survey questionnaire: For quantitative analysis, the field survey was conducted in 21 major potato growing districts of Bangladesh through face to face interview with 7,000 potato farmers using a set of pre-structured questionnaire (Appendix-questionnaire) encompassing the relevant study indicators. A field guide emphasizing on the comprehensive list and colourful photographs of *Globodera* spp. of potato was also prepared aiming to enhance the data enumerators and farmers to ease identification of the respective pests whether these were occurred in their field or not.

Focus Group Discussion (FGD): For qualitative analysis, 21 FGD meetings were organized considering one FGD for each sampled districts with the participation of at least 10 potato farmers for each. The FGD meetings were conducted using pre-structured FGD guidelines (Appendix-FGD guidelines).

Key Informant Interview (KII): The key informant interviews were conducted with the extension personnel at field and headquarters level of DAE, officials of Plant Quarantine Centres at Sea and land ports; officials of Ministry of Agriculture; Plant Pathologist of BARI, Agricultural Universities, officials of BADC, SCA and Potato importers and Exporters. A total of 21 key personnel were interviewed using a semi-structured KII Checklist (Appendix-Key Informants Interview) encompassing the qualitative issues of the study.

g. Field visit/observation:

In addition, the expert team of the study physically visited the sampled districts of the study area aiming to observe the physical status of the insect pests, diseases and other associated pest problems in field condition.

h. Recruitment and training of field staff:

The Junior Plant Pathologists having master's degree in Plant Pathology respectively were recruited as data enumerators. A total 21 data enumerators, of which 1 for each of 21 sampled districts were employed for field level data collection. A total 7 field supervisors for 7 administrative divisions of Bangladesh were also recruited and employed to supervise the activities of data enumerators. After recruitment, all the data enumerators and supervisors had been trained by three-day training course about data collection procedures for this PRA study.

i. Method of data collection:

Direct personal interview approach was adopted for primary data collection. The field enumerators personally contacted the farmers and filled up the each question of the questionnaire one by one to obtain desired information. In addition, qualitative information was collected through FGD meetings with potato farmers using FGD guidelines under supervision of supervisors. The field level data collection was conducted for a month started from 5 January 2016 to 19 May 2016.

k. Data analysis:

As soon as the filled up questionnaires received from the field, data entry of the questionnaires were completed using SPSS and MS Access computer packages and the data were analyzed for tabulation of the primary data into data tables.

l. Laboratory Investigation:

i. **Methodology followed**: During workshop with the data enumerators and data collectors, detailed procedure was given for taking records on field symptoms, collecting plants and soil samples. It was advised to collect the root and soil samples especially from the suspected fields having typical or very close symptoms shown in the Field Guide Book. In addition, some samples from the apparently healthy looking field were also collected in some cases.

ii. Collection of samples: The potato field showing stunted growth, yellow leaves and more preferably the symptoms were restricted in a particular place of the field or showing distinct patchy pattern which is the more precise diagnostic symptoms of cyst nematodes (*Globdera rostochiensis* and *G. pallida* and other *Globodera* species). Several samples were collected from a suspected field from a depth of 20 cm with trowel and finally made a composite sample of 500 gram for each sample. The roots and soil samples were kept in zip-lock polythene bag with proper labelling and immediately send to the laboratory for subsequent analysis. During survey, samples of potato, tomato, and brinjal were collected following the same procedure.Samples from imported seed potato andWeeds samples of the Solanaceae family (black night shade: *Solanum nigrum* and Gurki: *Phycalis heterophylla*.) were also collected for determining the association of *Globodera* spp. in Bangladesh.

iii. Working procedure: Three standard methods with slight modifications were followed for isolation of cyst and other nematodes from roots and soil samples.

Nematode isolation from infested root system: Baermann Funnel Technique- The root samples were chopped into small pieces having 1-2 inches length. The chopped roots were placed on clean cheese cloth and keep it on the funnel. The funnel and rubber tube were filled with water and cheese cloth with root pieces were kept on the funnel and set it for 24 hours for isolation. If the roots contained any nematodes will swim to the water and gradually deposit at the bottom of clipped rubber tube. After 24 hours, the water suspension were taken out in a petridish and directly observed under stereo-binocular microscope.

Isolation of cyst nematodes from soil samples: Flegg's sieving and sedimentation technique was followed for isolation of nematodes. To especially collect the cyst nematodes the soil samples are mixed with enough tap water in a bucket and stir well with a stick. The soil suspension is then passed through a series of sieves consisting of 40, 60, 80 and 120 mesh so that nematodes of different size and shapes will be deposited on the sieves if any. Afterwards, the nematodes are collected by back flashing of water by wash bottle and collect the suspension in a beaker. The suspension of individual sieves are collected in different beaker and allowed to settle for 1-2 hour in room condition. After settling well the upper part of nematode suspension are siphoned out and the remaining suspension are examined under stereo-binocular microscope taking the suspension a petridish.

Isolation of vermiform nematodes from soil samples: Vermiform nematodes present in the soil were isolated following modified sieving and sedimentation technique. In this method a sieve is fit with a plastic bucket and a thin ply of facial tissue is carefully set over the sieve in such a way that the sieve and facial tissue just touch the upper surface of the water. Then the soil spread over the facial tissue and allowed overnight to allow the nematodes to pass through the facial tissue and settle down at the bottom of the bucket. The next day, the suspension are decanted into a beaker and allowed to settle for 1-2 hour. The upper part of the suspension is siphoned out and rest of the suspension was examined under stereo-binocular microscope.

Nematode fixation and preservation: The collected nematodes are immediately killed taking all nematodes in a test tube and dipping in hot water for short time. Dipping the test tube for 2-3 times the nematode become dead and ready for preservation in TAF (Trienthanol Amine Formalin (TAF) solution) for long time.

Procedure of identification of nematodes: The isolated, fixed nematodes were identified by observing different organs of nematodes like stylate, median bulb, salivary gland, ovary, vulval position, tail etc. and compared with appropriate keys.

Isolation and identification of Globodera spp.: Soil and plant samples from suspected locations were analysed in the laboratory. For cyst nematode isolation, modified sieving and sedimentation techniques was followed. No cyst nematodes were isolated, norany suspected structures were found during diagnosis (Appendis Table 1-4). Therefore, it is indicated that the existence of PCN (*Globodera* spp.) still not detected in surveyed area

m. Approach of the PRA study:

A step-wise systematic approach was followed for the present PRA study considering field survey and formal and informal discussion with relevant stakeholders aiming to identify the existence of *Globodera* spp. of potato, and their alternate hosts like tomato, brinjal and solanaceous weed hosts present in the potato field like Black nightshade (*solanum nigrum*) and Clammy ground cherry (*Physalis heterophylla)* potential hazards, the risk and management options. An additional task was also considered to find out other plant parasitic nematodes in the potato field and the risk associated with them.The study involved the use of (i) field survey through structured questionnaire, (ii) semi-structured interviews by means of Focus Group Discussions (FGD), (iii) formal and non-formal interviews through Key Informant Interview (KII); (iv) collection of primary data by laboratory analysis ofimported tuber, plant and soil sampled and secondary information by reviewing the available reports and (v) physical field visits to the sampled area.

Chapter 2

Review of literatures

2.1 Identity of the pests

Globodera rostochiensis **(Wollenweber, 1923) Behrens**

Identity of the pest:

<u>Preferred Scientific Name</u>

Globodera rostochiensis (Wollenweber, 1923) Behrens, 1975

<u>Preferred common name</u>

Yellow potato cyst nematode

<u>Other Scientific Names</u>

Globodera rostochiensis (Wollenweber, 1923) Mulvey& Stone, 1976

Heterodera (*Globodera*) *rostochiensis* Wollenweber, 1923 (Skarbilovich, 1959)

Heterodera rostochiensis Wollenweber, 1923

Heterodera schachtii rostochiensis Wollenweber, 1923

*Heterodera schachtii solan i*Zimmerman, 1927

<u>International Common Names</u>

English: eelworm; golden eelworm; golden nematode; golden nematode eelworm; golden nematode of potato; golden potato cyst nematode; potato cyst nematode; potato golden nematode; potato root eelworm

Spanish: nematodo dorado; nematodo dorado de la papa

French: anguillule a kyste de la pomme de terre; anguillule des racines de la pomme de terre; nématodedoré; nématodedoré de la pomme de terre

<u>Local Common Names</u>

Denmark: kartoffelal; kartottelcystenematod

Finland: peruna-ankeroinen

Germany: Aelchen, GoldfarbenesKartoffelzysten-; Aelchen, Kartoffel-; Nematode, Kartoffel-

Iran: nematode sibsamini

Italy: Anguilluladellapatata

Netherlands: Aardappelcystenaaltje

Norway: potetcystenematode

Sweden: potatiscystnematod

EPPO code

HETDRO (*Globodera rostochiensis*)

Invasiveness

*Globodera rostochiensis*is a worldwide pest of temperate areas, including both temperate countries and temperate regions of tropical countries, for example India's Nigrilis region. Distribution is linked to that of the potato crop. Potato cyst nematode is considered to have originated from the Andes region of South America, from where it spread to Europe with potatoes. The ease with which it has been transported across continents proves what a resilient pest it is. The cyst form which adheres to host roots, stolons and tubers and to soil particles during transportation gives rise to new infestations where climate and food source are both available and favourable.

Secondary means of dispersal is through the movement of contaminated farm machinery, farming implements and contaminated footwear. Cysts are also successfully spread by wind dispersal, during winter storms or sand storms where top soil is redistributed. Rain which causes flooding and water to run off fields into trenches or irrigation channels also redistributes cysts into adjoining areas.

Taxonomic Tree

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Nematoda
Order: Tylenchida
Family: Heteroderidae
Genus: *Globodera*
Species: *Globodera rostochiensis*

Description

Eggs

The eggs of *G. rostochiensis* are always retained within the cyst body and no egg sacs are produced. The eggshell surface is smooth and no microvilli are present.

Length=101-104 µm; width=46-48 µm; L/W ratio=2.1-2.5

Females

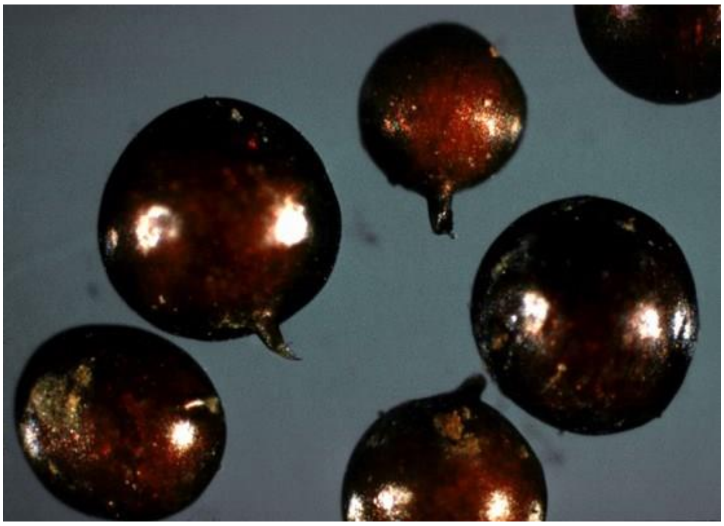
The females emerge from the root cortex about one month to six weeks after invasion by the second-stage juveniles. They are pure white initially, turning golden yellow on maturation. Mature females are approximately 500 µm in circumference without a cone. The cuticle of the female sometimes has a thin sub-crystalline layer

Stylet length=23 µm ± 1 µm; stylet base to dorsal oesophageal gland duct=6 µm ± 1 µm; head width at the base=5.2 µm ± 0.7 µm; head tip to median bulb=73 µm ± 14.6 µm; median bulb valve to excretory pore=65 µm ± 2.0 µm; head tip to excretory pore=145 µm ± 17 µm; mean diameter of the median bulb=30µm ± 3.0 µm; mean diameter of the vulval basin=22 µm ± 2.8 µm; vulval slit length=9.7 µm ± 2.0 µm; anus to vulval basin=60 µm ± 10 µm; number of cuticular ridges between the anus and vulva=21 ± 3.0.

The female head bears one to two annules and the neck region has numerous tubercules, which can be seen using a scanning electron microscope. The head skeleton is hexaradiate and weak. The stylet is divided equally in length between the conus and the shaft. An important diagnostic feature is the backward slope of the stylet knobs. The median bulb is large and circular and well developed. The large paired ovaries often displace the oesophageal glands. The excretory pore is well defined at the base of the neck. The posterior of the female, at the opposite pole to the neck and head, is referred to as the vulval basin and is contained within a rounded depression. The vulval slit is located in the centre of this region flanked on either side by papillae, which usually fill the translucent areas of cuticle in crescentic shape, from the slit to the edge of the fenestra. The anus is distinct and is often seen at the point in the cuticle where the 'V' shape tapers to an end. The number of cuticular ridges found in the area between the anus and the edge of the fenestra is counted as an aid to identification of *Globodera* species. The entire cuticle is covered in small subsurface punctations.

Cyst

Length without neck=445 µm ± 50 µm; width=382 µm ± 60 µm; neck length=104 µm ± 19 µm; mean fenestral diameter=19.0 µm ± 2.0 µm; anus to fenestra=66.5 µm ± 10.3 µm; Granek's ratio=3.6 ± 0.8.



(Fig. 2 Cyst of *Globoderarostochiensis*, source:**http://www.cabi.org/isc/datasheet/27034**)

Cysts contain the eggs, the progeny for the next generation, and are formed from the hardened dead cuticle of the female. Newly produced cysts may still show an intact vulval basin but older cysts, particularly those which have been in the soil for many seasons, will have lost all signs of their genitalia with only a hole in the cuticle to show the position of the fenestral basin

Males

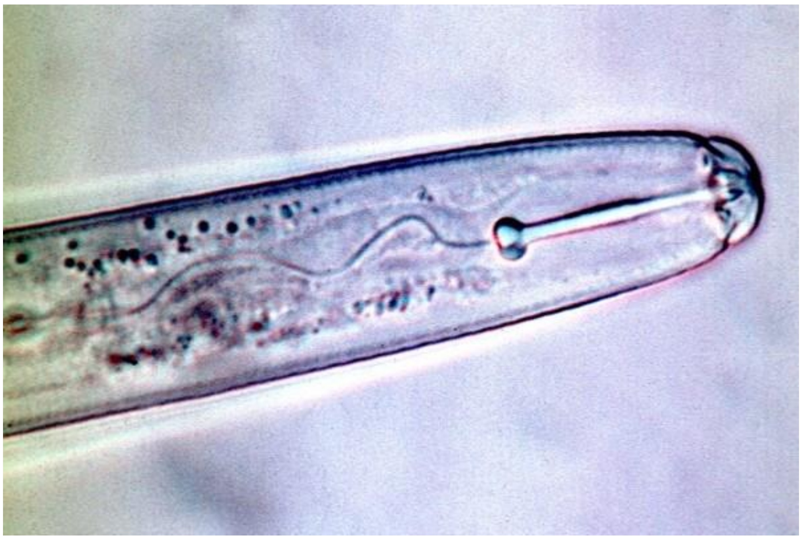
Length=0.89 -1.27 mm; width at excretory pore=28 µm ± 1.7 µm; head width at base=11.8 µm ± 0.6 µm; head length=7.0 µm ± 0.3 µm; stylet length=26 µm ± 1.0 µm; stylet base to dorsal oesophageal gland duct=5.3 µm ± 1.0 µm; head tip to median bulb valve=98.5 µm ± 7.4 µm; median bulb valve to excretory pore=74 µm ± 9µm; head tip to excretory pore=172 µm ± 12.0 µm; tail length=5.4 µm ± 1.0 µm; tail width at anus=13.5 µm ± 0.4 µm; spicule length=35.0 µm ± 3.0 µm; gubernaculum length=10.3 µm ± 1.5µm.

The male is vermiform in shape with a short tail and no bursa. On fixation, the body assumes a curved shape with the posterior region twisted at a 90 degree angle to the remainder of the body. There are four incisures in the mid-body i.e. three bands which terminate on the tail. The rounded head isoffset and bears 6-7 annules. The head is strongly developed having a hexaradiate skeleton. The cephalids are located at body annules 2-4 and 6-9, respectively. The stylet is strong and hasbackward sloping knobs.

The median bulb is well developed and has a large crescentic valve.The nerve ring islocated around the oesophagus between the median bulb and the intestine. The hemizonid is found 2-3 annules anterior to the excretory pore and is itself two annules in length. The hemizonion is approximately nine body annules posterior to the excretory pore and is one annule in length. The single testis fills half the body cavity. The paired spicules are arcuate and end with single tips. The gubernaculum is around 10 µm in length and 2 µm in thickness and lies in a position dorsal to the spicules.

Juveniles

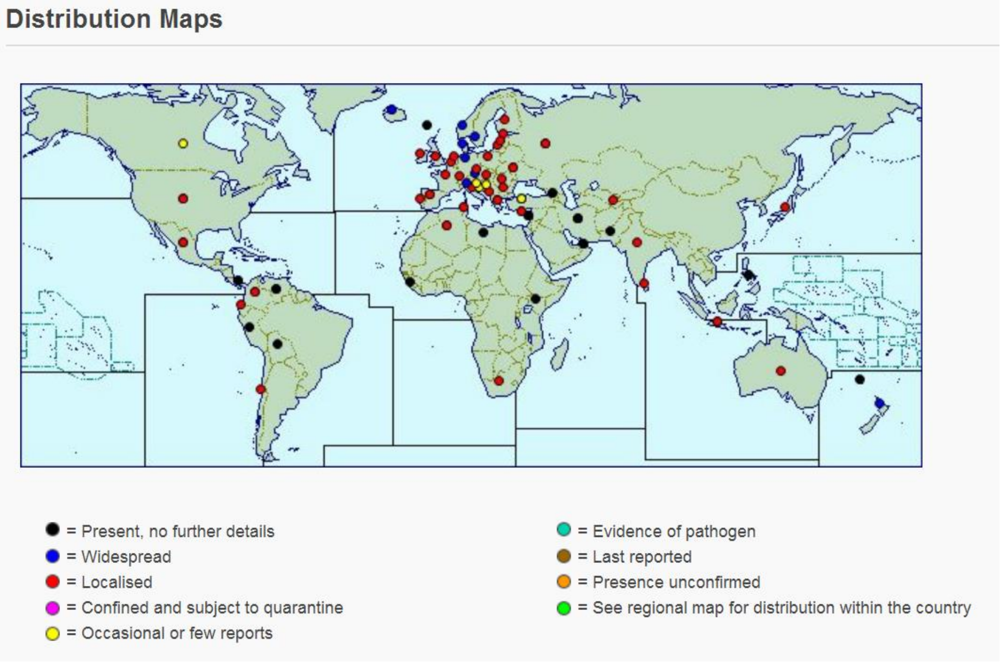
Body length=468 µm ± 100 µm; width at excretory pore=18 µm ± 0.6 µm; head length=4.6µm ± 0.6 µm; stylet length=22 µm ± 0.7 µm; head tip to median bulb valve=69 µm ± 2.0 µm; median bulb valve to excretory pore=31 µm ± 2.0 µm; head tip to excretory pore=100 µm ± 2.0 µm; tail length=44 µm ± 12 µm; tail width at anus=11.4 µm ± 0.6 µm; hyaline tail length=26.5 µm ± 2.0 µm.



(Fig.3 Juvenile of *Globodera rostochiensis*, source: **http://www.cabi.org/isc/datasheet/27034**)

The second-stage juvenile hatches from the egg, the first moult taking place within the egg. The juvenile, like the male, is vermiform with a rounded head and finely tapered tail. The hyaline portion of the tail represents about two thirds of its length. The lateral field has four incisures in the mid-body region reducing to three at the tail terminus and anterior end. The head is slightly offset and bears four to six annules. The head skeleton is well developed and hexaradiate in form. The cephalids are located at body annules 2-3 and 6-8, respectively. The stylet is strong, the conus being about 45% of the total length. The stylet knobs are an important diagnostic feature and typically slope backwards. The median bulb is well developed and elliptical in shape, having a large central valve. The nerve ring encircles the oesophagus between the median valve and the intestine. The hemizonion is about one body annule in width and is located five body annules posterior from the excretory pore. The hemizonid is around two body annules in width and is found just anterior to the excretory pore. The gonad primordium is four-celled and located at around 60% of the body length.Other measurements can be found in Granek (1955), Spears (1968), Green (1971), Greet (1972), Golden and Ellington (1972), Hesling (1973, 1974), Mulvey (1973), Behrens (1975), Mulvey and Golden (1983), Othman et al. (1988) and Baldwin and Mundo-Ocampo (1991).

Distribution:



(Fig. 4 Distribution map: Source **(http://www.cabi.org/isc/datasheet/27034)**

Symptoms

Sign

Leaves: Abnormal colours, wilting

Root: Cysts on root surface, reduced root system

Vegetative organs: Surface cracking

Whole plants: Dwarfing, early senescence

Globodera pallida (Stone, 1973) Behrens

Preferred Scientific Name: *Globoderapallida* (Stone, 1973) Behrens, 1975

Preferred Common Name: White potato cyst nematode

Other Scientific Names

Heterodera pallida Stone, 1973

International Common Names

English: pale potato cyst nematode

Spanish: nematodoquisteblanco de la papa

French: nématode blanc de la pomme de terr

Taxonomic Tree

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Nematoda
Order: Tylenchida
Family: Heteroderidae
Genus: *Globodera*
Species: *Globodera pallida*

Eggs

The eggs of *G. pallida* are retained within the cyst and no egg sac is produced. The surface of the eggshell is smooth; no microvilli are present.Measurements of the egg fall within the range: 108.3 ± 2.0 µm × 43.2 ± 3.2 µm.

Female

Female measurements: stylet length = 27.4 ± 1.1 µm; head width at base = 5.2 ± 0.5 µm; stylet base to dorsal gland duct = 5.4 ± 1.1 µm; head tip to median bulb valve = 67.2 ± 18.7 µm; median bulb valve to excretory pore = 71.2 ± 22 µm; head tip to excretory pore = 139.7 ± 15.5 µm; mean diameter of the vulval basin = 24.8 ± 3.7 µm; length of vulval slit = 11.5 ± 1.3 µm; anus to vulval basin = 44.6 ± 10.9 µm; number of ridges ontheanal-vulval axis = 12.5 ± 3.1.

The female has an almost spherical body from which the neck and head protrude. *G. pallida*females are either white or cream, depending on pathotype, when they break through the cortical root cells, and this phase lasts for 4-6 weeks. There is never a golden or yellow stage as in *Globodera rostochiensis*. The head bears one to two annules and the neck is covered in ridges between which tubercles are found when viewed by SEM. The head skeleton is hexaradiate and weak. The stylet is equally proportioned (50% conus and 50% shaft), and the basal knobs reflex in an anterior direction. The median bulb is very well developed and has a large crescentic pump. The oesophageal gland lobes are often displaced into a forward position as the large paired ovaries expand in the body cavity. The excretory pore is located at the base of the neck. The vulval slit of the female is found in the vulval basin, a round depression at the opposite pole of the body to the neck. The vulval slit is surrounded by a translucent area of thin cuticle which bears papillae.

Cyst

The tough, hardened cuticle of the dead female tans to a deep brown colour and acts as a protective bag around the embryonated eggs, which will form the next generation of *G. pallida*. The dimensions of the cyst are almost identical to those of the female, although the head is usually lost. The features of the cyst fenestrae are important in morphologically-based identification. The fenestrae have normally been lost by the mature cyst stage so that only a hole remains. The anus is generally conspicuous. New cysts may retain the remnants of a thin sub-crystalline layer. All *Globodera* species are abullate, but occasionally small rounded brown pigmented bodies are found, and these are termed vulval bodies.

(Fig.5 Adults of *Globodera pallida*, source: **http://www.cabi.org/isc/datasheet/27034)**

Cyst measurements: width = 534 ± 66 µm; length, excluding neck = 579 ± 70 µm; neck length = 188 ± 20 µm; mean fenestral diameter = 24.5 ± 5.0 µm; anus to fenestra distance = 50 ± 13.4 µm; Granek's ratio (Granek, 1955) = 2.2 ± 1.0.

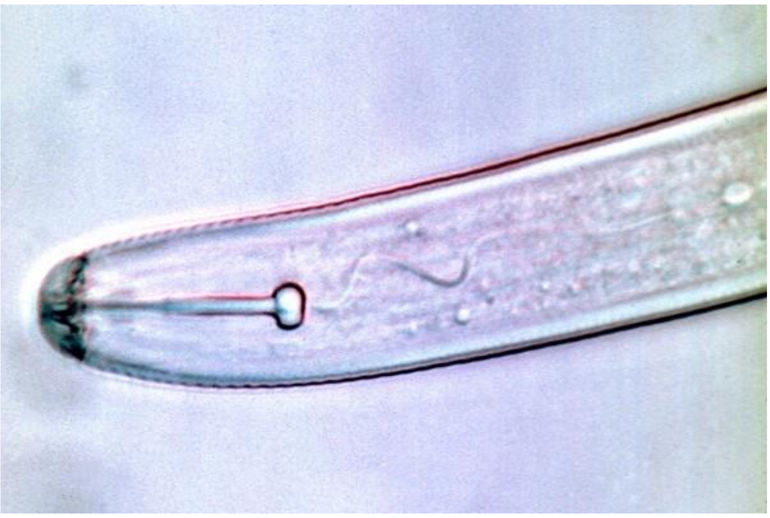
Male

The male is vermiform and usually assumes an open C shape upon death and fixation, the short rounded tail twisting through 90-180°. The body annules are regular along the body and there are three bands in the lateral field, which narrows both posteriorly and anteriorly. The head skeleton of the male is heavily sclerotized and hexaradiate, and the head is offset and bears 6 or 7 annules. The anterior cephalids are located at head annules 2 and 4 and posteriorly at annules 6 to 9. The stylet is strong with backward sloping knobs. The median bulb is rounded and the crescentic valve strong. The oesophagus is encircled by the nerve ring. The dorsal oesophageal gland nucleus is the most prominent of the three gland nuclei and the lobe itself extends almost to the excretory pore. The hemizonid is two body annules wide and two body annules behind the excretory pore. There is a single testis which extends for about 60% of the body length. The arcuate spicules have single points and some workers have separated the species *G. pallida* and *G. rostochiensis*using spicule measurements (Behrens, 1975). The gubernaculum is small and without ornamentation.

Male measurements: body length = 1200 ± 100 µm; body width at excretory pore = 28.4 ± 1.0 µm; head width at base = 12.3 ± 0.5µm; head length = 6 ± 0.3µm; stylet length = 27.5 ± 1.0 µm; stylet base to dorsal gland duct opening = 3.0 ± 1.0 µm; head tip to median bulb = 66 ± 7.1 µm; median bulb to excretory pore = 81.0 ± 11 µm; head tip to excretory pore = 176.4 ± 14.5 µm; tail length = 5.2 ± 1.4 µm; tail width at anus = 13.5 ± 2.1 µm; spicule length = 36.3 ± 4.1 µm; gubernaculum length = 11.3 ± 1.6µm.

Juvenile

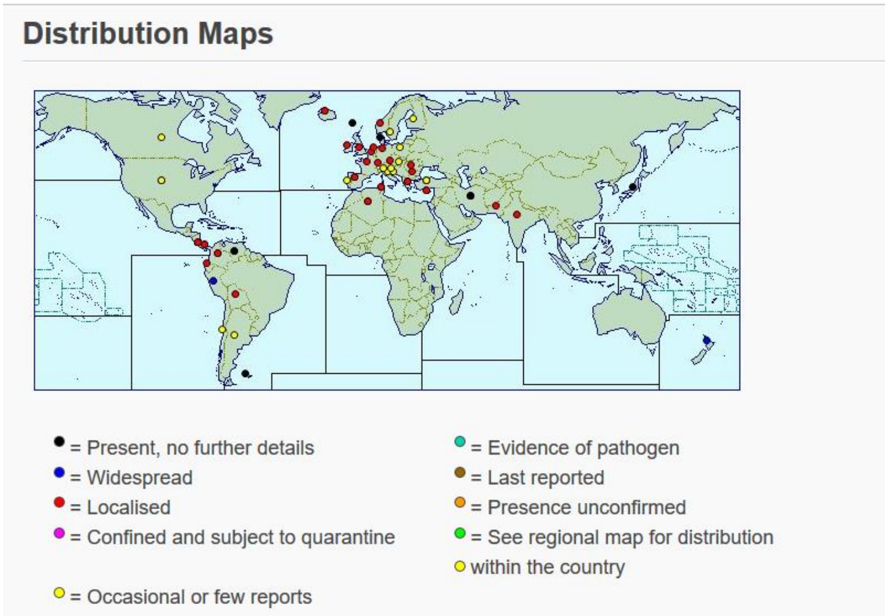
The second-stage juvenile, the infective stage in the life cycle, hatches directly from the egg where it has already undergone a moult. The juveniles of *G. pallida* and *G. rostochiensis*are very alike, but *G. pallida* juveniles are generally larger: the body length is greater and the stylet is longer and more robust, with anteriorly facing stylet knobs as opposed to those of *G. rostochiensis*, which have smaller, backward-sloping knobs. The juvenile is folded four times within the egg and the tail tapers to a rounded point. The body cavity extends half way along the tail, ending at the anus. The hyaline tail region is about 20 µm in length. There are four incisures (*i.e.* 3 bands) along the body length. The head is offset, rounded and bears 4-6 annules. The head skeleton is strongly sclerotized and hexaradiate. The cephalids are located at body annules 2 and 3 and posteriorly at annules 6 to 8. The stylet is well developed as are its basal knobs, which project anteriorly in lateral view. The nerve ring encircles the oesophagus and the excretory pore is about 110 µm from the head. The hemizonid is found just before the excretory pore and is about 2 annule widths long. The hemizonion is 5-6 annules behind the excretory pore. The genital primordium is located 60% of the body length from the head tip.



(Fig. 6 Juveniles of *Globodera pallida*, source: **http://www.cabi.org/isc/datasheet/27034)**

Juvenile measurements: body length = 486 ± 2.8 µm; body width at excretory pore = 19.3 ± 0.9 µm; stylet length = 23.0 ± 1.0 µm; stylet base to dorsal gland duct = 5.3 ± 0.9 µm; head tip to median bulb valve = 68.7 ± 2.7 µm; head tip to excretory pore = 108.6 ± 4.1µm; tail length = 51.1 ± 2.8 µm; tail width at anus = 12.1 ± 0.4 µm; length of hyaline terminus = 26.6 ± 4.1µm.

<u>Distribution</u>:



(Fig.7 Distribution map Source: **http://www.cabi.org/isc/datasheet/27034**)

2.2 Symptoms of damage:

There are no specific above-ground symptoms of diagnostic value associated with potato cyst nematode infection. Small immature females of white and yellow stages can be seen on the roots (Brown, 1969). Females of *G. rostochiensis* will go through yellow stage while *G. pallida* remain white until dead (Guile, 1970). Females can also be observed on the tuber surface, but with less frequency (Franco, 1981). When the female die they become cyst and their cuticle become brown and leathery, and contain as many as 500 nematode eggs.

2.3 Biology:

Eggs remain viable in soil for a long period of time and encysted eggs are stimulated to hatch by root exudates. Juveniles become active at 10^{o}C and maximum invasion takes place at 16°C (Franco, 1979). The mature, enlarged females rupture the root tissue, but remain attached to the root by their heads and producing necks which stay inserted in the root tissue. The fertilized females become large and subtropical and go through a sequence of color change prior to dying and becoming cysts. Potato cyst nematodes generally complete one generation during a growing season (Morris 1971).

2.4 Distribution:

The nematodes damage the roots and reduce yield, even when infestations produce no obvious symptoms in the haulm. With severe infestations, roots are more seriously damaged and may be killed. Severely infested plants are stunted, often chlorotic and typically occur in patches. *Rhizoctonia* and other fungal diseases associated with nematode feeding may also contribute to the yield loss.

Barker and Koenning (1998) revealed that yield losses incurred in *G.rostochiensis* susceptible potato averaged 38% (12-76%), compared to 18.3% (12-34%) in resistant potato. Hajihassani *et al*. 2013 reported up to 50% yield loss in untreated cyst affected potato field in Iran. Wide spread in Europe, including the UK; also South America, where they originated. Present to amore limited extent in all other continents. The numberand ranges of pathotypes vary from country to country.

The golden potato cyst nematode, *G. rostochiensis* is widespread in tropical and subtropical climates as well as intemperate regions (Marks and Brodie, 1998) with significant potential to reduce potato tuber yields up to 80% in heavilyinfested and uncontrolled fields (Spears, 1968)

2.5 Other hosts:

Tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena*) are agronomic crops other than potatoes attacked by both species of potato cyst nematodes (Brodie, 1984).

2.6 Climatic requirement:

The potato cyst nematode *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone, despite being less wide-spread than previous nematode groups, appear to be the most damaging nematodes of potato in the subtropics. They are adapted to a wide range of soil texture (sandy to 35% clay) (Greco and Moreno, 1992; Greco and Moreno 1992; Greco et al. 1982; Greco et al. 1988) and temperature (Greco and Moreno 1992; Greco et al. 1988); e.g. they infect potato and develop well at 20° C as well as 27° C. the most common root-knot nematode species develop poorly if soil temperature is below 25° C.

Pathotype Ro_1 of *G. rostochiensis* seems to be prevalent in the subtropics (Di Vito 1981) and most potato cultivars possessing resistance to potato cyst nematodes are resistant to this pathotype. However, some *G. rostochiensis* populations contain specimens that reproduce on resistant cultivars; hence continuous use of resistant cultivars might select for virulence in *G. rostochiensis* populations.

2.7 *Globodera tabacum*

The species *Globodera tabacum* was first described by Lownsbery, B.F and Lownsbery, J.W. in 1954 from solanaceous plants in Connecticut, USA. Stone (1983) suggested that *G. tabacum* (Lownsbery and Lownsbery, 1954) should be considered a species complex, to include *G. solanacearum* (Miller and Gray, 1972) and *G. virginiae* (Miller and Gray, 1968) as subspecies. The second-stage, vermiform juvenile hatches from the egg, the moult from first to second stage having been completed before hatching. There are three bands in the lateral field. The head is offset and bears 3-4 head annules. The stylet has three anchor-shaped, anteriorly facing knobs. The stylet conus and shaft are equal in length. One third of the body length is occupied by the oesophageal glands, which overlap the anterior part of the intestine. The median bulb is robust, rounded and approximately 70 µm from the head tip. The excretory pore is about 110 µm from the head tip. The tail is finely pointed and is 50-58 µm long. The hyaline portion of the tail is usually half the tail length.

This species is well distributed around the world. In Asia, it is reported to be present in China (Shepherd & Barker, 1990; CABI/EPPO, 2004), Japan (Uehara et al., 2005), Korea

(Shepherd & Barker, 1990; CABI/EPPO, 2004) and Pakistan (Brown, 1962; CABI/EPPO, 2004). In Europe this species are reported to be present in Bulgeria, Itali, France, Greece, USSR, Spain, Slovenia and former Yogoslavia.

G. tabacum can be present in the uninfested areas where tobacco, tomatoes and other solanaceous plants are grown are very important. Many solanaceous plants are hosts of this cyst nematode complex and will act as sources of infestation. Tobacco cyst nematodes can cause severe stunting of the aerial parts of tobacco plants and the root system is also much smaller than normal (Milne, 1972). Females and cysts, coloured yellow to dark brown, can normally be seen with the naked eye on infected plant roots, 6 to 8 weeks after planting.

Globodera tabacum has sub-groups like *Globodera tabacum tabacum*, *Globodera tabacum solanacearum* and *Globodera tabacum virginiae*.

The life cycle and the biology of the *G. tabacum* complex are very similar to those of the potato cyst nematodes. This nematode can survive the tropical weather. It is reported that at 26°C two generations could be produced on crops of aubergine, each taking around 36 days to complete. At 20°C the time to complete one generation was 72 days and at 30°C the cycle was shortened to 32 days (Ambrogioni *et al.* 1995)

Taxonomic Tree

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Nematoda
Order: Tylenchida
Family: Heteroderidae
Genus: *Globodera*
Species: *Globodera tabacum*

2.8 *Globodera ellingtonae*

A new species of cyst nematode, *Globodera ellingtonae*, is described from soil.*Cysts*: Light brown to brown in color, spherical to subspherical with a protruding neck.

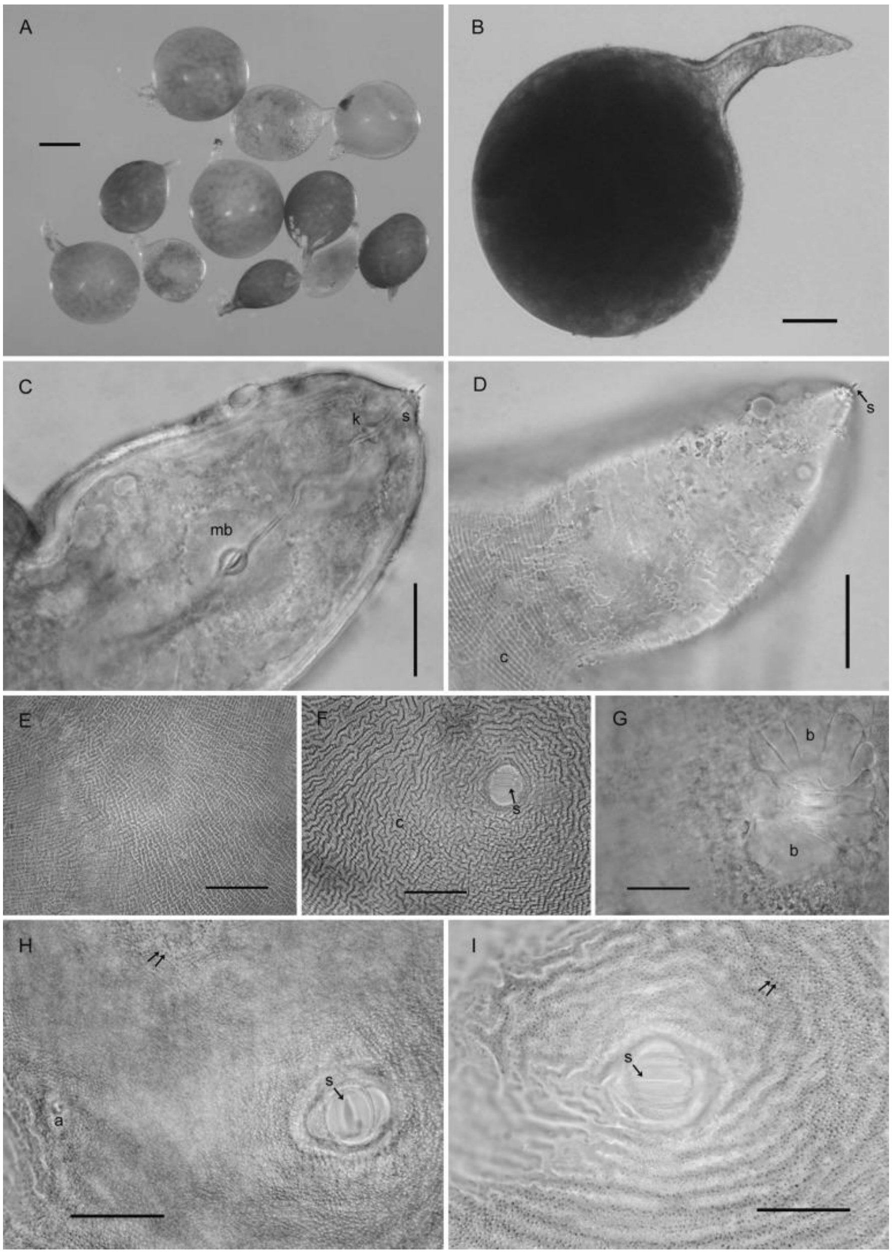


Fig. 8 Different structures of *Globodera ellingtonae* Sources: Handoo *et al.* 2012)

2.9 *Globodera mexicana*

It develops on tobacco, tomato and various solanaceous plants including *Solanum nigrum*. It is geographically separated, coming from Mexico. The species is very close to *Globodera pallida*. It can mate and form viable hybrids on tomato but usually develop on different solanaceous plants.

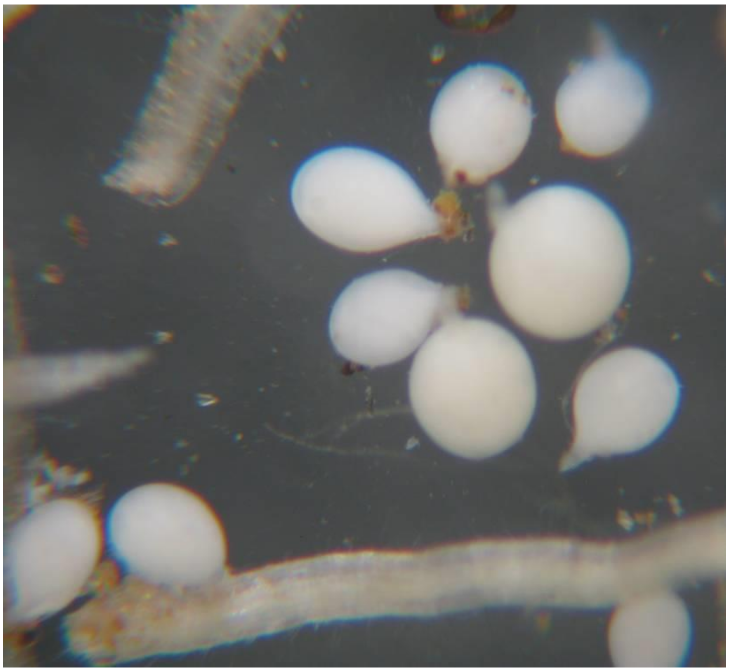


Fig. 9 Adult nematodes of *Globodera mexicana*

2.10 Other common nematodes of potato found in the tropical and sub-tropical areas

Meloidogyne incognita: *M. incognita* is found worldwide in tropical and subtropical regions, in particular in the warmer areas. *M. incognita* is found on many soil types. Damage and yield losses caused are generally more severe on coarse-textured sandy soils (Van Gundy, 1985). *Meloidogyne* spp. are generally intolerant of flooded soil conditions.

Field symptoms are typically of stunted, poorly growing plants with yellowing leaves. Infected root systems show characteristic knots or galls, the severity of which varies with the degree of nematode infection and species and variety of plant parasitized.

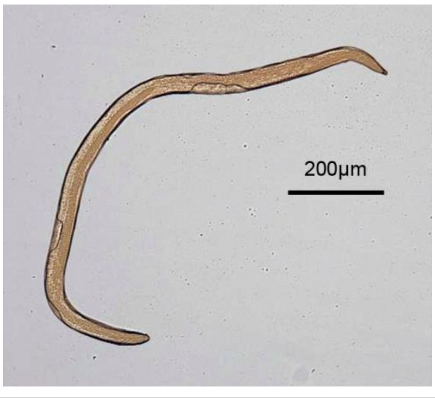


Fig.10 Juveniles of *Meloidogyne incognita*

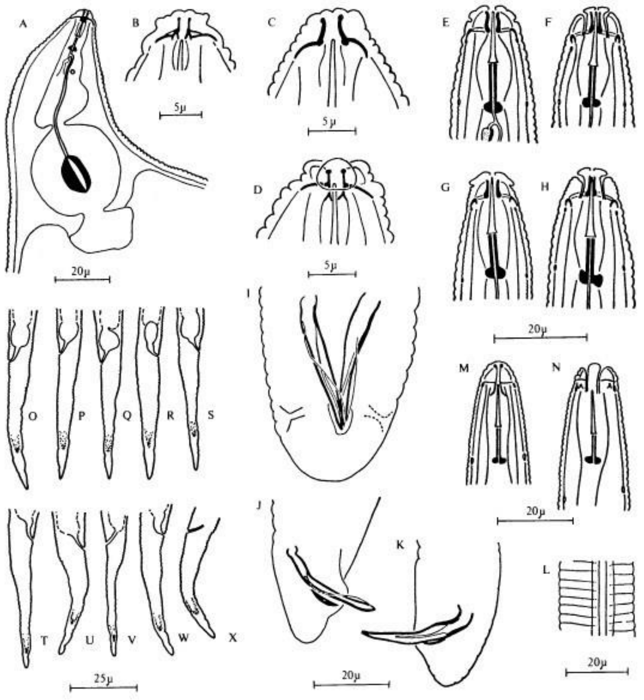


Fig.11 Different parts of female *Meloidogyne incognita*

Female: Endoparasitic, body spherical with projecting neck. Head with 2 or occasionally 3 annules behind head-cap.Cuticle thickening abruptly at base of relaxed stylet.Stylet knobs rounded or drawn out laterally.Excretory pore at level of or posterior to stylet knobs, 10-20 annules behind head. Posterior cuticular pattern displays bewildering variation. Typical pattern 'incognita type' with striae closely spaced, very wavy to zig-zag especially dorsally and laterally. Dorsal arch high, rounded. Lateral field not clear, sometimes marked by breaks in striae, broken ends often forked, pattern merging into body striae. 'Acrita type' with striae smoother, more widely spaced (or with coarse widely spaced striae separated by fine closely spaced striae visible for short distances). Dorsal arch variable, may be flattened at top or trapezoid. Striae often forked along a 'lateral line'. Limits of pattern more or less well-defined. Aberrant patterns occur.

<u>Juvenile</u>

Head not offset, truncate cone shape in lateral view, sub-hemispherical in dorso-ventral view. Head-cap wide followed by 2 clear annules on sublateral head sectors (lateral view), 3 annules on lateral head sectors (dorsal or ventral view). Stylet knobs prominent, rounded. Hemizonid 3 annules long just anterior to excretory pore. Lateral field with 4 incisures, outer bands cross striated. Rectum inflated. Tail tapering to subacute terminus, striae coarsening posteriorly.

Distribution: *Meloidogyne incognita* has been reported wide spread in Bangladesh (Shepherd and Barker, 1990; CABI/EPPO, 2002)

Hosts: *M. incognita* is probably the most widely distributed and economically important species of plant parasitic nematode in tropical and subtropical regions. Two-thirds of the root-knot nematode samples obtained from a number of tropical countries were of *M. incognita* (Sasser, 1979). In India alone, 232 plant genera have been reported as hosts to *M. incognita* (Krishnappa, 1985) and worldwide the species is a parasite of a wide range of crop plants.

Biology and ecology: The life cycles of *Meloidogyne* spp. are well studied and in their essentials differ little between the major species (De Guiran and Ritter, 1979). At 21°C *M. incognita* took 37 days to complete its life cycle on *Antirrhinummajus*, a similar time to that reported on soyabeans (temperatures not published) (Ibrahim and El-Saedy, 1987). Juveniles penetrate root tips, occasionally invading roots in the zone of root elongation. Invaded nematodes initiate the development of giant cells in the meristematic, cortical and xylem tissues of the root and galling of roots occurs. Third- and fourth-stage juveniles and young females occur after about 6-8 and 15 days, respectively. Adult females were observed after 20 days and egg laying commenced after 25 days (Ibrahim and El-Saedy, 1987).

Helicotylenchus dyhistera:

Preferred Scientific Name: *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961

Preferred Common Name: common spiral nematode

Taxonomic Tree

Domain: Eukaryota
- Kingdom: Metazoa
  - Phylum: Nematoda
    - Family: Hoplolaimidae
      - Genus: *Helicotylenchus*
        - Species: *Helicotylenchus dihystera*

Distribution: *Helicotylenchus dihystera* has been reported to be present in Bangladesh

(Khan *et al*. 2006; CABI/EPPO, 2010)

Symptoms: On the roots cortex with lesions, necrotic streaks or lesions, reduced root system, soft rot of cortex, leaves become abnormal in colour like yellow or dead.The whole plants become dwarf, dead or die back.

Potentiality: The nematode is potentially important to cause disease symptoms in various crops and they can be invaded through eggs, adults and juveniles in the seedlings, micro propagated plants, roots etc.

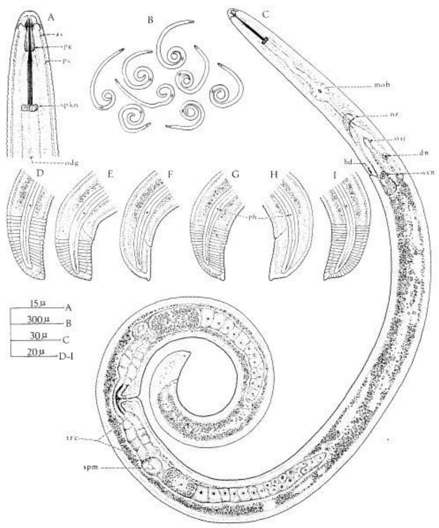


Fig. 12 Different morphological structures of *Helicotylenchus dihystera*

Chapter 3

Weed hosts

3.1 Kaakmachi or Gurki or Black nightshade (*Solanum nigrum*, family: Solanaceae) is a perennial herb with soft stem and many branches. It is native to Eurasia and introduced to the tropical and sub-tropical regions of the world.

Taxonomic Tree

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Solanales
Family: Solanaceae
Genus: *Solanum*
Species: *Solanum nigrum*

Description

Tit Begun (Black Nightshade)

Solanum nigrum is a very variable ephemeral, annual or sometimes biennial herb, 0.2-1.0 m tall, reproducing only by seed. It has a strong white taproot, with many lateral roots being produced in moist and fertile surface soils.

Fig.13 Adult plant of *Solanum nigrum*

Stems vary from prostrate to ascending or erect, and from herbaceous in ephemeral plants to rather woody or even shrubby in those that survive long enough to be biennial. Stems are round or angular, smooth or sparsely hairy, and green to purplish.

Leaves are alternate, ovate and are carried on short stalks, 2-8 cm long, and vary between plants from smooth-edged to shallowly lobed. They are opaque, matt and dark green both above and below, and either smooth or finely hairy.

The small, white, star-shaped flowers are carried in umbels on slender stalks developing directly from the stems between the leaves. Each cluster usually carries from 5-10 flowers, which open sequentially over several days. The flowers are 5-8 mm across, and have prominent yellow centres. Fruits are globular, dark green, matt berries 5-13 mm across, matt black when ripe, which contain many flattened, finely pitted, yellow to dark brown woody seeds approximately 1.5 mm long.

Seedlings of *S. nigrum* exhibit epigeal germination. The hypocotyl is commonly slender, about 1 cm long, green or purplish and distinctly hairy. The spreading cotyledons are slender, about 5 mm long, and taper towards the tips. The epicotyl is slender, smooth to finely hairy, and carries small, ovate, juvenile leaves that gradually assume the adult shape and size.

3.2 Foska Begun (Clammy ground cherry)

Physalis heterophylla, colloquial name clammy groundcherry, is a herbaceous plant that is a member of the Solanaceae family. It is native to North America, occurring primarily in the eastern United States and Canada. It is known to occur in all contiguous states except for Nevada and California. It is found mainly in habitats such as dry or mesic prairies, gravel hills and rises, sandy or rocky soils, and waste places such as roadsides.

Physalis heterophylla is a perennial, and is one of the taller-growing North American members of the genus, reaching a height up to 50 cm. The leaves are alternate, with petioles up to 1.5 cm, ovate in shape, usually cordate at the base (this is especially true of mature leaves), 6–11 cm long at maturity. Each member of the *Physalis* genus has at least one characteristic that makes it easy to differentiate in the field. For *P. heterophylla*, the stems and leaves are glandularly pubescent, giving it the "clammy" feel from which its name is derived. The plant also has distinctive thick rhizomes that run horizontal to the stem. Some sources recognize four distinct subspecies based primarily on leaf variation:

P. heterophylla var. *heterophylla*, with thin leaves that have dentate margins; *P. heterophylla* var. *clavipes*, with thick, conspicuously veined leaves and sparingly tooth-like protrusions on otherwise entire margins; *P. heterophylla* var. *ambigua*, with thick, conspicuously veined leaves and dentate margins;*P. heterophylla* var. *nycangienea*, with thin leaves that have sparingly tooth-like protrusions on otherwise entire margins.

Fig. 14 Adult plant of *Physalis heterophylla*

The flowers are on simple inflorescences that emerge from leaf apexes. The petals are yellow on the exterior, and yellow on the interior with purple highlights emanating up each petal from the base. They are funnelform in shape, with five fused petals. There are five reticulated sepals, which enlarge after flowering to eventually protect the maturing fruit. Stamens are five, with yellow anthers and purple filaments. The flowers face downwards when open, and are about 2.5 cm in diameter. The fruits are typical for the family (appearing like a tomatillo), and have a slightly bitter taste, though they are perfectly edible. The rest of the plant is poisonous.

Kingdom: Plantae
Angiospermus
Eudicots
Asterids
Order: Solanales
Family: Solanales
Genus: *Physalis*
Species: *P. heterophylla*

Chapter 4

Study Findings

4.1 Potato growers' perception regarding *Globodera* spp.: Study report of survey of potato growers

Seven thousand farmers of 21 districts namely Chittagong , Comilla, Chandpur, Laxmipur, Comilla, Chandpur, Jessore, Jhenaidha, Rajshahi, Gaibandha, Manikganj, Narayanganj, Munshiganj, Dinajpur, Thakurgaon, Panchagor, Noagaon, Joypurhat, Lalmonirhat, Kurigram, Bogra, Rangpur, and Nilphamary were interviewed by a structured questionnaire in order to understand their perception regarding *Globodera* infestation in the potato field, any abnormal symptom except the known ones, collect information of seed potato source, any activities that can accelerate the persistence and spreading, any activities that can also suppress the growth of invading nematode.

4.1.1 Age of potato growers: According to the questionnaire most of the potato farmers aged between 30-50 years (77.57%) and 16.40% farmers are over 50 year of age and the rest below 30 years meaning that a various age group are involved in potato cultivation. Observing problems in the potato field critically and adopting new technologies also dependent on the age group.

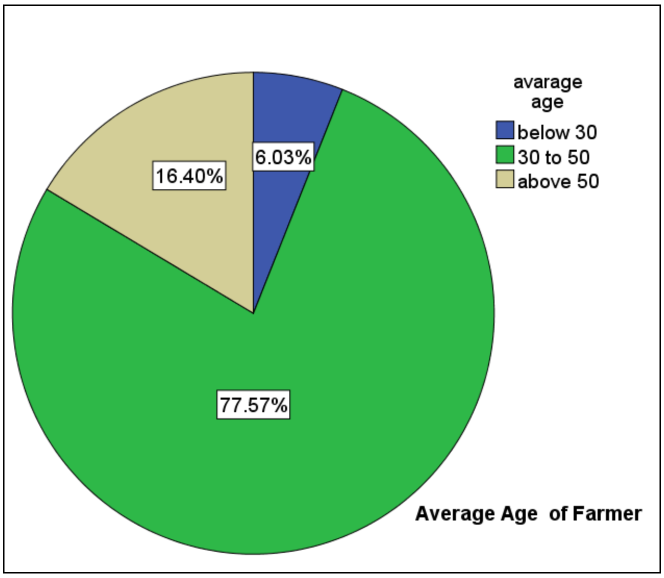
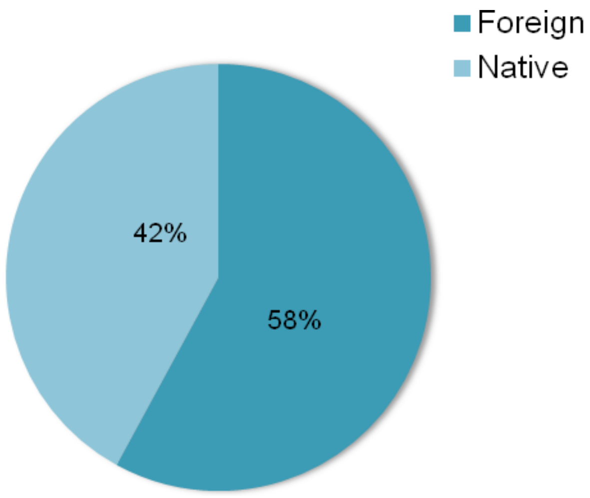


Fig.15 Average age of potato farmers

4.1.2 Sources of seed potato and origin: Seed potato source is an important issue to find out the possible pathway of *Globodera* spp. in Bangladesh. According to the survey, most of the farmers (58%) prefer foreign variety (High Yielding Variety- most of the seed locally produced in Bangladesh by BADC and local company), while 42% farmer like native variety. On the other hand, major part of the farmers collect the seed tubers from local businessmen (54.11%), 30.60% farmers collect the seeds that were kept in the cold storage last year, BADC can supply only 8.92% of seed potato (Table-appendix 2 and 3).



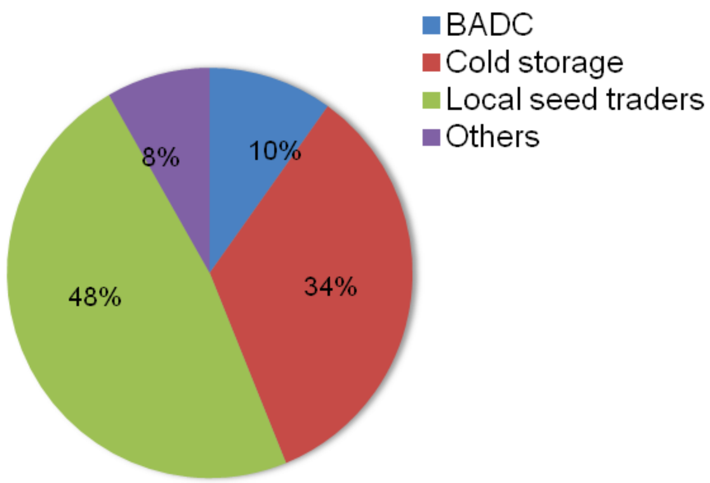


Fig. 16 Source of seed potato and their origin

4.1.3 Pre-harvest cultural practices: Pre-harvest practices are important for persistence of cyst nematode as well as other nematodes. Weeding are followed by most of the farmers, seed potato treatment and carbamate pesticide like Furadan are followed by 71.63% and 39.51 % respectively, while only 11.67% farmers use weedicide in their field (Table appendix 4).

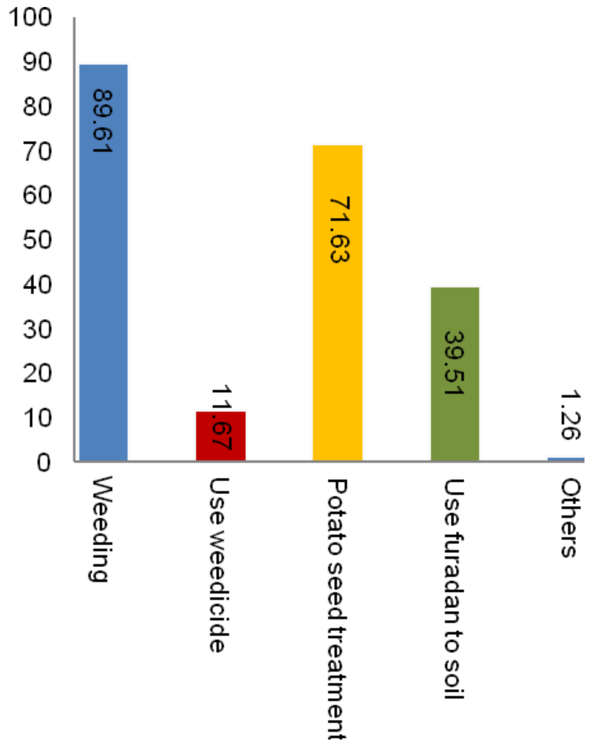


Fig.17 Pre-harvest cultural practices in the farmers' field

4.1.4 General conception of diseases of potato: To understand the knowledge of diseases of potato, farmers were asked question during interview. Although they do not know the pathogen/causal organisms directly, but some abnormalities in potato field are familiar with the farmers. Most farmers are experienced with late blight of potato, and wilting. A few farmers (11.69%) have idea about yellowing of potato plants and only 3.81% have idea about most important and diagnostic field symptom of cyst nematode disease in the potato field (Table appendix 5).

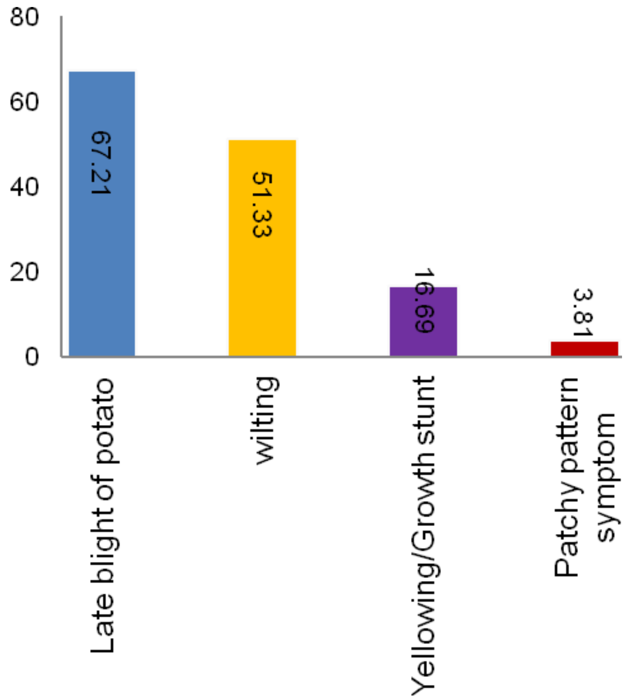


Fig. 18 General conception of farmers regarding potato disease

4.1.5 General conception of nemic diseases of potato: In most the cases farmers do not have any knowledge about nemic diseases of potato. A least number of farmers have idea about some nemic problems of crops. Farmers (8.78%) have idea about root-knot symptoms of potato, some farmer (7.94%) have experienced with red or black spot on the roots, only 4.96% farmers have idea about lesions on root of potato (Table Appendix 6).

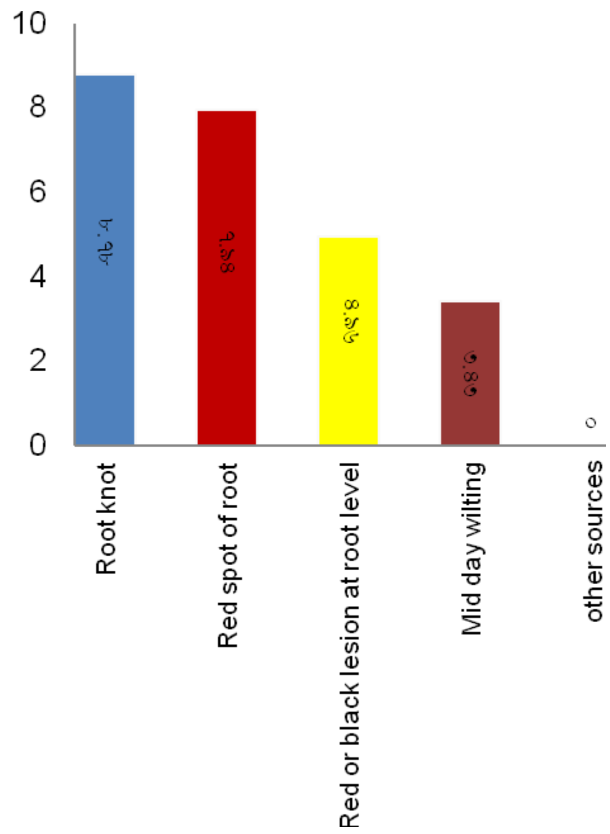


Fig. 19 General concept of nemic diseases of potato

4.1.6 Disease symptoms of nematode: Sometimes symptoms of nemic diseases can be seen at different time of the day. Specially wilting due to nematode can be seen at noon or mid-day. Most of the farmers have no idea about mid-day wilting of potato. It was analyze and found that the wilting was occurred by *Ralstonia solanacearum*. Only a few (8.53%) have experienced with this kind of symptoms in the potato field (Table Appendix 7).

4.1.7 Conception about patchy pattern symptoms: Potato Cyst Nematode (PCN) as well as other plant parasitic nematodes are generally restricted in a particular place of the field and gradually spread by various activities throughout the year. Therefore, PCN often cause symptom in a patch or distinct area of field. Most of the farmers (86.98%) have no idea about patchy pattern symptoms in the potato field and only 14.9% farmers told about the experience of this particular symptom in the field (Table Appendix 8).

4.1.8 Cultivation practices in potato field: Farmers are acquainted with the conventional to modern cultivation practices in the potato field. According to the nature of cultivation practice the spreading of nametode depends. Conventional farming with cattle and country plough are decreasing and only 4.76% farmers follow this method, 37.08% farmers use power tiller and 32.77% farmers use modern tractor in their potato field. The data reveal that if there is PCN in the potato field, then there is enormous scope of spreading faster than the conventional method (Table Appendix 9).

4.1.9 Cultural management of potato field: A number of cultural management are very common in the potato field like weeding, fertilizer management, water management, earthing up, sanitation practices etc. All these methods are very much related to establishment, spreading and perpetuation of the nematodes. Most of the farmers follow the common practices in the potato field. Only a few farmers do the sanitation practices in their potato field like taking out the affected plants from the field (Table Appendix 10).

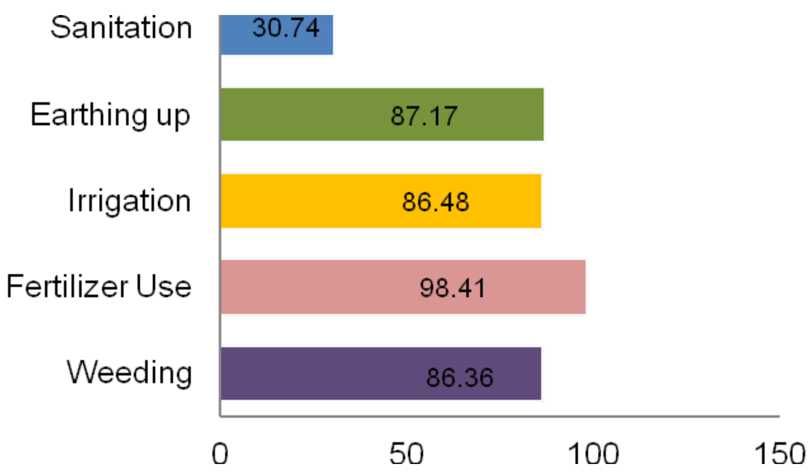


Fig. 20 Cultural management in the farmers' field

4.1.10 Water management in the potato field: Irrigation water can play role in disseminating the cyst nematode from one field to another field. Most of the farmers (85.47%) use shallow tube well (STW) and 10.72% farmers use DTW while 3.81 % farmers use other irrigation source. It can be speculated that there is enough chance to spread the cyst nematode if present in the field (Table Appendix 11)

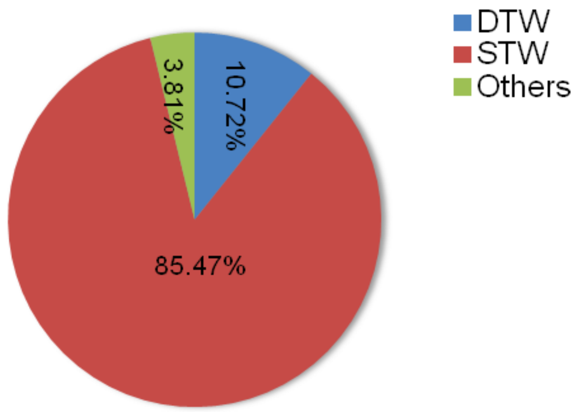


Fig. 21 Water management in the farmers' potato field

4.1.11 Harvesting procedure followed in potato filed: In all surveyed location farmers (92.22%) harvest the tuber by hand picking and 96.22% farmers left the tuber few time on the field by stacking or piling them in a place. It is often found that sudden rain deteriorate the tuber directly in the field and potato become rotten which often decompose and may harbour the nematode if any (Table Appendix 12).

4.1.12 Post harvest cultural practices in potato field: Farmers generally practices activities related to post harvest like potato grading, putting tubers in bag, leaving tubers in the field, potato cleaning etc. All these activities are generally practiced by farmers under the survey (Table Appendix 13).

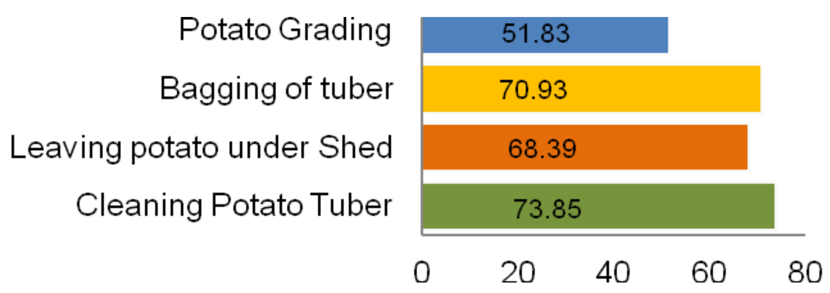


Fig.22 Post-harvest cultural practices in the farmers' field

4.1.13 Farmers' perception about cyst nematode: Farmers do not have any idea or conception about potato cyst nematode. Only a few (0.32%) farmers have theoretical idea about PCN while 98.23% farmers do not have any idea about PCN and their damage in potato tuber (Table Appendix 14).

4.1.14 Farmers' perception of cyst nematode in other crops: Cyst nematode can survive in other solanaceous crop varieties like tomato and brinjal. All farmers (100%) do not have any idea about alternate host of cyst nematode (Table Appendix 15). It is already reported that *Globodera* spp. can infect tomato and brinjal plants in some temperate countries. Therefore, it is likely to have cyst nematode infestation in tomato and potato plants in Bangladesh.

4.1.15 Farmers' perception of management of cyst nematode in potato field: Farmers in the surveyed area have no clear idea about this notorious pest of potato and their management (Table Appendix 16).

4.1.16 Farmers' perception about diseases of tomato and potato: Although farmers have no idea about nematode disease of potato, but a number of farmers have some abnormalities/diseases of tomato and brinjal. Farmers (42.47%) have idea

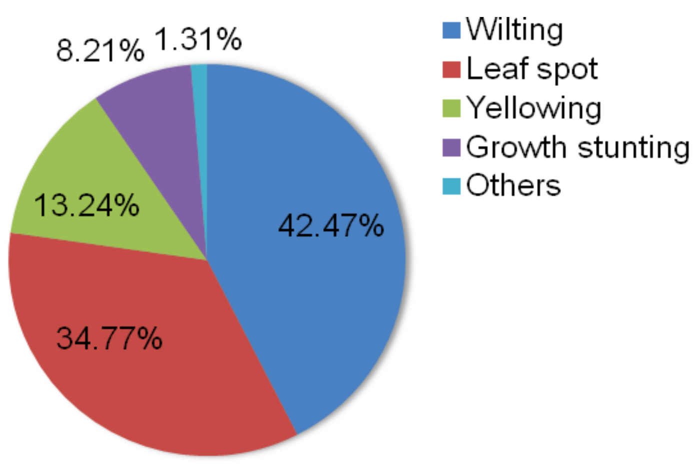


Fig.23 Farmers' conception about tomato diseases

About wilting symptoms of tomato and potato, 34.77% have idea about leaf spot, while 13.24% and 8.21% have idea on yellowing and growth stunting symptoms of tomato and brinjal (Table Appendix 17)

4.1.17 Farmers' perception about management of tomato and brinjal diseases: Farmers were interviewed whether they have any idea about management of diseases in the tomato and potato field (Table Appendix 18). Most of the farmers (45.82%) are dependent on chemical application, 28.66% farmers have idea about rouging out of affected plants while 23.49% farmers followed flood irrigation.

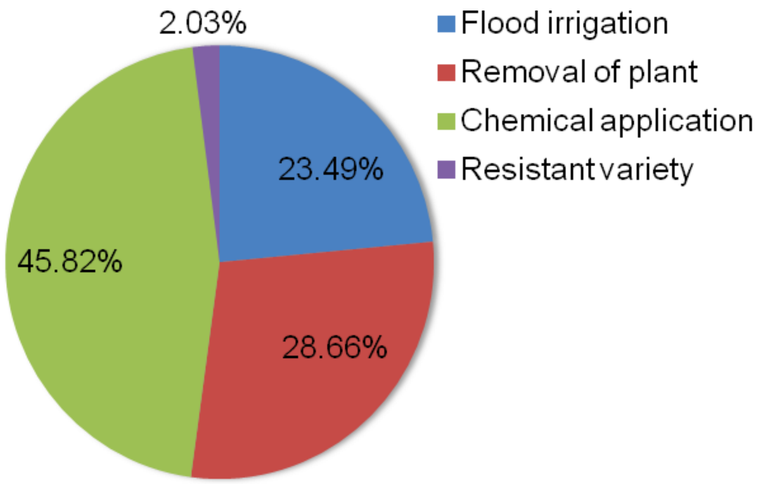


Fig.24 Farmers' conception about management of tomato and brinjal diseases

4.1.18 Weed association in potato field: Almost all farmers have the experiences on weed association in potato field. Weeds of many species are very common in the potato field like tit begun, kanta begun, bon begun, bothua, foska begun, bishkatali, and durba. Among the weeds tit begun, and foska begun are the members of solanaceae family and may have the chance of harbouring *Globodera* spp. Among the weeds, tit begun, kanta begun, bon begun, foska begun cover 37.5, 21.10, 18.5, 13.1 % of the total weeds association during survey respectively.

Farmers mostly (34.0%) collect the weeds by hands, but 30.0 % farmers use chemicals for controlling weeds. On the other hand mulching is common practice for moisture management and weed control and 26% farmers believe that weeds are controlled by earthing up of soil, while 7% farmers believe control weeds by mulching.

4.2 Findings of FGDs of major potato growing regions regarding *Globodera* spp.

The respondents of the FGDs more or less in the similar ways as bellows-

1. They have never seen the presence of cyst nematodes in their potato field.
2. They have no idea/conception about the stage of the potato crops which is vulnerable for cyst infestation.
3. No clear idea about the seasonal variation regarding cyst infestation.
4. The respondents do not follow any particular management practices for cyst/nemic diseases in their potato field.
5. Most of the farmers use Furadan 3G (a granular carbamate pesticide) during land preparation in their field.
6. Some farmers recognise about the patchy pattern symptoms in their field, but they have no idea about the cause.
7. They have never consulted with any extension worker or any one for having serious problem like cyst nematode.
8. They understood the magnitude of the problem.

4.3 Findings of Key informants' Interview (KII) regarding *Globodera* spp.:

In total 21 informants were interviewed with structured questionnaires. None of the informants got any kind of experience at the field level regarding any disease caused by *Globodera* spp. in Bangladesh. Some researchers and university teachers have practical experiences during their higher studies in abroad. Some scientists and university teachers have wide experiences with nematode diseases in Bangladesh, but none of them had any experience of PCN. Some of the informants are quite confident about recognizing the disease symptoms in the field.

Some key informants especially university teachers and researchers have knowledge and experiences on different hosts of *Globodera* spp. According to their responses, it is revealed that *Globodera* spp. are polyphagous nematode that include soybean. But, none of the respondents had any information regarding the existence of *Globodera* spp. in any oil crops in Bangladesh. They added that potato fields are rotated by oil seed crops in some areas of Bangladesh.

Some respondents have clear idea about the weed hosts of *Globodera* spp. They stated that mostly two weed hosts are associated namely Black nightshade and Clammy groundcherry which are very common weeds though out the country under the solanaceae family. None of the key informants ensured about the association of *Globodera* spp. in Bangladesh. According to the responses of the informants that famers do not usually apply chemicals/pesticide in the potato field. Moreover, due to use of natural mulches weed hosts often get more chances to establish in the potato field.

Although some respondents have very clear conception about the typical symptoms of *Globodera* spp. in potato field but none of them had noticed any special symptoms like stunted growth and yellowing in a patch in potato field. Respondents like agriculture officers/DD ensured the nemic problems in tomato, brinjal, ginger and garlic, but according to them they never got any problem statement like stunting, yellowing and patchy symptoms.

The key informants stated that they had neither any personal experience nor they have any information of existence of *Globodera* spp. in Bangladesh. They have no idea of species of *Globodera* can be existed in Bangladesh.

Respondents are not aware about the existence of *Globodera* spp. in Bangladesh and therefore they have no idea whether this nematode exist in Bangladesh year after year.

According to the key informants, they have no information regarding the management of *Globodera* spp. in Bangladesh. They supplemented that in general the farmers in Bangladesh use the chemical pesticides of carbamate group namely Furadan 3G/ 5G during last land preparation to manage the soil inhabiting insects and nematodes.

Key informants in different port areas were interviewed to specially investigate the existence of *Globodera* spp. during importing potato tubers and other agricultural materials. The respondents had no record of interception and return of any consignment to the exporting country due to presence of *Globodera* spp.

Some of the key informants informed that there are possibilities of introduction of *Globodera* spp. in Bangladesh by various ways. The most important passage is potato tuber during importing from the countries where *Globodera* spp. is available. There are many agencies who are importing seed potato tuber for multiplication though which *Globodera* spp. can be introduced. They further added that the border areas are also vulnerable of introduction of *Globodera* spp. by smuggling seed potato tuber.

According to the key informants, prevention is more important especially during importing seed potato tubers. They further stressed about the regular vigilance of potato field during cultivation time to recognize the typical symptoms in the field. They further suggest strengthening training at the root-level employees of DAE for proper identification potato cyst nematodes.

Chapter 5

Findings of laboratory analysis of the plant and soil samples from selected areas

5.1 Methodology followed:

During workshop with the data enumerators and data collectors, detailed procedure was given for taking records on field symptoms, collecting plants and soil samples. It was advised to collect the root and soil samples especially from the suspected fields having typical or very close symptoms shown in the Field Guide Book. In addition, some samples from the apparently healthy looking field were also collected in some cases.

5.2 Collection of samples:

The potato field showing stunted growth, yellow leaves and more preferably the symptoms were restricted in a particular place of the field or showing distinct patchy pattern which is the more precise diagnostic symptoms of cyst nematodes (*Globdera rostochiensis* and *G*. *pallida*). Several samples were collected from a suspected field from a depth of 20 cm with trowel and finally made a composite sample of 500 gram for each sample. The roots and soil samples were kept in zip-lock polythene bag with proper labelling and immediately send to the laboratory for subsequent analysis. During survey, samples ofimported and fieldpotato, tomato, and brinjal were collected following the same procedure. Weeds samples of the Solanaceae family (black night shade: *Solanumnigrum* and Gurki: *Phycalis* sp.) were also collected for determining the association of *Globodera* spp. in Bangladesh.

5.3 Working procedure:

Three standard methods with slight modifications were followed for isolation of cyst and other nematodes from roots and soil samples.

5.3.1 Nematode isolation from infested root system:

Baermann Funnel Technique: The root samples were chopped into small pieces having 1-2 inches length. The chopped roots were placed on clean cheese cloth and keep it on the funnel. The funnel and rubber tube were filled with water and cheese cloth with root pieces were kept on the funnel and set it for 24 hours for isolation. If the roots contained any nematodes will swim to the water and gradually deposit at the bottom of clipped rubber tube. After 24 hours, the water suspension were taken out in a petridish and directly observed under stereo-binocular microscope.

5.3.2 Isolation of cyst nematodes from soil samples:

Flegg's sieving and sedimentation technique was followed for isolation of nematodes. To especially collect the cyst nematodes the soil samples are mixed with enough tap water in a bucket and stir well with a stick. The soil suspension is then passed through a series of sieves consisting of 40, 60, 80 and 120 mesh so that nematodes of different size and shapes will be deposited on the sieves if any. Afterwards, the nematodes are collected by back flashing of water by wash bottle and collect the suspension in a beaker. The suspension of individual sieves are collected in different beaker and allowed to settle for 1-2 hour in room condition. After settling well the upper part of nematode suspension are siphoned out and the remaining suspension are examined under stereo-binocular microscope taking the suspension a petridish.

5.3.3 Isolation of vermiform nematodes from soil samples:

Vermiform nematodes present in the soil were isolated following modified sieving and sedimentation technique. In this method a sieve is fit with a plastic bucket and a thin ply of facial tissue is carefully set over the sieve in such a way that the sieve and facial tissue just touch the upper surface of the water. Then the soil spread over the facial tissue and allowed overnight to allow the nematodes to pass through the facial tissue and settle down at the bottom of the bucket. The next day, the suspension are decanted into a beaker and allowed to settle for 1-2 hour. The upper part of the suspension is siphoned out and rest of the suspension was examined under stereo-binocular microscope.

5.3.4 Nematode fixation and preservation:

The collected nematodes are immediately killed taking all nematodes in a test tube and dipping in hot water for short time. Dipping the test tube for 2-3 times the nematode become dead and ready for preservation in TAF (Trienthanol Amine Formalin (TAF) solution) for long time.

5.3.5 Procedure of identification of nematodes:

The isolated, fixed nematodes were identified by observing different organs of nematodes like stylate, median bulb, salivary gland, ovary, vulval position, tail etc. and compared with appropriate keys.

5.3.6 Isolation and identification of *Globodera* spp.:

Soil and plant samples from suspected locations were analysed in the laboratory. For cyst nematode isolation, modified sieving and sedimentation techniques was followed. No cyst nematodes were isolated, nor any suspected structures were found during diagnosis (Appendix Table 1-4). Therefore, it is indicated that the existence of PCN (*Globodera rostochiensis, Globodera pallida, Globodera tabacum, Globodera ellingtonae,* and *Globodera mexicana*) still not detected in surveyed area.

5.3.7 Isolation and identification of plant parasitic nematodes: Nematodes under two genera *viz*. *Meloidogyne* and *Helicotylenchus* were isolated. Isolated nematodes were fixed and preserved for detailed study. Different morphological features and published reports were considered for identification.

Chapter 6

Risk Assessment for *Globodera* spp.

The pest risk assessment was carried out with the aim to record the existence of *Globodera* spp. in potato and other alternate hosts like tomato and brinjal as well as associated weed hosts in order to revise Bangladesh's phytosanitary measure regarding the potatoes imported from any potato exporting countries including the Netherlands, Belgium, Germany, Denmark and USA into Bangladesh and also exporting potatoes from Bangladesh. Additionally the occurrences of other plant parasitic nematodes are also listed from the plant and soil samples from the surveyed areas. Based on the previous PRA reports and research reports nematodes associated with seed potatoes *(Solanum tuberosum)*, tomato and brinjal in Bangladesh, the Netherlands, Germany, Denmark, USA and EU countries are listed in Table 1-5.

6.1 Risk Assessment :

The risk assessment of *Globodera* spp. and other associated nematodes of potato, tomato, brinjal and weed hosts were done in accordance with International Plant Protection Convention (IPPC) and the International Standards for Phytosanitary Measures (ISPMs) including ISPM 2 and ISPM 11. The import risk assessment consists of two main components, the consequence of the introduction and the introduction potential of pests to the importing country. The consequences of introduction evaluate the economic and environmental impact of the pest while the introduction potential measures the entry, establishment and dispersal to the importing country. Each risk is then assigned a qualitative value and a risk rating value (Table 4). The risk values are combined to give an overall estimate of the risk.

Table 4: Risk rating and corresponding risk value for risk assessment of quarantine pests

Risk rating	Risk value
High	3
Medium	2
Low	1

6.1.1. Assess Consequences of Introduction of *Globodera* spp. (Table 5 & 6)

The undesirable outcomes being considered are the negative impacts resulting from the introduction of *Globodera* spp. After identifying *Globodera* spp. that could reasonably be expected to follow the pathway, the assessment of risk continues by considering theconsequences of introduction. For each of these quarantine pests, the potential consequences of introduction are rated using five **Risk Elements**. These elements reflect the biology, host ranges and climatic/geographic distributions of the pests. For each Risk Element, pests are assigned a rating of **low** (1 point), **medium** (2 points) or **high** (3 points). A Cumulative Risk Rating is then calculated by summing all Risk Element values.

Risk Element 1: Climate-Host Interaction

When introduced to new areas, *Globodera* spp. can be expected to behave as they do in their native areas if host plants and climates are similar. Ecological zonation and the interactions of the nematodes and their biotic and abiotic environments are considered in the element. Estimates are based on availability of both host material and suitable climate conditions. To rate this Risk Element, the 30 defined agriculture ecological zones are used. Due to the availability of both suitable host plants and suitable climate, the pest has potential to establish a breeding colony:

Rating scores are as follows:

Low : In a single ecological zone……………........…..........…… **1 point.**
Medium : In two or three ecological zones……............…….…**2 points**.
High : In four or more ecological zones…...................……..... **3 points**

Risk Element 2: Host Range

The risk posed by a plant pest depends on both its ability to establish a viable, reproductive population and its potential for causing plant damage. For pathogens, risk is more complex and is assumed to depend on host range, aggressiveness, virulence and pathogenicity; for simplicity, risk is rated as a function of host range.

Rating scores are as follows:

Low : Pest attacks a single species or multiple species within a single genus...**1 point**
Medium : Pest attacks multiple species within a single plant family…………….**2 points**
High : Pest attacks multiple species among multiple plant families……............**3 points**

Risk Element 3: Dispersal Potential and Pathway

A pest may disperse after introduction to a new area. The following items are considered:

- Reproductive patterns of the nematode pest
- Inherent powers of movement

Rating scores are as follows:

Low : Pest has neither high reproductive potential nor rapid dispersal capability.....**1 point**
Medium : Pest has either high reproductive potential or the species is capable of rapid dispersal………………………………………………………………………….........**2 points**
High : Pest has high biotic potential, *e.g.*, many generations per year, many offspring per reproduction ("r-selected" species), and evidence exists that the pest is capable of rapid dispersal, *e.g.*, over 10 km/year under its own power; via natural forces, wind, water, vectors, *etc*., or human assistance…………………………………...……**3 points**

Risk Element 4: Economic Impact
Introduced pests are capable of causing a variety of direct and indirect economic impacts.
These are divided into three primary categories (other types of impacts may occur):
- Lower yield of the host crop, *e.g.*, by causing plant mortality, or by acting as a disease vector.
- Lower value of the commodity, *e.g*., by increasing costs of production, lowering market price, or a combination.
- Loss of foreign or domestic markets due to presence of new quarantine pest.

Rating scores are as follows:

Low: Pest causes any one or none of the above impacts…......................**1 point**
Medium: Pest causes any two of the above impacts…………………...........**2 points**
High: Pest causes all three of the above impacts………………................**3 points**

Risk Element 5: Environmental Impact

The assessment of the potential of each pest to cause environmental damage proceeds by considering the following factors:

- Introduction of the pest is expected to cause significant, direct environmental impacts, *e.g.*, ecological disruptions, reduced biodiversity.
- Pest is expected to have direct impacts on plant species as endangered or threatened in Bangladesh.
- Pest is expected to have indirect impacts on plant species as endangered or threatened by disrupting sensitive, critical habitat.
- Introduction of the pest would stimulate chemical or biological control programs.

Rating scores are as follows:

Low: None of the above would occur…......................**1 point**
Medium: One of the above would occur…..................**2 points**
High: Two or more of the above would occur......…....**3 points**

6.1.2 Assessment of Risk Rating of Consequences of Introduction of *Globodera* spp. and other plant parasitic nematodes

Consequences of Introduction of *Globodera rostochiensis* (Wollenweber) (Golden Potato Cyst Nematode or Golden Nematode)	**Risk Rating**
Climate-Host Interaction *Globodera rostochiensis* distributes large in the world, including the temperate regions to tropical countries (CABI, 2006). The worldwide distributions (EPPO, 1978) of *Globoderarostochiensis* are EPPO region: Albania, Algeria, Austria, Belarus, Belgium, Bulgaria, Czech Republic, Cyprus, Denmark, Egypt, Estonia, Faroe Islands, Finland, France, Germany, Greece (including Crete), Hungary (one locality only), Iceland, Ireland, Latvia, Lebanon, Libya, Lithuania, Luxembourg, Malta, Morocco, Netherlands, Norway, Poland, Portugal (including Madeira; unconfirmed in Azores), Spain (including Canary Islands), Russia (Central Russia, Eastern Siberia, Far East, Northern Russia, Southern Russia, Western Siberia), Slovakia, Sweden, Switzerland, Tunisia, UK (England, Channel Islands), Ukraine, Yugoslavia (unconfirmed). Found in Israel on only two occasions in 1954 and 1965 in a small area in the Sharon region, and was successfully eradicated. Asia: Cyprus, India (Kerala, Tamil Nadu), Japan (Hokkaido), Lebanon, Pakistan, Philippines, Sri Lanka, Tajikistan, Russia (Eastern Siberia, Far East, Western Siberia). Africa: Algeria, Egypt, Libya, Morocco (intercepted only), Sierra Leone, South Africa, Tunisia. North America: Canada (Newfoundland, British Columbia Vancouver Island only), Mexico,	High (3)

USA (New York; eradicated in Delaware). Central America and Caribbean: Costa Rica, Panama. South America: Throughout the high Andean region: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru, Venezuela. More southerly in range than G. pallida. Oceania: Australia (two outbreaks, one in Western Australia in 1986, the other in Victoria in 1991; both are subject to official eradication programmes), New Zealand, Norfolk Island. EU: Present. The optimum temperature for the hatch of *Globodera rostochiensis* is about 15°C (Evans, 1968). Therefore, it can be established in all ecological zones of Bangladesh. One or more of its potential hosts occurs in these zones.	
Host Range *Globodera rostochiensis* has been recorded on a wide range of Solanaceae including: *Lycopersicon esculentum* (tomato), *Solanum melongena* (aubergine), *Solanum tuberosum* (potato), *Datura stramonium* (jimsonweed), *Lycopersicon pimpinellifolium* (currant tomato), *Oxalis uberosa* (oca), *Solanum* (nightshade), *Solanum aviculare* (kangaroo apple), *Solanum gilo* (gilo), *Solanum indicum*, *Solanum marginatum* (white-edged nightshade), *Solanum mauritianum* (tree tobacco), *Solanum nigrum* (black nightshade), *Solanum quitoense* (Narangillo), *Solanum sarrachoides* (green nightshade) (UK) (CABI, 2006).	Medium (2)
Dispersal Potential and Pathway The potato cyst nematodes are dispersed with soil debris and plant material contaminated by the cysts and by infected or contaminated potato tubers. (http://nematode.unl.edu/pest6.htm). In general, the potato cyst nematodes will survive in any environment where potatoes can be grown. A period of 38-48 days (depending on soil temperature) is for a complete life cycle of the potato cyst nematodes (Chitwood and Buhrer, 1945). Potato cyst nematode eggs can remain dormant and viable within the cyst for 30 years (Winslow and Willis, 1972). After mating, each female produces approximately 500 eggs (Stone, 1973b).	High (3)
Economic Impact Crop losses induced by the golden nematode range 20-70% (Greco, 1988). It has been estimated that approximately 2 t/ha of potatoes are lost for every 20 eggs/g soil (Brown, 1969). Up to 80% of the crop can be lost when nematode populations are raised to very high levels by repeated cultivation of potatoes. In Chile, yield losses of 20, 50 and 90% were obtained with population densities of 9, 28 and 128 eggs/g soil (Moreno et al., 1984; Greco and Moreno, 1992). *Rhizoctonia* and other fungal diseases associated with nematode feeding may also contribute to the yield loss. In Canada, *Globodera rostochiensis* was found in Newfoundland in 1962 and 800,000$Can /year has been spent on control and research of golden cyst nematode (Miller, 1986). Besides that, affected plants suffer tubers are smaller (CABI, 2006). This	High (3)

means it effects on quality of potato tubers as well as seed potatoes. Control on golden cyst nematode, *Globodera rostochiensis* is major by soil fumigants but fumigant nematicides are toxic and expensive (Mazin, 1991). Moreover, golden cyst nematode (*Globodera rostochiensis*) is quarantine pest for EPPO, APPPC, NAPPO (OEPP/EPPO, 1978; 1981). Therefore, the presence of the potato cyst nematodes in potato growing areas prevents the export of potatoes to international markets due to the restrictions imposed by many countries against this pest. Based on these Economic Impacts, the Potato cyst nematodes could become established in Bangladesh with High (3) Risk potential.	
Environmental Impact Introduction of *Globodera rostochiensis* into Bangladesh is likely to initiate chemical, because it is a serious pest of economically important crops. The fumigation to control this nematode may also harm to the beneficial organisms available to the soils. This species has the potential to attack plants (Tomato, brinjal, black nightshade and clammy ground cherry) that are main crop in Bangladesh. As a large chemical will be used for its controlling. Therefore, it can impacts on ecological system.	High (3)
Consequences of Introduction of *Globodera pallida* (Stone, 1973) Behrens, 1975 (White Potato Cyst Nematode)	Risk Rating
Climate-Host Interaction The centre of origin of *Globodera pallida* is in the Andes Mountains in South America, then they were introduced to Europe with Potatoes and spread with seed potatoes to other areas. The present distribution covers temperate zones down to sea level and in the tropics at higher altitudes (many countries in EPPO region, Asia including India, Africa, America and Oceania including Scotland). In these areas, distribution is linked with that of the potato crop (www.eppo.org/quarantine/nematodes/globodera-_pallida/HETDSP_ds.pdf, (CABI, 2006)). Potato crop distributes in most of the agro-ecological areas of Bangladesh such as High Land of North (Tista River Floodplain) and North-West regions (Ganges River Floodplain), Brahmaputra-Jamuna Flood plain regions, Meghna River and Estuarine Flood plain regions of Bangladesh. Based on all information available, we estimate this nematode could become established in all agro-ecological regions in Bangladesh.	High (3)
Host range The hosts of *Globodera pallida* are restricted to the Solanaceae. Major hosts are *Lycopersicon esculentum* (tomato), *Solanum melongena* (aubergine) and *Solanum tuberosum* (potato). In addition to many other hosts in the family Solanaceae listed as following: *Datura tatula, Lycopersicon glandulosum, L. hirsutum, L. mexicanum, L. esculentum peruvianum, L. pyriforme, Physalis philadelphica, Physochlaina orientalis, Salpiglossis sp., S. acaule, S. aethiopicum, S. ajanhuiri, S. alandiae, S. alatum, S. anomalocalyx, S. antipoviczii, S. armatum, S. ascasabii, S. asperum, S. berthaultii, S. blodgettii,*	High (3)

S. boergeri, S. brevimucronatum, S. bulbocastanum, S. calcense, S. calcense, S. cardenasii, S. caldasii, S. canasense, S. capsicibaccatum, S. capsicoides, S. carolinense, S. chacoense, S. chaucha, S. chloropetalum, S. citrillifolium, S. coeruleiflorum, S. commersonii, S. curtilobum, S. curtipes, S. demissum, S. demissum x S. tuberosum, S. dulcamara, S. durum, S. elaeagnifolium, S. famatinae, S. garciae, S. gibberulosum, S. giganteum, S. gigantophyllum, S. gilo, S. glaucophyllum, S. goniocalyx, S. gracile, S. heterophyllum, S. heterodoxum, S. hirtum, S. hispidum, S. indicum, S. intrusum, S. jamesii, S. jujuyense, S. juzepczukii, S. kesselbrenneri, S. kurtzianum, S. lanciforme, S. lapazense, S. lechnoviczii, S. leptostygma, S. longipedicellatum, S. luteum, S. macolae, S. macrocarpon, S. maglia, S. mamilliferum, S. marginatum, S. melongena, S. miniatum, S. multidissectum, S. nigrum, S. nitidibaccatum, S. ochroleucum, S. ottonis, S. pampasense, S. parodii, S. penelli, S. phureja, S. pinnatisectum, S. platypterum, S. polyacanthos, S. polyacanthos S. polyadenium, S. prinophyllum, S. quitoense, S. radicans, S. rostratum, S. rybinii S. salamanii, S. saltense, S. sambucinum, S. sanctae-rosae, S. sarrachoides, S. schenkii S. schickii, S. semidemissum, S. simplicifolium, S. sinaicum, S. sinaicum, S. sisymbrifolium, S. sodomaeum, S. soukupii, S. sparsipilum, S. stenotomum, S. stoloniferum, S. subandigenum, S. sucrense, S. tarijense, S. tenuifilamentum, S. toralopanum, S. triflorum, S. tuberosum ssp. andigena, S. tuberosum ssp. tuberosum, S. tuberosum 'Aquila', S. tuberosum 'Xenia N', S. utile, S. vallis-mexicae, S. vernei, S. verrucosum, S. villosum, S. violaceimarmoratum, S. wittmackii, S. wittonense, S. xanti, S. yabari and *S. zuccagnianum* (Ellenby, 1945, 1954; Mai, 1951, 1952; Winslow, 1955z; Stelter, 1957, 1959, 1987; Roberts and Stone, 1981; http://plpnemweb.cudavis.edu/nemaplex/Taxadata/G053S2.htm).	
Dispersal Potential and Pathway The cyst of *Globodera pallida* contain as many as 500 eggs, the eggs can remain viable for many years in the absence of solanaceous (25-30-40 years) before gradually deteriorating. This nematode adapts to develop at cool temperatures range of 100C to180C. Most juveniles go into a form of dormancy known as diapause, in this state; they can remain viable for many years. The lifecycle takes 38-48 days to complete (depending on soil temperature) (Chitwood and Buhrer, 1945; Franco,1979; Stelter, 1971; Stone, 1973b; Jones and Jones, 1974; Golinowski et al.,1997; www.Scotland.gov.uk/consultations/agriculture/pcn_technical_paper_seerad.pdf). *Globodera pallida* have no natural means of dispersal, and can only move the short distances traveled by juveniles attacked towards root in soil. However, Potato Cyst Nematode (*G. pallida*) are usually spread by cysts for long distance by contaminated soil, attached to tuber, plants for transplanting or to farm machinery and other pathway as transport vehicles, non-host plant material, containers and packing andso on. (CABI, 2006); www.eppo.org/quarantine/nematodes/globodera_pallida/HETDSP_ds.pdf	High (3)
Economic Impact *Globodera pallida* are major pests of the potato crop in cool-temperate areas. Damage is related to the number of viable eggs per unit of soil, and is reflected the weight of tubers produced. It has been estimated that	High (3)

approximately 2 tons/ha of potatoes are lost for every 20 eggs/g soil. Up to 80% of the crop can be lost when nematode populations are raise to very high levels by repeated cultivation of potatoes (Brown, 1969). In the UK, potato yields lost 6.25 tons/ha per 20 eggs/g soil (Wood et al., 1995), etc. Besides that, affected plants suffer tubers are smaller (CABI, 2006). This means it effects on quality of potato tubers as well as seed potatoes. Control on *Globodera pallida* is major by soil fumigants but fumigant nematicides are toxic and expensive (Mazin, 1991). Moreover, *Globodera pallida* is quarantine pest for EPPO, APPPC, NAPPO (OEPP/EPPO, 1978; 1981).	
Environmental Impact Apart from using nematicide, methyl bromide was the most effective fumigant available to control *Globodera pallida*. But methyl bromide is an ozone-depleting substance (Thomas, 1996). The fumigation to control this nematode may also harm to the beneficial organisms available to the soils. This species has the potential to attack plants (Solanum) that are main crop in Bangladesh. As a large chemical will be used for its controlling. Therefore, it can impacts on ecological system.	High (3)
Consequences of Introduction of *Globodera tabacum*	Ratings
Climate-Host Interaction *Globodera tabacum* is limited in distribution; the major problem areas are Virginia and Connecticut, from where the tobacco cyst nematodes are beginning to radiate into adjoining States (Melton et al., 1991). It is distributed in Asia (China, Japan, Korea, Pakistan), Europe (Bulgaria, France, Spian, Greece, Italy, Slovenia, former USSR), North America (Canada, USA, Mexico, Virginia). The information is limited for *Globoderatabacum*, but we estimate that this nematode could become in different agro-ecological zones in Bangladesh.	High (3)
Host range The host of *Globodera tabacum* has vast host under the family Solanaceae. *Nicotiana rustica* (wild tobacco), *Nicotiana tabacum* (tobacco), *Solanum gilo* (gilo), *Solanum indicum*, *Solanum lycopersicum* (tomato), *Solanum mauritianum* (tobacco tree), *Solanum melongena* (aubergine), *Solanum nigrum* (black nightshade), *Solanum quitoense* (Narangillo), *Solanum tuberosum* (potato).	High (3)
Dispersal Potential and Pathway Within *Globodera* spp. the first stage juvenile moults inside the egg and the second stage juvenile hatches. Recent experiments by Ambrogioni et al. (1995) on G. tabacum in Italy suggest that at 26°C two generations could be produced on crops of aubergine, each taking around 36 days to complete. At 20°C the time to complete one generation was 72 days and at 30°C the cycle was shortened to 32 days. The maximum number of generations produced on aubergines in Italy is three, whereas in the USA, Barker and Lucas (1984)	High (3)

reported four to five generations from tobacco fields. *Globodera tabacumvirginiae* and *G. tabacum solanacerum* have temperature requirements and life cycles similar to those reported in Italy (Miller and Gray 1968, 1972). *Globodera tabacum* have no natural means of dispersal, and can only move the short distances travelled by juveniles attacked towards root in soil. However, *G. tabacum* are usually spread by cysts for long distance by contaminated soil, attached to tuber, plants for transplanting or to farm machinery and other pathway as transport vehicles, non-host plant material, containers and packing andso on. (CABI, 2006).	
Economic Impact *G. tabacum tabacum* and *G. tabacum solanacearum* both have the potential to reduce crop yield significantly. In Virginia, USA, in 1983, the state lost $0.7 million due to an estimated yield loss of 15% of tobacco, the most important cash crop in Virginia (Miller, 1986). In Connecticut, USA, large areas of its' river valley are infested with tobacco cyst nematodes, causing large yield losses and poor quality plants. Nematicides are used extensively in this area and the estimated cost in 1982 was $60,000 per annum (Miller, 1986).	High (3)
Environmental Impact The control of any cyst nematode is difficult as cysts containing viable eggs can survive in the soil for many years. The build up to large, damaging population densities may require only a single susceptible host crop, depending on the original density and the environmental conditions (e.g. temperature). In the USA, LaMondia (1996) and Johnson (1995) have conducted many studies into the control and suppression of the G. tabacum complex. Methods used include trap-cropping, chemical control, crop rotation and the use of resistant tobacco genotypes. At the present time there is no information on biological control of the *G. tabacum* complex.	High (3)
Consequences of Introduction of *Globodera ellingtonae*	Ratings
Climate-Host Interaction *Globodera ellingtonae* is a new cyst nematode which was described from Oregon (US) in 2012. A molecular diagnostic method has been developed to identify *G. ellingtonae* and differentiate it from other *Globodera* species. This new species was first found in soil samples collected in May 2008 at Powell Butte in Oregon, from a field in which potato (*Solanum tuberosum*) and other crops had been grown. We estimate that this nematode could become in	High (3)

different agro-ecological zones in Bangladesh.	
Host range Preliminary experiments have demonstrated that potato and tomato (*S. lycopersicum*) can be host plants of *G. ellingtonae*, but the pathogenicity of the nematode to both crops remains to be studied.	High (3)
Dispersal Potential and Pathway Although *Globodera ellingtonae* are comparatively less studied genus of *Globodera*, but it is speculated that the movement will be through the cysts for long distance dispersal. Contaminated soil, farm machinery and equipment are the common means of dispersal.	High (3)
Economic Impact *Globodera ellingtonae* cause potential economic damage in potato and tomato in Idaho and Oregon state. Therefore, it is assumed that if these nematodes are exposed in Bangladesh could be a potential threat.	High (3)
Environmental Impact All cyst nematodes develop numerous eggs and viable eggs remain soil for long time. If the nematodes get any susceptible host they can build large, damaging population densities if the environment is favourable.	High (3)
Consequences of Introduction of *Globoderamexicana*	Ratings
Climate-Host Interaction *Globodera mexicana* is very closely related with, and produces viable offspring with, *Globodera pallida*. However, the two differ in host range and Subbotin et al (2010) found that *G. mexicana* is molecularly and biochemically different from all other *Globodera* species.	High (3)
Host range The host range is very sililar to that of other cyst nematodes of potato. One important difference is *Globoder amexicana* develops on *Solanum nigrum*, but do not develop on *Solanum tuberosum*.	High (3)
Dispersal Potential and Pathway Although *Globodera mexicana* are supposed to be disseminated by contaminated soil, farm equipment and machinery through cysts.	High (3)
Economic Impact *Globodera mexicana* could be a potential threat because it can develop on a	High (3)

very common solanaceous weed (*Solanum nigrum*) which is very common in the potato field.	
Environmental Impact All cyst nematodes develop numerous eggs and viable eggs remain soil for long time. If the nematodes get any susceptible host they can build large, damaging population densities if the environment is favourable.	High (3)
Consequences of Introduction of *Meloidogyne incognita* (Root knot Nematode)	Ratings
Climate-Host Interaction They develop on the extensive root systems of both annual and perennial crops, damage the root system severely. Soil moisture is an important factor affecting nematode development and infection (Wallace 1963; Duncan et al. 1998; Hunter 2000). Increasing water stress decreases the water potential from −1 to −10 bars around the root-knot nematode,*Meloidogyne javanica* (Treub 1885; Chitwood 1949), which reduces the percentage of eggs hatching and increases the percentage of second - stage juvenile mortality (Mohwesh & Karajeh 2013). Four major species of root knot nematodes occur in upland rice ecosystems: *Meloidogyne graminicola*, *M*. *incognita*, *M*. *javanica*, and *M*. *arenaria*. *M*. *graminicola* is mainly distributed in South and Southeast Asia (Bridge et al 1990).Different species of *Meloidogyne* (*Meloidogyne javanica*, *M. incognita*, *M. graminicola* etc.) are reported to present widely in Bangladesh (Page et al. 1979; CABI/EPPO 2001; EPPO 2014; CABI/EPPO 2002).	High (3)
Host range Global distribution of nematodes varies greatly based on species, some having a cosmopolitan trend like *Meloidogyne* spp. while others are confined to specific geography especially more from the tropical climate than temperate regions or are highly host targets like *Nacobbus* spp (Nicol, et al., 2011b;Perry,et al, 2009b). Root knot nematodes are widely distributed worldwide than any other genus of plant parasitic nematodes and attributed to biological and favourable environmental conditions (Adegbite, 2011; Pmas, 2013;Sasser, 1977).The parasites are capable of surviving in the tropics, subtropics and temperate climates over a wide geographical range where they parasitize nearly all higher plants species(de Almeida Engler, et al., 2011).Differential presence of root knot nematode has been reported in terms of distribution;*M. incognita* Chitwood,*M.hapla* Chitwood and *M.arenaria* Chitwood have an even presence around the globe while*M.javanica* Chitwood is mainly localized to warmer climatesas described by Chitiwood from *Meloidogyne exigua* Goldi(Tiwari,et al., 2009). Previous reports also indicate that *M. Incognita* and *M. Javanica* occur world wide(Luc,et al., 2005) while in recent developments *Meloidogyne minor* Karssen et al was discovered and reported in Netherlands, Ireland, Portugal, United Kingdom Chile and the United States of America (Wesemael,et al.,2014). However because this species was recently described,information about its geographical distribution isyetto be studied extensively (Prior ,2015). *Meloidogyne enterolobii* a tropical and subtropical species has also been reported in Brazil, Venezuela,China, Cuba, France, Guatemala, Puerto Rico, Martinique, Malawi, Senegal, South	High (3)

Africa, Switzerland, Trinidad and Tobago, United States, West Africa (Ivory Coast and Burkina Faso) and recently in Kenya on parasitizing African nightshade vegetables(Chitambo, et al., 2015;Rito,et al., 2015).	
Dispersal Potential and Pathway The life cycles of *Meloidogyne* spp. are well studied and in their essentials differ little between the major species (De Guiran and Ritter, 1979). At 21°C *M. incognita* took 37 days to complete its life cycle on Antirrhinum majus, a similar time to that reported on soyabeans (temperatures not published) (Ibrahim and El-Saedy, 1987). Juveniles penetrate root tips, occasionally invading roots in the zone of root elongation. Invaded nematodes initiate the development of giant cells in the meristematic, cortical and xylem tissues of the root and galling of roots occurs. Third- and fourth-stage juveniles and young females occur after about 6-8 and 15 days, respectively. Adult females were observed after 20 days and egg laying commenced after 25 days (Ibrahim and El-Saedy, 1987). There are possibilities of dispersal through Clothing/footwear and possessions, Containers and packaging (wood), Land vehicles, Mail/post, Soil, sand, gravel etc. by means of Eggs and galls in soil.	High (3)
Economic Impact *Meloidogyne* spp. causes an estimated annual loss of $157 billion globally (Abad et al., 2008). However, in most cases, the impact of *Meloidogyne* spp. is grossly underestimated. Based on the level of nematode populations, Meloidogy*ne* spp. can cause high levels of crop loss during growth, increase the cost of production through increased fertilizer application and control programmes, and also significantly reduce post-harvest yields. Crop losses of 30% or more in tobacco farms in some parts of Tanzania have been Reported (Whitehead, 1969). In addition, crop losses of 50% in pyrethrum flower yields and a decrease in pyrethrin content in Kenya has also been attributed to *Meloidogyne* spp. infection (IITA, 1981).	High (3)
Environmental Impact *M. incognita* is found worldwide in tropical and subtropical regions, in particular in the warmer areas. For example, in California (USA), M. incognita is found more commonly in the hot valleys of the interior (Ferris and Van Gundy, 1979) and in East Africa it is restricted to altitudes below 2000 m above sea level (Whitehead, 1969). *M. incognita* is found on many soil types. Damage and yield losses caused are generally more severe on coarse-textured sandy soils (Van Gundy, 1985). *Meloidogyne* spp. are generally intolerant of flooded soil conditions. Studies on *Meloidogyne* spp. of different crops like rice, vegetables, pulses are available in Bangladesh and generally this nematode can cause considerable economic loss under Bangladesh condition. (CABI)	High (3)
Consequences of Introduction of *Helicotylenchus dyhistera* – common spiral nematode	Ratings
Climate host interaction	Medium (2)

Helicotylenchus spp. is found in temperate and tropical regions on all continents (except Antarctica), on many islands, and throughout the United States. It is the second most commonly reported species of *Helicotylenchus* worldwide. Statewide in Florida, *Helicotylenchus spp.* is one of the most common plant-parasitic nematode species found in agriculture fields, nurseries, landscapes, pastures, and natural habitats. *Helicotylenchus spp.* is less affected by soil type than many other nematodes and can be found in heavy, sandy, and organic soils (Khan et al. 2006; CABI/EPPO 2010).	
Host range *Helicotylenchus spp.* has a wide host range including fruit crops, vegetables, agronomic crops, ornamental plants, forages, turfgrasses, weeds, and plants in natural habitats. On most of these hosts it is either considered a non pest or the amount of damage cause by *Helicotylenchus spp.* has not been explored. Plants that research has shown to be damaged by *Helicotylenchus spp.* include potato, soybean, cotton, corn, bermudagrass, seashore paspalum, and creeping bentgrass.	Medium (2)
Dispersal potential and pathway The main mode of long and short distance spread is through artificial means: movement of nematode-contaminated soil, run-off and irrigation water, cultivation tools, equipment and any human activity that can move soils from infested to non-infested sites.	Medium (2)
Economic impact While *Helicotylenchus spp.* is a parasite of many economically important plants, it is seldom considered a major pest on most of them. The exception is seashore paspalum, a turfgrass used in tropical and subtropical regions that is particularly susceptible to infestation by *Helicotylenchus spp.* On this grass, *Helicotylenchus spp.* is among the most common nematodes requiring nematicide application. *Helicotylenchus pseudorobustus* also has been associated with unthrifty corn, soybean, and other crops, and with declining bermudagrass, and bentgrass on golf courses. Plant damage caused by high populations of spiral nematodes may be more significant in small-area plant productions and/or containerized crops in nursery, residential and local situations than in large acreages and environments such as, pastures, parks, and cultivated fields. Crop damage under field conditions is difficult to assess as *Helicotylenchus* spp. are often mixed with other genera and/or two or more spiral nematode species occurring together (Norton, 1984). *Helicotylenchus* spp. are considered mild plant pathogens. Crop losses under field conditions are not reported, however, under experimental conditions, reductions in root and total plant weight have been observed in cereals and grasses (Griffin, 1984). There is no economic study about *Helicotylenchus* spp. in Bangladesh. As this pathogen is considered as mild pathogen, therefore heavy economic loss is not expected due to this pathogen.	Low (1)
Environmental impact The impact of *Helicotylenchus spp. o*n natural environments is most likely not significant as the species is already widespread without causing apparent detriment to ecological balances and processes, however, heavy infestations	Low (1)

of spiral nematodes could affect home/urban gardening. The nematodes can cause damage in solanaceous crops in environmental condition like Bangladesh.	

6.1.3 Cumulative Risk Rating for Consequences of Introduction:

The assessment of the consequences of introduction of *Globodera* spp. and other plant parasitic nemtodes have been summarized for each pest by summing the five Risk Elements to produce a Cumulative Risk Rating. This Cumulative Risk Rating is considered to be a biological indicator of the potential of the pest to establish, spread, and cause economic and environmental impacts. The cumulative Risk Rating should be interpreted as follows:

- Low : 5 - 8 points
- Medium : 9 - 12 points
- High : 13 - 15 points

A cumulative Risk Rating is then calculated by summing all risk element values. The values determined for the Consequences of Introduction for each pest are summarized in Table 5.

Table 5: Summary of Consequences of Introduction

Pest	Risk element 1	Risk element 2	Risk element 3	Risk element 4	Risk element 5	Cumulative risk rating
Globodera rostochiensis Order: Tylenchida Family: Heteroderidae	High (3)	Medium (2)	High (3)	High (3)	High (3)	14
Globodera pallida (Stone) Behrens Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	15
Globodera tabacum Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	15
Globodera ellingtonae Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	15
Globodera mexicana Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	15
Meloidogyne incognita Order Tylenchida Family Heterodoridae	High (3)	High (3)	High (3)	High (3)	High (3)	15
Helicotylenchus dihystera Order Tylenchida Family Hoplolaimidae	Medium (2)	Medium (2)	Medium (2)	Low (1)	Low (1)	8

6.1.4 Assessment of Introduction Potential of Quarantine Pests

The potential of introduction is a function of the quantity of the commodity to be imported as well as the opportunity provided for the quarantine pests to survive pre and post-harvest handling. The pest's opportunity is defined by six criteria that consider the potential for the pest survival along the pathway. These include the pest's opportunity to survive pre- and postharvest treatment and shipment, the possibility of avoiding detection at port of entry and the potential to find suitable host.

1. **Sub-element 1- Quantity of commodity imported annually**

   The rating for the quantity imported annually is based on the amount of commodity expected to be imported. For qualitative import risk assessments, the amount of the commodity imported is estimated in units of standard 40-foot long shipping containers. The rating assigned is as follows:

   Table 6: Showing the risk rating and value assigned to quantity of shipping containers imported annually

Quantity (Containers/year	Rating	Rating value
<10	Low	1
10-100	Medium	2
>100	High	3

Approximately 98% of the potato produced in Bangladesh is propagated from imported seed potatoes. In 2014-15, Bangladesh imported potatoes 5211.6 tons of seed potatoes from Netherlands, Belgium and Germany (DAE, 2015) which amounts to 208 forty-foot shipping containers annually, considering 25 tons capacity for each container. The probability of all pest entering as a direct result of the quantity of the commodity being imported is therefore high (3). Therefore, we can estimate that*Globodera* spp. and other nematode pests could be entered into Bangladesh. Thus, the risk is rated high (3) in this sub-element.

Sub-element 2: Survive post harvest treatment

The potato golden cyst nematodes (*Globodera rostochiensis*) are among the most difficult pests to control (Chitwood, 1951). Once established, they are difficult to eradicate because the potato cyst nematodes have one of the highest survival values for any organism, and can survive for over 30 years as eggs protected by the durable cyst wall (Chitwood, 1951; Winslow and Willis, 1972). Moreover, the build-up of nematode populations is slow, and their presence is not easily detected; once the nematode populations increase to high levels, drastic crop losses occur. This is rated High (3) for this risk element. Likewise, *Globodera rostochiensis* and *G. pallida*, other species of *Globodera* i.e. *G. tabacum*, *G. ellingtonae* and *G. mexicana* can survive post- harvest treatment due to cyst formation.

*Meloidogyne incognita*is damaging pest potato industry. Adult, eggs and juveniles can be spread by tuber, roots, growing medium along with plants, seedlings, micro propagated seedlings etc. and cannot be seen with naked eye but visible under light microscope. Although both species are reported in Bangladesh causing damage in various crops and various management methods including cultural, biological and chemical are followed. But, there is still risk of introduction of new virulent races of *Meloidogyne* spp. from the importing countries. Based on above mentioned information, they are rated Medium (2) for this risk element.

Adult, eggs and juveniles of *Helicotylenchus dihystera*can be spread by tuber, roots, growing medium along with plants, seedlings, micropropagated seedlings etc. and cannot be seen with naked eye but visible under light microscope. This nematode can be managed following some common techniques like crop rotation and selecting non host crops. Based on the above mentioned information *Helicotylenchus dihystrea* is rated to be Low (1).

Sub-element 3- Survival potential during shipment

Most seed potatoes are transported from Netherlands to Bangladesh by seaway. Therefore, the period of time taken for shipment through seaway from Netherlands to Chittagong Seaport of Bangladesh is about 25-30 days. Secondly, it is packed in wrapping (wooden boxes) and stored in normal conditions. So the pests could survive during transporting process. Therefore, all pests are rated High (3) for this risk sub-element.

Sub-element 4- Not are detected at the port of entry suitable for survival

Potato golden cyst nematodes (*Globodera rostochiensis*), in common with other cyst nematodes, do not cause specific symptoms of infestation (CABI, 2006). In fact, potato tubers infested with potato cyst nematodes often go unrecognized. To be confident that these symptoms are caused by potato golden cyst nematodes and to give an indication of population density, soil samples must be taken or the females or cysts must be observed directly on the host roots. Therefore, all cyst nematodes are rated High (3) for this risk element.

Meloidogyne incognita can sometimes develop blemishes on potato tuber which can be seen on the surface of potato tuber. Besides, eggs and juveniles can also survive on the roots and debris. They are rated medium (2) for this element.

Helicotylenchus dihystera usually remain undectable with naked eye but visible under light microscope. Eggs and juvelines can remain viable in tuber, roots and other living parts. They are rated medium (2) for this element.

Sub-element 5- Imported or moved subsequently to an area with an environment suitable for survival

The potato golden cyst nematode (*Globodera rostochiensis*) is recorded in the temperate regions of tropical countries, so it could be suitable climatic conditions in North and NorthWestern agro-ecological zones in Bangladesh for pest survival. This pest is rated High (3) for this risk element. On the other hand, other species of cyst nematode of potato can easily survive in Bangladesh condition.

Bangladesh annual imported more than 100 containers per year of potato tubers and they are sold in every regions of Bangladesh. In other words, the climate condition in growing potato crop areas is adapted to this nematode (*Globodera pallida)*. Therefore, these nematodes are estimated to present a High (3) risk of being moved to a habitat suitable for survival.

Globodera tabacum, G. *ellingtonae* and *G. mexicana* are also pest of tomato, potato and solanaceous crops. Therefore, these nematodes are estimated to present a High (3) risk of being moved to a habitat suitable for survival.

*Meloidogyne incognita*and *Helicotylenchus dihystera* are well established in different crops of Bangladesh. Therefore, if any new races of these two nematodes remain in the tuber or other living parts can be easily establish in soils of Bangladesh. These pests rated high (3) for this risk element.

Sub-element 6- Come into contact with host material suitable for reproduction

The most important host of the golden nematode (*Globodera rostochiensis*) is potato. According to the EPPO quarantine pest datasheet (EPPO/CABI, 1978), seed potato is the main means by which the cyst is introduced into new areas. With Bangladesh's reliance on imported seed potato and its wide distribution into the potato growing areas of the country, it is expected that the nematode cyst once introduced will find suitable host. The risk rating is therefore high (3).

Potato is a suitable host of *Globodera pallida*. Besides that, many other host plants among families Sonalaceae, Fabaceae, Brassicaceae, Asteraceae etc. (such as tomato, aubergine, onion, garlic, and some weed host like *Solanum nigrum*, *Physalis heterophylla* and so on) are also grown popularly in Bangladesh and very common weeds in the potato field. Therefore, they are considered to have a High (3) risk of coming into contact with host material via this pathway. *Globodera tabacum* can develop on tobacco and other solanaceous crops which are very common in Bangladesh. Similarly, G. *ellingtonae* and *G. mexicana* can easily reproduce in Bangladesh if they are exposed.

Meloidogyne incognita, *M. javanica* and *Helicotylenchus dihystera* are well established in Bangladesh. Potato is the good host for *Meloidogyne* spp. besides they have a number of good hosts including tomato, brinjal and other solanaceous and non-solanaceous crops in Bangladesh. Therefore, they are considered to have a High (3) risk of coming into contact with host material via this pathway.

Summary of Cumulative Risk Rating for Potential of Introduction

The assessment of the Potential of Introduction of Quarantine Pests has been summarized for each pest by summing the six Sub-elements to produce a Cumulative Risk Rating for Potential of Introduction. The cumulative Risk Rating should be interpreted as follows:

- Low : 6 - 9 points
- Medium : 10 - 14 points
- High : 15 - 18 points

A cumulative Risk Rating for Potential of Introduction is then calculated by summing all risk Sub-element values. The values determined for the Potential of Introduction for each pest are summarized in Table 7.

Table 7: Risk Rating for Potential of Introduction of Quarantine Pests

Pest	Sub-element 1	Sub-element 2	Sub-element 3	Sub-element 4	Sub-element 5	Sub-element 6	Cumulative risk rating
Globodera rostochiensis (Wollenweber) Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	High (3)	18
Globodera pallida (Stone) Behrens Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	High (3)	18
Globodera tabacum Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	High (3)	18
Globodera ellingtonae Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	High (3)	18
Globodera mexicana Order: Tylenchida Family: Heteroderidae	High (3)	High (3)	High (3)	High (3)	High (3)	High (3)	18
Meloidogyne incognita Order Tylenchida Family Heterodoridae	High (3)	Medium (2)	High (3)	Medium (2)	High (3)	High (3)	16
Helicotylenchus dihystera Order Tylenchida Family Hoplolaimidae	High (3)	Low (1)	High (3)	Medium (2)	High (3)	High (3)	15

6.2. Conclusion: Pest Risk Potential and Pests Requiring Phytosanitary Measures

The Pest Risk Assessment (PRA) is based on the International Standard for Phytosanitary Measures No 11 (2004) and the PRA scheme developed by European and Mediterranean Organization (1997). To estimate the pest risk potential for each pest, the cumulative risk rating for the Consequences of Introduction and Potential of Introduction is summed. The risk potential ratings are assigned as follows:

Low : 11-18 points

Medium : 19-26 points

High : 27-33 points

Table 8: The Overall Pest Risk Potential Rating

Pest	Consequences of Introduction	Potential of Introduction	Pest Risk Potential
Globodera rostochiensis (Wollenweber) Order: Tylenchida Family: Heteroderidae	High (14)	High (18)	High (32)
Globodera pallida (Stone) Behrens Order: Tylenchida Family: Heteroderidae	High (15)	High (18)	High (33)
Globodera tabacum Order: Tylenchida Family: Heteroderidae	High (15)	High (18)	High (33)
Globodera ellingtonae Order: Tylenchida Family: Heteroderidae	High (15)	High (18)	High (33)
Globodera mexicana Order: Tylenchida Family: Heteroderidae	High (15)	High (18)	High (33)
Meloidogyne incognita Order Tylenchida Family Heterodoridae	High (15)	High (16)	High (31)
Helicotylenchus dihystera Order Tylenchida Family Hoplolaimidae	Low (8)	High (15)	Medium (23)

Potential ratings:

- Low: Pest will typically not require specific mitigations measures;

- Medium: Specific phytosanitary measure may be necessary.

- High: Specific phytosanitary measures are strongly recommended.

Port-of-entry inspection is not considered sufficient to provide phytosanitary security. Identification and selection of appropriate sanitary and phytosanitary measures to mitigate risk for pests with particular Pest Risk Potential ratings is undertaken as part of the risk management phase.

Chapter 7

Pest Risk Management

The risk management/mitigation options have been proposed for the nematode pests of potato either identified in this study or considered as having high risk potential that includes the available options for managing the risk. The measures are recommended which can be refined by the Chief Plant Quarantine Officer, DAE for taking decision once the measures are considered to be appropriate.

Management Options	**Category**	
Phytosanitary Measures	Pest free areas	As a sole mitigation measure, the establishment of pest-free areas or pest-free places of production may be completely effective in satisfying an importing country's appropriate level of phytosanitary protection (IPPC, 1996b, 1999).Establishment and maintenance of pest-free areas or production sites should be in compliance with international standards (e.g., IPPC, 1996b, 1999, 2006). Potatoes grown in an area that has not been determined to be free of high risk pests would be required to be grown in approved production sites registered with the National Plant Protection Organization (NPPO) of the Netherlands, Belgium, Germany, Denmark and USA. Initial approval of the production sites would be completed jointly by the NPPO of exporting countries and Plant Quarantine Wing (PQW) under the Department of Agriculture Extension (DAE) of Bangladesh. Thus, Bangladesh must require from importing countries that potatoes be produced in a pest free area. This will ensure that the specific pests of concern are removed from the pathway. This measure is highly effective where it is feasible to implement.
	Pre export inspection and treatment	The NPPOs of exporting countries will inspect all consignments in accordance with official procedures in order to confirm those consignments are satisfied with import requirements on phytosanitary of Bangladesh. The consignment may be treated by pure (100%) methyl bromide (CH3Br) at 48g/m3/2 hours. Beside, at 21-25°C temperature, these insects can be treated by methyl bromide fumigation at 15-18g/m3 in 5 – 6 hours (EPPO, 1998, www.eppo.org/Meetings/2006_meetings/treatments.htm). In *Globodera rostochinensis*, *Globodera pallida* are found the consignment should not be exported to Bangladesh.
	phytosanitary certification from country of origin	The phytopathological service of the country of origin should ensure the potato from which the consignment is derived was not grown in the vicinity of unhealthy potato crops and was inspected by a duly authorized official/phytopathological service and the seed potatoes have been produced in areas within the country free from all pests and diseases.

	Port-of-entry inspection and treatment	Upon arrival in the Bangladesh, each consignment of potato should be inspected to detect pests, with export phytosanitary certificate and seed certificate. Sampling of potato consignments at port-of-entry in Bangladesh should combine visual inspection and laboratory testing. Visual inspection is useful to verify that certain phytosanitary certification requirements have been met and consignment is generally free of contaminants. The efficacy of this measure depends on the statistical level of sampling and the ability to detect the pests or article of concern (e.g. soil). Laboratory testing requires that a portion of each sample taken for inspection be subjected to laboratory analysis for the detection of pathogens. The consignment could re-export or destroy if quarantine pests or regulated articles with high risk potential are found during an inspection.
Pre-harvest management	Strengthening inspection at port of entry	When the risk rating is high for any invading pest, then inspection at the point of entry is not enough. Further actions should be taken. Modern technologies like molecular-based/PCR based techniques should be available at all ports of entry of the country.
	Strengthening seed certification system	Inclusion of PCN testing in the Seed Certification System. Farmers can be advised to use only the PCN certified seeds.
	General surveillance of seed tuber	The seed potato importers usually multiply seeds and then they sell it next year. The field of seed potato growers should be considered under regular surveillance. The importers can be imposed to test their seed tubers and soil in a regular basis. Field data can be preserved digitally so that the invasion of any quarantine pest can be located easily.
	Crop rotation	Certain potato pest especially *Globodera rostochiensis* and *G. pallida* can survive longer time in soil. On the other hand PCN and other plant parasitic nematodes can survive in alternate crop host; therefore, for PCN a six year crop rotation may be useful. Depending on the type of pathogen, it may survive in the resting form either in the soil or in potato plant debris, or in a living form in surviving potato tubers. On occasion, diseased tubers survive the winter and grow the following spring as diseased volunteer plants. These volunteer potatoes are a source of contamination for the current season crops. (Western Potato Council, 2003).
	Chemical spray	Pre-harvest chemical sprays may be used to control pests within production fields, for example, the use of nematicides to control the golden nematode.

	Seed handling	Before handling seed tubers, all containers, tools, knives and mechanical cutters, planters, and other equipment should be thoroughly washed with a detergent solution, rinsed, and then sanitized with a disinfectant. When cutting seed tubers, the cutting tool should be periodically washed and sanitized. It is essential that this should be done before cutting seed tubers from a different source. To be effective, disinfectants must be present for a minimum of 10 minutes (preferably 20–30 minutes) on any surface being treated (Rowe *et al*.).
	Pre-harvest inspection	The relevant officers and inspectors from the importing country should inspect and verify the cleaning and disinfecting of equipment and storage used in potato production. Laboratory testing should be done periodically. Quarantine restrictions may be used to limit spread of diseases detected.
	Soil solarization	Soil solarisation is an effective control method for nematodes in hot climates like Bangladesh. The soil can be covered with layers of polyethylene sheeting and the soil beneath is heated by the sun to temperatures of 60°C or more. The high temperatures effectively kill nematodes and their eggs. Solarisation is most effective for killing nematodes in the top 10 cm of soil.
	Bio-fumigation	Field trials evaluated the effect of four plant-based bio-fumigants/stimulants on population levels of *G. pallida* and the resulting potato yields and quality. Three formulations contained seaweed bio-stimulants (Algifol, Nutridip and Metastim) and one bio-fumigant containing mustard and chilli pepper extracts (Dazitol). These were compared with the fumigant nematicide Nemathorin and untreated control plots. The effect of *G. pallida* on growing potato crops was assessed by recording haulm characteristics which indicated that the nematicide treatment gave most protection. Levels of PCN juveniles and migratory nematodes were assessed during the trial. Plots treated with Nemathorin and Dazitol had fewest PCN, whilst the highest number of migratory nematodes occurred in fallow plots. Sixteen weeks after planting the nematicide treatment produced highest yield and tuber numbers. Dazitol treatment produced a lower yield but the largest tubers (Martin et al. 2007).
Post-harvest management	Hygiene and waste disposal	All infected tubers and debris should be discarded from the filed or they can be burnt that will reduce the pest infestation in the coming years.

	Sanitization of equipment and material	All machinery, transport and storage surfaces that the seed will contact should be cleaned and disinfected prior to receiving new potato. Sanitation consists of cleaning and disinfecting all equipment, storage, tools and pallet boxes that contact the seed and ware potatoes. Since most disinfectants are inactivated by soil and plant debris, it is essential that this material be removed by thoroughly cleaning the equipment and storage with a pressure washer or steam cleaner before the disinfectant is applied (Western Potato Council, 2003).

Risk Management Conclusions

It is imperative to take mitigation measures for the nematodes. Due to diverse natural conditions, a single measure will not be adequate to reduce the risk to acceptable levels. Rather, combinations of measures or integrated management are being suggested as a feasible approach.

Chapter 8

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