PATHOGEN RISK ANALYSIS OF MAIZE IN BANGLADESH

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DHAKA-1207

JUNE, 2013

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A Thesis
Submitted to the Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

SEMESTER: JAN-JUN, 2011

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CERTIFICATE

This is to certify that the thesis entitled, ***"Pathogen Risk Analysis of Maize in Bangladesh"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE*** *in* ***PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by* ***Muhammad Morshed Alam, Registration No. 06-02050,*** *under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.*

I further certify that any help or sources of information has been availed of during the course of this investigation has duly been acknowledged.

Dated:
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ACKNOWLEDGEMENT

All praises to Almighty and kind-full "Allah Rabbul Al-Amin" who enabled me to pursue my higher study and to complete the research work as well as to submit the thesis for the degree of Master of Science (M.S.) in Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

The author avails the opportunity of conveying his heartfelt respect, deepest sense of gratitude, immense indebtedness and profound honor to his most reverend teacher and research supervisor, Dr. M. Salahuddin M. Chowdhury, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for his scholastic guidance, valuable suggestions, constructive criticisms, continuous inspiration and constant encouragement during the entire period of the research work and in the preparation of the manuscript.

The author wishes to express his sincere appreciation and extreme gratitude to his co-supervisor, Abu NomanFaruqAhmmed, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for his precious advices, instructions, inspirations and cordial help to complete the research work.

Cordial thanks and honors to Dr. F. M. Aminuzzaman, Associate Professor, Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for his inspiration and co-operation throughout the period of the research work.

The author is especially thankful to "Eusuf and Associates" and Department of Agricultural Extension (DAE) for conducting research under the project "Pest Risk Analysis (PRA) of maize and listing of Quarantine Pests".

The author is pleased to extend thanks to his respected teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh for their inspiration and co-operation throughout the period of the research work.

The author remains indebted to the officers and the staffs of the Plant Pathology Department, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh for their continuous assistance during the research period.

The author is thankful to his friends especially Atiq, Mamun, Prince, Roky, Noman,Keya, Mitu, Roni, Susmita, Rabeya, Bobita,Shakila,Samia and Bithi for their co-operation and assistance during the course of the research work.

Finally, the author expresses his deepest gratitude to his beloved parents and siblings for their patients, blessing inspiration, sacrifice and dedicated efforts in completion of his higher education.

June, 2013 *The Author*

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ABSTRACT

A field survey was conducted in 20 major maize growing districts of Bangladesh for pathogen risk analysis of maize and listing of quarantine diseases in 2012 by the Department of Plant Pathology under Sher-e-Bangla Agricultural University through a project of QSSP, DAE. Pre-designed questionnaire was used in recording data of maize diseases based on the opinion of farmers and policy level officers of DAE under studied areas. Focus group discussion was arranged in each district and opinion of the respondents about maize diseases were recorded. Total 100 seed samples were collected throughout the country. Significant seed borne pathogens viz. *Aspergillus flavus, A. niger*, *Penicillium spp., Rhizopus spp.* and *Fusarium moniliforme* and *Xanthomonas sp*. have been detected through seed health testing. Altogether 30 maize diseases of which 23 fungal, 2 bacterial, 3 viral and 2 nemic diseases have been identified as maize diseases of Bangladesh. Maize diseases of exporting countries were documented and a list of pathogens prepared to conduct pathogen risk analysis of Bangladesh. Pathogen risk analysis was done following the guideline of ISPM-2, ISPM-11 and ISPM-21 constituted by FAO. A total sixteen pathogens were identified on maize grain from the maize exporting countries which have quarantine risk to Bangladesh agriculture and ten pathogens viz. *Peronosclerospora sorghi*, *Cercospora zeae-maydis,* Maize dwarf mosaic potyvirus, High Plains virus, Wheat streak mosaic rymovirus, *Sclerospora graminicola*, *Phymatotrichopsis omnivore*, Maize chlorotic mottle machlomovirus, *Pantoea stewartii* subsp. Stewartii, *Clavibacter michiganensis* subsp. nebraskensis were found as highly quarantine concern for maize farming in Bangladesh.

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CHAPTER I

INTRODUCTION

Bangladesh is a small developing country with an area of 147570 square kilometer inhabited by 156.6 million people. It is located in the North Eastern part of South Asia between $20^{o}34^{\prime}$ and $26^{o}38^{\prime}$ North latitude and $88^{o}01^{\prime}$ and $92^{o}41^{\prime}$ East. The country is bordered by India on the West, the North, and the Northeast, Burma on the Southeast and the Bay of Bengal on the South. It is primarily an agricultural country dominated by crop production. Contribution of crop sector to Gross Domestic Product (GDP) is 13.44 percent (DAE, 2013). Maize (*Zea mays*) is grown from 58^{0}N to 40^{0}S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rain fall per year (Anon. 2009) and with a growing cycle ranging from 3 to 13 months. In terms of area of production it is the third most important cereal crop in Bangladesh (Moniruzzaman *et al.*, 2009). The condition of maize has been gaining popularity in recent years in Bangladesh. It is now becoming an important cereal crop for its high productivity and diversity (Alom *et al.,* 2010). In Bangladesh maize has been cultivated in 2.02 lakh hectares with a production of 13.17 lakh metric tons in 2010 (BBS, 2011). Bangladesh grows moderate quantities of maize, which do not meet domestic demand. To meet the local demand Bangladesh has to import a good quantity of maize from exporting countries like India, Australia, China, USA and so on. Under the increasing trend of cultivation, the demand for hybrid seed is increasing rapidly .Bangladesh imported huge quantities of hybrid maize seed since 2000-01fiscal year. In 2010-11, fiscal year Bangladesh imported 2071.970 metric ton maize seed while it was 167.920 metric ton in 2000-01 fiscal year (DAE).

A number of pathogens are likely to be associated with maize seed (Bari and Alam, 2004). Six different diseases-leaf blight, yellow spot, stalk rot, stem rot, cob sheath rot/cob blight and mosaic were also reported to occur on three varieties of maize-Barnali, Mohor and Shuvra (Siddique, 1996). Fakir (2001) listed 11 seed-borne diseases on the crop. New pathogen may introduce in the country through imported maize seed and could pose a great threat to domestic agriculture. Listing of quarantine pathogens are also a vital factor to make a good trade of maize worldwide. Besides Bangladesh is surrounded by India and Burma from three sides – west, north and east leaving Bay of Bengal on the south. The pathways of the pathogen may be both inter and intra country and threats of massive spread of quarantine pathogens through illegal carrying, boarder belt informal trade and illegal black marketing and formal imports of seeds, plant materials, herbs, flood and rain waters, wind and natural disaster etc. As such, there are potential risks of the presence and entry of harmful quarantine maize diseases in our country.

World Trade Organization (WTO) has proposed a set of rules and obligations in trading agricultural commodities. As a member state, Bangladesh must have to follow those rules and regulations. To satisfy the prerequisite of WTO for maize trade, it is necessary to conduct pathogen risk analysis of maize in Bangladesh.

The purpose of this study is to:

(i) Survey analysis for the idea about existing maize diseases in the country;

(ii) Study of the pathogens associated with maize seeds and

(iii) Prepare a list of quarantine pathogens of maize.

CHAPTER II

REVIEW OF LITERATURE

The main purpose of this chapter is to review the previous studies, which are related to the present study. Little work has been done on the prevalence and pathogenic potential of fungi associated with maize seeds in Bangladesh. Therefore, on attempt has been made here to compile the research carried out on the subject elsewhere.

Cao *et al.* (2013) conducted an experiment in Southern Europe where whole maize kernels are ground and used for making bread and other food products, infection of the kernels by *Fusarium verticillioides* and subsequent fumonisin contamination pose a serious safety issue. The influence of environmental factors on this fungal infection and mycotoxin accumulation as the kernel develops has not been fully determined, especially in such food grade maize. Three maize hybrids were planted at two different sowing dates and kernel samples were collected 20, 40, 60, 80 and 100 days after silking. The percentage of kernels infected, and ergosterol and fumonisin contents were recorded for each sampling. *F.verticillioides* was the most prevalent species identified as the kernels developed. Temperature and moisture conditions during the first 80 days after silking favored natural kernel infection by *F. verticillioides* rather than by *Aspergillus* or *Penicillium* species.

Reddy *et al.* (2013) conducted an intensive roving survey in major maize growing areas of Andhra Pradesh of India during the *kharif* 2011-2012 for turcicum leaf blight identification. Northern corn leaf blight or turcicum leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs is one of the important diseases affecting photosynthesis with severe reduction in grain yield to an extent of 28 to 91%. Diseases symptoms first appear on the leaves at any stage of plant growth, but usually at or after anthesis. The studies on survey reveals that high intensity of the disease was noted in the district where mean maximum temperature was below 32^0C and relative humidity was above 85 percent during the cropping season.

Liu, K. J. and Xu. D. (2013) conducted an experiment on Gray leaf spot of maize (*Zea mays* L.) in China. The causal organism of gray leaf spot in China is generally regarded as *Cercospora zeae-maydis*. In October 2011, symptoms similar to gray leaf spot were observed on 77% of maize plants in 25 locations (about 3,000 ha.) of Yunnan Province, China, and the disease could cause yield losses of 35 to 50%. The symptoms of leaf spot were different from those caused by *C. zeae-maydis*. The lesions on leaves were oblong, pale gray to pale brown, 2 to 3 × 5 to 40 mm, and confined by leaf veins that eventually coalesced. To identify the pathogen, 75 leaf samples were collected from 25 fields (three leaf samples for each field) at the kernel maturity stage. Single, well-separated lesions were excised and surface-sterilized by placing them in 75% ethanol for 5 s, then disinfested in 2% sodium hypochlorite for 5 min and rinsed with sterilized water. The lesions were incubated on water agar (WA) at 24°C for 48 to 72 h to allow sporulation. Seventy-five single-conidial isolates were obtained and cultured as described in Crous. Morphology of the isolates was determined on plates containing maize leaf powder agar (MLPA). After 5 days, isolates produced pale brown mycelia that consisted of 3- to 4-μm-wide, septate, branched hyphae. Conidiophores were 5 to 7 × 55 to 100 μm, straight to slightly flexuous, and usually 1- to 5-septate. Conidia were average 7.5 × 68 μm, fusiform, apex sub-obtuse, base sub-truncate, and 3- to 6-septate. These characteristics are similar to *C. zeina*. The internal transcribed spacer (ITS) region of rDNA was amplified from each of the 75 isolates using primers ITS1/ITS4 and sequenced. The same sequences were obtained and the sequence of isolate YNGLS was submitted to Gene Bank (Accession No. KC878692). BLAST analysis of the sequence showed 100% confirmation to *C. zeina* (DQ185081). Additionally, a PCR-based diagnostic test using species-specific primers confirmed the identification of the 75 isolates as *C. zeina*. The pathogenicity of the isolates was tested on greenhouse grown maize variety Huidan 4. The test was performed on 40 plants and replicated three times.

The plants were inoculated at the 10 leaf stage by injecting 2 ml of conidial suspensions (2,500 conidia ml^{-1}) into leaf whorl using a hypodermic syringe, and control plants were injected with sterile water. Conidia were collected from 5-day-old cultures grown on MLPA and suspended in sterile water. Forty days after inoculation, all inoculated plants showed characteristic lesions on leaves, but control plants remained asymptomatic. *C. zeina* was re-isolated from the lesions, and the identity of the re-isolates was confirmed by the morphological and molecular characteristics as stated above.

Wang *et al.* (2013) conducted surveys to determine population structure of *Fusarium* species on maize were conducted in 22 provinces in China during September 2009 to October 2012, where the disease incidence ranged from 5 to 20% in the individual fields. Maize ears with clear symptoms of Fusarium ear rot (with a white to pink- or salmon-colored mold at the ear tip) were collected from fields. Symptomatic kernels were surface-sterilized (1 min in 0.1% $HgCl_2$, and 30 s in 70% ethanol, followed by three rinses with sterile distilled water), dried and placed on PDA. After incubation for 3 to 5 days at 28°C in the dark, fungal colonies displaying morphological characteristics of *Fusarium* spp. were purified by transferring single-spores and identified to species level by morphological characteristics, and DNA sequence analysis of translation elongation factor-1α (TEF) and β-tubulin genes. A large number of *Fusarium* species (mainly *F. graminearum* species complex, *F. verticillioides* and *F. proliferatum*) were identified. These *Fusarium* species are the main causal agents of maize ear rot. Morphological characteristics of six strains from Anhui, Hubei and Yunnan provinces were found to be identical to those of *Fusarium kyushuense*, which was mixed with other *Fusarium* species in the natural infection in the field. Colonies grew fast on PDA with reddish-white and floccose mycelia. The average growth rate was 7 to 9 mm per day at 25°C in the dark. Reverse pigmentation was deep red. Microconidia were obovate, ellipsoidal to clavate, measuring 5.4 to 13.6 (average 8.8) µm in length. Macroconidia were straight or slightly curved, 3- to 5-septate, with a curved and acute apical cell, 26.0 to 50.3 (average 38.7) µm in length. No chlamydospores were observed. Identity of the fungus was further investigated by sequence comparison of the partial TEF gene (primers EF1/2) and β-tubulin gene (primers T1/22) of one isolate. BLASTn analysis of the TEF amplicon (KC964133) and β-tubulin gene (KC964152) obtained with cognate sequences available in GenBank database revealed 99.3 and 99.8% sequence identity, respectively, to *F. kyushuense*. Pathogenicity tests were conducted twice by injecting 2 ml of a prepared spore suspension (5×10^5 spores/ml) into maize ears (10 per isolate of cv. Zhengdan958) through silk channel 4 days post-silk emergence under field conditions in Wuhan, China. Control plants were inoculated with sterile distilled water. The ears were harvested and evaluated 30 days post-inoculation. Reddish-white mold was observed on inoculated ears and the infected kernels were brown. No symptoms were observed on water controls. Koch's postulates were fulfilled by reisolating the pathogen from infected kernels. *F. kyushuense*, first described on wheat in Japan, has also been isolated from rice seeds in China. It was reported to produce both Type A and Type B trichothecene mycotoxins, which cause toxicosis in animals. This is the first report of *F. kyushuense* causing maize ear rot in China and this disease could represent a serious risk of yield losses and mycotoxin contamination in maize and other crops.

Orio *et al.* (2012) reported that Stewart's wilt is a serious disease of corn (*Zea mays* L.) caused by the bacterium *Pantoea stewartii* subsp. *stewartii* (*Pss*). Typical symptoms of infected fields and dent corn are longitudinal streaks with irregular or wavy margins, which are parallel to the veins and may extend the length of the leaf. These pale to green yellow lesions become dry and brown as the disease progresses producing a leaf blight. During the growing seasons 2010 to 2011 and 2011 to 2012, symptoms of bacterial leaf blight of corn were observed in central Argentina maize fields, with an incidence of 54% in Córdoba province. To identify the pathogen, leaves from 10 symptomatic maize plants per field were collected from 15 fields covering a representative geographical area. High populations of morphologically uniform bacteria were isolated from leaf tissues by conventional methods using King's medium B agar. Ten representative facultatively anaerobic gram-negative, non-fluorescing, non-motile, catalase positive and oxidase negative rod-shaped and yellow-pigmented bacterial isolates were evaluated further. The biochemical profile obtained was: fermentative metabolism, negative indol, acetoin and hydrogen sulfide production, negative gelatin hydrolysis (22°C), positive acid production from D-glucose and lactose, negative gas production from D-glucose, and negative nitrate reduction. All the isolates produced a 300-bp band with PCR using the species specific primer pair PST3581/PST3909c. The *Pss* ATCC 8199 and *Pseudomonas fluorescens* ATCC 13525 strains were used as positive and negative controls for the PCR assays, respectively. The pathogenicity test was performed by stem inoculation of five to ten P2069 YR maize plants (one to two leaf growth stage) grown in growth chamber. Plants were inoculated by syringe with a 10^7 to 10^8 cell/ml bacterial suspension and kept in a humid chamber at 25 to 27°C. Plants inoculated with *Pss* ATCC 8199 or with sterile water were used as positive and negative control treatments, respectively. The development of symptoms similar to those originally found in the field was observed on all the plants inoculated with the different isolates at 7 to 10 days post inoculation. In addition, symptoms on inoculated plants were similar to those observed for the positive control treatment. No symptoms were found on negative controls. Koch's postulates were fulfilled since bacteria isolated from symptomatic tissue had identical characteristics to isolates used to inoculate plants and to the reference *Pss* strain for biochemical tests and PCR reaction mentioned above. This is the first report of *P. stewartii* subsp. stewartii isolated from diseased maize in Argentina.

Alippi and Lopez (2010) observed an uncharacterized disease of maize (*Zea mays* L.) in commercial fields of Laguna Blanca, Formosa, Argentina and from different fields of Santa Fe and Catamarca provinces of Argentina between 2007 to 2008. Symptoms included light-colored necrotic streaks on leaves and tan or white irregular blotches that sometimes were surrounded by reddish purple-to-dark brown margins. Severity of symptoms varied greatly from one field to another. Abundant bacterial streaming was observed from lesions when examined at ×150. Gram-negative, facultatively anaerobic bacteria were consistently isolated from lesions. These formed light yellow-to-orange, glistening, convex colonies on yeast dextrose calcium carbonate agar incubated at 30°C. Ten isolates from ten different symptomatic plants were selected for further study. All isolates were motile, induced a hypersensitive response in tobacco plants, and were oxidase negative. Colonies developed at 37°C. Physiological and biochemical characterization with the API 20E test strips and database (bioMerieux, Buenos Aires, Argentina) showed that the strains belonged to the genus *Pantoea*. All strains were positive for β-galactosidase, utilized citrate and tartrate, and produced acid from D-glucose, D-mannitol, D-melibiose, L-arabinose, sucrose, meso-inositol, glycerol, D-sorbitol, and amygdalin. All were negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophane deaminase, H2S production, urease, and reduction of nitrate to nitrite. Variable results were obtained for indole, gelatinase, and L-rhamnose. Their identity was confirmed by sequencing the 16S rRNA gene strain F327 (Gene Bank Accession No. GU068363). A Blast N search of Gene Bank revealed 99% nt identity with strains LMG 20103 (AF364847.1), LMG 20105 (AF364845.1), and LMG 2665 (FJ611815.1) of *Pantoea ananatis*. Pathogenicity was verified on *Z. mays* (EM 6079 HX, Dow Morgan) by injection-infiltration of bacterial suspensions at 10^5 CFU/ml. Controls were infiltrated with sterile distilled water. Plants were kept at 26 ± 3°C in a greenhouse. Symptoms were first detected 15 to 17 days after inoculation and then lesions expanded to resemble natural infections within 30 days. Bacteria were reisolated and the original and reisolated strains were compared by using repetitive sequence-based (rep)-PCR with ERIC primers (1) and fingerprints of the reisolated strains were identical to those of the original strains, thereby fulfilling Koch's postulates. No lesions were observed on controls. Known strains of *P. stewartii* from the United States (SW2, DC400, DC441, and DC283) were also tested for comparison. On the basis of sequencing data, pathogenicity, and physiological tests, the pathogen was identified as *P. ananatis*.

Mukanga *et al.* (2010) conducted a survey to determine the prevalence of the ear rot problem and levels of mycotoxins in maize grain. A total of 114 farmsteads were randomly sampled from 11 districts in Lusaka and southern provinces in Zambia during 2006. Ten randomly picked cobs were examined per farmstead and the ear rot disease incidence and severity were estimated on site. This was followed by the standard seed health testing procedures for fungal isolation in the laboratory. Results indicated that the dominant ear rots were caused by *Fusarium* and *Stenocarpella*. Incidence of *Fusarium verticillioides* ranged from 2 to 21%, whereas that of *Stenocarpella maydis* reached 37% on ear rot diseased maize grain. In addition, 2–7% *F. verticillioides*, and 3–18% *Aspergillus flavus*, respectively, were recovered from seemingly healthy maize grain. The mean rank of fungal species, from highest to lowest, was *F. verticillioides, S. maydis, A. flavus, Fusarium graminearum, Aspergillus niger, Penicillium* spp., *Botrydiplodia* spp., and *Cladosporium* spp.

Niaz and Dawar (2009) conducted an experiment on different species of maize seed to detect seed borne mycoflora. Seed borne mycoflora of maize was tested by using blotter, agar plate and deep freezing methods as recommended by ISTA. Of the 100 samples collected from different places of Pakistan, a total number of 56 species belonging to 23 genera of fungi were isolated and identified. About 70% of the samples were infested with *Aspergillus flavus, A. niger, A. wentii* and *Penicillium* spp. Of the three methods used, agar plate method yielded the highest number of fungi as compared to blotter and deep freezing methods. Deep freezing method was the best for the detection of *Drechslera* spp., *Fusarium* spp., and *Penicillium* spp., while agar plate method was suitable for the detection of *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., *and Rhizopus* spp. Out of 56 species, 22 species viz., *Arthrinium phaeospermum*, *Aspergillus foetidus*, *A. tubingensis*, *Curvularia clavata*, *C. intermedia*, *C. pallescens*, *Bipolaris maydis*, *Drechslera carbonum*, *Diplodia zea*, *Fusarium crockwellense*, *F. cladosporium*, *F. culmorum*, *F. graminearum*, *F. nivale*, *F. proliferatum*, *Penicillium citrinum*, *P. funiculosum*, *Phoma herbarum*, *Rhizopus oligosporum*, *Rhizoctonia solani*, *Syncephalastrum racemosum* and *Trichoderma harzianum* are new reports from Pakistan on maize seeds. However, the same fungal species have been reported on maize seed from various countries of the world such as USA, Thailand, India, Canada, Australia, France, Nepal, United Kindgdom, Western Romania and Hungary.

Zitter (2009) Sweet corn is widely grown in New York State from large, commercial fresh market acreages, to extensive processing vegetable acreages, to the ever-popular backyard garden plots. The diseases affecting sweet corn in New York State are numerous, and are caused by three major groups of plant pathogens — bacteria, fungi, and viruses. Thirteen diseases were discussed in this report. All the diseases listed are not found every year because their occurrence is influenced by environmental factors (temperature, humidity, soil moisture), previous cropping histories, crop location within the state, and availability of insect vectors. In addition, most corn seed sold is treated with a fungicide/insecticide mixture to reduce the risk of seed rot and seedling blights. Most of the diseases discussed occur sometime after plant establishment.

Gonzalez (2008) conducted a laboratory experiment in Asturias to determine the efficacy of 75% chlorothalonil, 25% azoxystrobin, 50% carbendazim, 12.5% epoziconazole, 0.5% flusilaole + 1% carbendazim, 9.4% flutriafol + 20% carbendazim and 16% cyproconazole + 30% carbendazim against *Exserobilum tucicum* (*Setosphaeria turcica*) casual organism of Northern corn leaf blight on corn. Flusilazole + carbendazim was the most effective followed by epoiconazole m flutriafol + carbendazim and cyproconazole + carbendazim.

Herrera *et al.* (2008) reported that *P. stewartii* colonizes the xylem of maize as sessile, cell-wall-adherent biofilms. Biofilm formation is a developmental process that generally involves some form of surface motility. For that reason, we reexamined the motility properties of *P. stewartii* DC283 based on the assumption that the organism requires some form of surface motility for biofilm development. Here, we show that the organism is highly motile on agar surfaces. This motility is flagella dependent, shown by the fact that a *fliC* mutant, impaired in flagellin subunit synthesis, is nonmotile. Motility also requires the production of stewartan exopolysaccharide. Moreover, surface motility plays a significant role in the colonization of the plant host.

Harlapur *et. al* (2008) conduct a survey in Karnataka and found Turcicum leaf blight (TLB) of maize caused by *Exserohilum turcicum* was major production constraint of maize crop. The symptoms were observed at different stages of growth. Elongated spindle shaped necrotic deep grey lesions on leaves and straw coloured centre with dark margins giving plant scorched appearance and leading to premature killing of plants with small sized, curved, partially filled malformed chaffy cobs with shriveled grains were observed. Survey indicated that, the disease was noticed in all the maize growing areas of the state in a low to severe form. The highest disease incidence (55.89%) was observed in Belgaum district and the lowest disease incidence (27.64%) in koppal district.

Parsons (2008) reported that Fusarium ear rot, caused by *Fusarium verticillioides*, is one of the most common worldwide diseases of maize, causing yield and quality reductions as well as contamination of grain by fumonisins and other mycotoxins. Drought stress and various insects have been implicated as factors affecting disease severity. Three separate field studies were conducted to evaluate the relative influence of drought stress at different stages of crop development, ear infestation by two species of insects, upon severity of Fusarium ear rot disease and fumonisin B_1 contamination of commercial maize hybrids.

Partridge (2008) reported that Ear rot, stalk rot, root rot and kernel rot disease caused by the fungus *Fusarium moniliforme*. This species and other *Fusarium* species also causes ear, kernel and root rot and seedling blight. Corn and sorghum are the most economically important hosts of *Fusarium moniliforme*. It is important to note that the fungus has a very broad host range influencing crop production in many areas of the world. Stalk rot is generally thought of as a problem of senescing plants. A higher incidence of stalk rot is common when conditions that tend to encourage early senescence occur. As with many stalk rots, lodging is another common symptom.

Goszczynska *et al.* (2007) observed an unreported disease of maize (*Zea mays*) on commercial fields in the Northwest and Mpumalanga Provinces of South Africa. Infected plants were stunted, with a vertical crack at the first internode. Inside the stem, a dark-brown, narrow lesion was present along the crack. Internal browning inside the stem extended upward, reaching the top internode in some plants. Seed cobs were underdeveloped. Diseased plants were scattered in the fields and 10 to 70% of the crop was affected. Gram-negative, facultatively anaerobic bacteria were consistently isolated from diseased tissues. Pathogenicity tests established that representative strains induced disease symptoms similar to those observed on maize plants in the field. Physiological and biochemical characterization using the API 20E and API 50CHE systems and 16S rRNA gene sequence analyses showed that the strains belonged to the genus *Pantoea*. The results of these tests also separated the strains into two groups. The first group, giving a positive reaction in the indole test, was similar to *Pantoea ananatis*. The second group of strains was indole negative and resembled *P. agglomerans*. The fluorescent amplified fragment length polymorphism (F-AFLP) genomic fingerprints generated by the indole-positive strains and *P. ananatis* reference strains were similar and clustered together in the dendrogram, confirming that the indole-positive bacteria causing brown stalk rot on maize were *P. ananatis*. The F-AFLP fingerprints produced by the indole-negative strains were distinctly different from those generated by *P. ananatis, P. agglomerans, P. dispersa, P. citrea, P. stewartii*subsp. *stewartii,* and *P. stewartii* subsp. *indologenes*. The results indicated that indole-negative bacteria causing brown stalk rot onmaize might belong to a previously undescribed species of the genus *Pantoea*. This is the first report of a new disease on maize, brown stalk rot, caused by two bacterial species, *P. ananatis* and an undescribed *Pantoea* sp.

Hossain (2007) presented the survey report in the national workshop programme on "Strategic Intervention on Plant Pathological research in Bangladesh" and stated that six seedling diseases viz. leaf blight i.e.,maydis leaf Blight (*Drechslera maydis*) and turcicum leaf blight (*Drechslera* *turcicum*), bipolaris leaf spot (*Bipolaris maydis*), stalk rot (*Fusarium spp. and Diplodia spp*.), seedling blight (*Aspergillus spp.*, *Penicillium spp*.), foot and root rot (*Fusarium spp*.) and maize dwarf mosaic (maize dwarf mosaic virus) were mostly found in Bangladesh. Incidence levels of those diseases also investigated.

Wilke and Bronson (2007) reported that *Fusarium verticillioides* can be seed transmitted and cause systemic infection of maize; however, the frequency of these phenomena has varied widely among and within individual studies. In order to better understand this variability, we evaluated the effect of temperature on the first step in the systemic infection process, the transmission of *F. verticillioides* from seed to seedling. Seed of a commercial maize hybrid were inoculated with a strain of *F. verticillioides* that had been transformed with a gene for green fluorescent protein (GFP). The seed were planted in a greenhouse potting mix and incubated in growth chambers. Plants were incubated at one of three temperature regimes designed to simulate average and extreme temperatures occurring in Iowa during the weeks following planting. Root, mesocotyl, and stem tissues were sampled at growth stages V2 and V6, surface disinfested, and cultured on a semi-selective medium. At V2, >90% of root and mesocotyl tissues was infected by the GFP-expressing strain at all three temperature regimes. Also at V2, infection was detected in 68 to 75% of stems. At V6, infection of root and mesocotyl tissues persisted and was detected in 97 to 100% of plants at all three temperature regimes. Plants also had symptomless systemic infection of belowground and aboveground internodes at V6. Infection of the three basal aboveground internodes was 24, 6, and 3% for the low-temperature regime; 35, 9, and 0% for the average-temperature regime; and 46, 24, and 9% for the high-temperature regime. Seed transmission and systemic infection occurred at all temperatures and did not differ significantly among treatments. These results indicate that, if maize seed is infected with *F. verticillioides*, seed transmission is common and symptomless systemic infection can be initiated under a broad range of temperature conditions.

Yasmin (2007) reported so far 28 different diseases of maize in Bangladesh and most of these diseases are caused by fungi. Twenty species of fungi were recorded on maize in Bangladesh.

Askun (2006) conducted experiment on twenty retail and bulk maize samples parallel for each group, surface disinfected and non-disinfected maize kernels, in Balikesir, Turkey. He reported that three species of *Rhizopus* spp. were commonly isolated; *R. oligosporus* Saito (19.0%), *R. oryzae* Went and Prinsen Geerlings (8.1%) and *R. stolonifer* (Ehrenb.) Lind. (22.0%). *Aspergillus* was the second most frequent genus isolated from non-disinfected maize kernels. Predominant species isolated were *Aspergillus tubingensis* (Schöiber) Mosseray (4.6%) and *A. niger* Van Tieghem (23%). In the disinfected group, *Aspergillus* spp. (25%), *Fusarium* spp. (21%), *Rhizopus* spp. (21%) and *Penicillium* spp. (13%) were commonly isolated. *Aspergillus tubingensis* (5.0%), *A. foetidus* var. *acidus* Naka, Simo and Wat (5.0%), *Fusarium proliferatum* (Matsushima) Nirenberg (17.1%), *Rhizopus oligosporus* (57%) and *Penicillium oxalicum* Currie and Thom (7.6%) were most frequently species isolated. Decrease of the *Rhizopus* genus by chlorine disinfection caused significantly increase of the *Fusarium* (21%), *Trichoderma* (8%) and *Aspergillus* (25%) rates. *Fusarium proliferatum* was also found dominant and potential mycotoxigenic storage fungi in the samples of corn maize.

Casa *et al* .(2006) reported that *Stenocarpella macrospore* and *S. maydis* might be responsible for causing seed rot, seedling blight, stem and car rot and leaf spot in maize. Normally these fungi are the main causal agent of grain rot when ears are infected. The damage caused exclusively by *Stenocarpella* has not yet been determined. The pathogens are found in practically all maize-growing regions of Brazil. The major disease intensity occurs under maize is monoculture, mainly in small farms and fields for seed production where maize is continuously cultivated.

Kar (2006) conducted an experiment in Orissa, India to evaluate the yield losses due to bipolaris leaf spot (*Bipolaris maydis*) in three popular high yielding cultivars of maize (Deccan-103, Navjot and VL-16). Plots were sprayed with 0.3% Mancozeb at 30 and 45 days after germination. The results revealed that the fungicidal sprays were effective in reducing disease incidence and increasing yield of 1000-grain weight. The mean disease intensities under protected conditions were 1.87, 1.78 and 2.12 in Deccan-103, Navjot and VL-16 respectively, and that under unprotected conditions were 3.32, 3.60 and 4.42 respectively. Grain yield in protected plots were 47.43, 44.43 and 30.82 q/ha in Deccan-103, Navjot and VL-16 respectively, and 42.30, 39.10, and 24.97 q/ha in unprotected Plots. The yield loss was maximum in VL-16 (18.98%) followed by Navjot (12%) and Deccan-103 (1.52%).

Taiwo *et al.* (2006) conducted a survey between April 2001 and February 2002 to determine the identity, prevalence, and incidence of maize viruses in 18 local government areas (LGAs) in and around Lagos by visual examination and serodiagnostic screening of symptomatic plants. All 112 fields surveyed during the dry season (September to December) and 18fields surveyed during the late dry season (December to February) had plants infected by *Maize streak virus* (MSV), whereas 97.1% of the 170 fields surveyed during the wet season (April to August) had plants infected by MSV. Maize mottle/chlorotic stunt virus (MMCSV) was prevalent in 99.1, 88.9, and 67.4% of the fields surveyed during the dry, late dry, and wet seasons, respectively. The incidence of MSV was higher in 16 of the LGAs. The highest incidence of MSV was 18.9%, whereas that of MMCSV was 7.4%. Serodiagnostic screening of leaf samples showing virus-induced symptoms, using antigen-coated plate enzyme-linked immunosorbent assay, indicated that 1,192/1,475 (80.8%) and 949/1,210 (78.4%) of the samples were positive for MSV and MMCSV, respectively. Vector transmission and host range studies confirmed the identity of the viruses. The results confirm the presence of MSV and MMCSV in Lagos and suggest that the use of MSV-susceptible cultivars is still widespread.

Amaral *et al.* (2005) conducted an experiment to identify and characterize the pathogens associated with symptoms similar to Phaeosphaeria leaf spot (PLS) of maize in different environmental conditions in Brazil. During the last decade, PLS became an important disease of maize in Brazil. However, doubt persists about the causal agent. Maize leaves with PLS-like lesions were collected from two locations (Cristalina, Goiás State [GO] and Vila Maria, Rio Grande do Sul State [RS]) in two growing seasons. Fungi associated with leaf lesions were isolated and cultured for taxonomic identification. Pathogenicity tests were carried out and the results indicated that three fungi (a *Phyllosticta* sp., *Phoma sorghina*, and a *Sporormiella* sp.) caused leaf spot similar to PLS on maize. The composition of pathogenic fungi in PLS-like lesions varied depending on locations and growing seasons. The fungi *P. sorghina* and a *Phoma* sp. (*Plenodomus* section) occurred in all environments, but the *Sporormiella* and *Phyllosticta* spp. were restricted to GO and RS, respectively. The results support the hypothesis that various pathogens are involved in PLS-like symptoms of maize and environmental conditions may influence the predominance of a specific agent.

Harlapur (2005) conducted survey during 2003 and 2004 in all the maize growing areas of Karnataka. Among the 11 districts surveyed, maximum disease incidence was observed in Belgaum district (55.89%) followed by Mysore (54.76), Davanagere (53.86), Mandya (53.85%) and Haveri (53.85%).

Paul and Munkvold (2005) conducted an experiment in controlled environment to determine the effects of temperature on the expansion of lesions of gray leaf spot, and the effects of temperature and relative humidity on the sporulation of *Cercospora zeae-maydis* on maize (*Zea mays*). For the lesion expansion experiment, potted maize plants were spray inoculated at growth stage V6, bagged, and incubated at 25 to 28°C and 100% relative humidity for 36 to 40 h. Symptomatic plants were transferred to growth chambers and exposed to constant temperatures of 25, 30, and 35°C. Lesion area (length by width) was measured at 4-day intervals for 17 days. For sporulation studies, lesions were excised from naturally infected maize leaves, measured, and incubated at constant temperature (20, 25, 30, or 35°C) and relative humidity (70, 80, 90, or 100%) for 72 h. Sporulation was estimated as the number of conidia per square centimeter of diseased leaf tissue. A quadratic function was used to model the relationship between log-transformed conidia per square centimeter at 100% relative humidity and temperature. Temperature had a significant effect on lesion expansion ($P \leq 0.05$). At 25 and 30°C, the rate of lesion expansion was significantly higher than at 35°C ($P \leq 0.05$). The largest lesions and the highest mean rate of lesion expansion were observed at 30°C; however, the mean lesion expansion rate at this temperature was not significantly different from that at 25°C. The interaction effect of temperature and relative humidity on the log of conidia per square centimeter of diseased tissue was significant ($P \leq 0.05$). At 100% relative humidity, the effect of temperature on sporulation was significant ($P \leq 0.05$), with maximum spore production occurring at 25 and 30°C. The quadratic model explained between 49 and 80% of the variation in the log of conidia per square centimeter at 100% with variation in temperature. These results suggest that the rapid increase in gray leaf spot severity generally observed during mid- and late summer may be due to favorable conditions for lesion expansion during this period. When relative humidity is >95%, expanding lesions may serve as a source of inoculum for secondary infections.

Bari and Alam (2004) reported 28 diseases of maize. They mentioned seed-borne diseases like leaf blight (*Bipolaris turcicum*), leaf spot (Bipolaris *maydis*), banded leaf and sheath spot (Rhizoctonia solani), cob rot (Aspergillus spp), foot and root rot (*Fusarium spp.)* damping off (Fusarium *moniliforme*) and anthracnose (Colletotrichum *spp*.). However there was no proof about the association of these fungal pathogens with the seeds of maize in Bangladesh.

CIMMYT (2004) reported that seedling blight, stalk rot, brown spot, gray leaf spot, smut, ear rot, anthracnose, sheath blight, turcicum leaf blight, bacterial leaf blight caused by *Fusarium spp.* or *Pythium spp.* or *Rhyzoctonia spp., Pythium aphanidermarum* or *Fusarium moniliformae*, *Bipolaris maydis, Cercospora spp., Ustilago maydis*, *Aspergillus spp.* or *Penicillium spp., Colletotrichum graminicola, Rhizoctonia solani, Helminthosporium turcicum* and *Pseudomonas rubrilineans* respectively.

Jha *et al*. (2004) evaluated thiram, emissan (2 methoxyethylmercury chloride), captafol 50% wp (captan) and bavistin 50% WP (carbendazim), alon or in combination for their effects on maize leaf blight (*B. maydis*) *in vitro*. The fungicide was applied at 0.01, 0.02, 0.05 and 0.10%, except bavistin, which was applied at 0.01, 0.02, 0.03 and 0.04%. All fungicide showed inhibitory effect on the spore germination at all contentrations. Bavistin at 0.03% showed 100% inhibition of spore germination. Thiram+ Bavistin, Captafol + Bavistin, thiram + cmissan, cmisan + indofil M- 45, captafol+ indofil M-45 and indofil M-45 +Bavistin were statistically similar to their efficacy in controlling fungal sporulation.

Li *et al*. (2004) observed that sugarcane mosaic virus (SCMV) is an important seed-borne virus in maize. SCMV was detected in maize seeds by ELISA, electron microscopy, biological assay and tissue culture. The SCMV particles or inclusions were found in the testa, aleuronic layer of endosperm and embryonic tissue, but not in the starch layer of the endosperm. The in aleuronic layer and embryo invade the growing maize seedling.

Ares *et al*. (2003) conducted an experiment to test the pathogenicity of *Fusarium graminearum* isolates on maize cultivars and found that *Fusarium graminearum* was an important pathogen of maize and caused seed rot and seedling blight as well as foot and root rot, stalk rot and ear rot. In growth chamber experiments, inoculation of corn cv. 'Loyel' seeds with six different *F. graminearum* isolates reduced emergence of seedlings and caused seedling death of varying degrees.

Dharanendra Swamy (2003) conducted survey in different villages of Dharwad and Belgaum districts, and observed maximum disease incidence in Mugad (45%) followed by Madihal (43%) and Devarahubballi (38%) of Dharwad taluks.

King and Hagood (2003) conducted a field experiment in 2000 and 2001 in Virgiana to evaluate the maize dwarf mosaic virus (MDMV) in response to post emergence Johnson grass control in two corn hybrids. The results showed that increased disease incidence resulted from greater transmission of MDMV by insect vectors which moves from dying Johnson grass to the crop. The results also revealed that little or no disease incidence occurred in the virus tolerant hybrid. With the virus susceptible hybrid, significance increases in disease incidence were observed in response to any herbicide treatment applied to Johnson grass-containing plots relative to the same treatment applied to weed free plots.

Mathur and Kongsdal (2003) reported that Southern leaf blight disease is caused by *Bipolaris maydis*. Leaves show grayish, tan, and parallel straight sided or diamond shaped 1-4 cm long lesions with buff or brown borders or with prominent colour banding or irregular zonation. Symptoms may be confined to leaves or may develop on sheaths, stalks, husks, ears and cobs. The lesions are longitudinally elongated typically limited to a single inter vascular region, often coalescing to form more extensive dead portions. Young lesions are small and diamond shaped. As they mature, they elongate. Growth is limited by adjacent veins, so final lesion shape is rectangular and 2 to 3cm long. Lesions may coalesce, producing a complete burning of large areas of the leaves. Southern maize leaf blight is prevalent in hot, humid, maize growing areas. The fungus requires slightly higher temperatures for infection.

Pataky and Ikin (2003) conducted an analysis of the risk of introducing *Erwinia stewartii* in maize seed based on the request of the International Seed Federation (ISF) as an initiative to promote transparency in decision making and the technical justification of restrictions on trade in accordance with international standards. The *Erwinia stewartii* is seed-borne in maize; the role of infected seed is insignificant in the epidemiology of Stewart’s wilt in areas of North America where the disease is endemic. The pathogen appears to have become specialized to exist in two specific hosts, *Zea mays* and *C. pulicaria*. Levels of Stewart’s wilt infection in US fields under good agricultural practice (GAP) are affected by the resistance of the host plant (i.e., the particular variety) and the prevalence of the corn flea beetle vector, *Chaetocnema pulicaria,* in which the bacteria also overwinters. In terms of international trade in seed for planting, the probability of introducing (entry and establishment) *E. stewartii* to a new area as a result of seed transmission is much lower than previously reported. Previous calculations of rates of transmission in the general plant pathological literature from 1940 to 1990 are based on a small number of experiments in which relatively few samples of seed from highly susceptible, open-pollinated cultivars were tested. This report provides the technical justification and an assessment of the risk level that may be posed by trade in commercial seed, and suggests field and laboratory phytosanitary risk management procedures (measures) that can be applied in accordance with international standards under the IPPC and the WTO SPS Agreement.

Wang and Ma (2003) noted that maize dwarf mosaic virus (MDMV) was one of the world's main viral diseases in producing areas. The domestic and overseas research progress on the epidemiology of MDMV was reviewed. Topics include occurrence and damage, cultivar resistance, pathogen, viral transmission, cultivation management, environmental conditions, temporal and spatial analysis of epidemics, and forecasting methods.

Zhu *et al.* (2002) conducted a survey in mid-September 2001 and reported that a sporadic symptom typical of gray leaf spots (*Cercospora zeae maydis*) was found in nine fields in Southern Ontario, Canada. Leaf samples with symptoms were placed in Petri dishes and clustered conidiophores arose from stomata on both leaf surfaces. Slightly curved, hyaline conidia with 3 to 5 septa appeared on the top of conidiophores. Upon further testing, gray leaf was re-isolated from inoculated plants. Fulfilling Koch s postulates. This is thought to be the first confirmation report of this pathogen in Canada.

Bohra *et al*. (2001) conducted an experiment during 1995 and 1996 in Udaipur, Rahashtan, India to evaluate the efficacy of different fungicides against Fusarium stalk rot (F. *moniliforme* [*Gibberella fujikuroi*]) in maize under *in vivo* and *in vitro* conditions. In laboratory bioassays, 6 different fungicides, i.e. bavistin (carbendazim), bayleton (triadimefon) , kitazin (iprobenfos), captafol (captan), thiram and dithane M-45 (mancozeb) were used at 50, 100, 200, 400 and 800 ppm concentrations. The results of in vitro bioassays showed that all treatments significantly inhibited the growth of *F. moniliforme.* Maximum growth inhibition (~ 100%) was observed at 50 ppm concentration of bavistin and bayleton. Both the fungicides were evaluated at lower concentrations, i.e.

5, 10, 20, 40 and 50 ppm. Bavistin was highly effective in inhibiting the mycelial growth of *F. moniliformae* even at 5 ppm concentration. While bayleton gave approximately 100% inhibition 40 ppm concentration. The efficacy of bavistin (0.1%) and captaf (0.2%), as soil application, against *F. moniliformae* was evaluated in the field. Bavistin and cartaf exhibited 54.5 and 46.9% efficacy of disease control.

Egein and Arinze (2001) discovered a new fungal disease of maize on a rubbish dump at Choba, Port Harcourt (Nigeria). The causal agent was identified as *Fusarium oxysporum* causing damping off of seedling with disease manifestation after 9 days after emergence were observed. The damping off disease incidence was found sporadically where domestic and some industrial wastes were damped.

Fakir (2001) listed 11 seed-borne diseases occurring on maize in Bangladesh. The diseases along with their pathogens were kernel mould (*Aspergillus flavus, Penicillium spp*.), cob rot (*Aspergillus spp, Gibberella zeae*), kernel rot (*Botryodiplodia theobromae*), seed rot (*Fusarium moniliforme, F. oxysporum, penicillium spp*), germination failure (*Aspergillus spp*.), seedling blight (*Gibberella zeae, penicillium spp*), blue eye (*Penicillium spp*.), brown spot (*Physoderma zeae-maydis*), scutellum rot (*Rhizopus spp.*) and smut (*Ustilago zeae*). However, no attempt was made to detect these fungal organisms in seeds of the crop.

Harlapur *et al.* (2000) conducted survey during 1995-1996, 1996-1997 and 1997-1998 to obtain recent information on maize disease in north Karnataka, India. Turcicum leaf blight (*Exserohilum turcicum*) was the major disease (53.5% disease incidence) affecting maize particularly during the kharif season of 1995-1997. Carcoal stalk rot (*Macrophomina Phaseolina*) appeared in major proportions during the rabi season (16.5% disease incidence). The incidence of other disease like maydis leaf spot (*Bipolaris maydis*), brown spot *(Physoderma maydis*) and phaeophaeria leaf spot (*Phaeosphaeria maydis)* incidence were observed in traces. During rabi season charcoal stalk rot and fusarium stalk rot (*Fusarium moniliforme*) incidence found to be moderate to severe.

Kumar and Jha (2000) determined in vitro effectiveness of nine chemicals to control *Rhizoctonia solani* causing banded leaf and sheath blight of maize. Out of nine fungicides screened in the laboratory, Bavistin, Bengard and Topsin –M proved most effective as they caused the maximum inhibition of mycelial growth. Other fungicide viz., Kitazin, Captafol, Brassicol, Indofil M-45 and Thiram also showed better performance regarding inhibition of mycelial growth of the pathogen in comparison to check.

Achon (1999) surveyed commercial maize fields in Southeast Catalonia (Spain) for maize dwarf mosaic virus (MDMV) during spring-summer, 1997. Maize dwarf mosaic virus was present in all surveyed fields, its average incidence in maize being 27.3%. The over wintering hosts of MDMV, Johnson grass (*Sorghum halepense*), was found in all surveyed fields, and 69% of the samples were infected with virus.

Wegary *et al*. (1999) carried out a survey of maize diseases in Ethiopia in 1997 and 1998. Gray leaf spot of maize caused by *Cercospora zeae maydis* was major diseases in the area studied. It is suggested that the development of resistant/tolerant varieties provides the most promising long-term means for controlling the disease, although crop sanitation and good crop management practices would also reduce infection. It is also suggested that fungicides could be used to control the disease when economically feasible.

White (1999) listed some seed-borne fungal diseases on maize plants and the diseases were seed rot, seedling blight and damping-off (*Fusarium moniliforme, Penicillium spp., Aspergillus spp*., *Bipolaris spp*., *Rhizoctonia spp*. and *Alternaria sp*.), stalk rots, ear rots and kernel rots (*Gibberella spp., Diplodia spp., Fusarium spp., Pythium spp., Penicillium spp. and Aspergillus spp*.). All the fungi were found associated with the recorded diseases were seed-borne.

Kumar and Agarwal (1998) conducted an experiment to locate the seed borne fungi in different parts of discolored maize seeds. Seed borne inoculums of *Bipolaris maydis, Botryodiplodia theobromae, Curvularia lunata, Fusarium moniliforme* were detected in all parts (tip cap, pericarp, embryo and endosperm) of maize seeds, where *C. pallescens* and *Bipolaris carbnum* were tip cap and pericarp; and tip cap, pericarp and endosperm, respectively.

Brunt *et al.* (1996) repoted that maize chlorotic mottle disease is caused by Maize chlorotic mottle machlomovirus (MCMV) which is first reported in maize from Peru. Spreads in Argentina, Mexico, Peru and the USA (Kansas, Nebraska and Hawaii). Maize is the natural host of this virus. Transmitted in a non-persistent manner by *Diabrotica spp.* and thrips, but the vectors are not known to move the virus over long distance. Level of seed transmission of this virus is low. Seed transmission would make MCMV a threat to the maize farming in Bangladesh.

El-Maghraby *et al*. (1995) isolated 63 species of fungi belonging to 21 genera from maize grains in Egypt. *Aspergillus* (15 species), *Penicillium* (17 species) and *Fusarium* (4 species) were the dominant genera isolated from the 3 types of maize. Of the four species of *Fusarium, F. moniliforme* was the dominant species. *F. oxysporum* and *F. subglutinans* were isolated exclusively from yellow corn (Maize).

FAO (1995) constituted International Standers for Phytosanitary Measure (ISPM) No 2 for framework for pest risk analysis. According to ISPM 2 three inter-related steps such as: (i) disease categorization, (ii) assessment of the probability of introduction and spread, and (iii) assessment of potential economic consequences (including environment and biodiversity at large) are involved in PRA. For disease categorization a list of diseases of targeted crop is required firstly. The list of diseases should be prepared through surveying targeted area. For disease categorization risk of diseases and their incidence should in consideration. During assessment of probability of introduction and spread survey report should in consideration. Finally assessment of potential economic consequence should be done.

Brown *et al.* (1994) reported that the disease High Plains disorder of maize is caused by High Plain Virus (HPV) which is distributed in different county of USA such as Texas, western Kansas, northern Colorado and central Idaho, Nebraska and Utah. The virus was first found in wheat and maize plants from Texas and Idaho, and in 1994 the disease was observed in Kansas and Colorado. By the end of 1995, HPV had been confirmed in maize and wheat samples from nearly 100 countries in an area extending from the Texas panhandle to eastern Nebraska, to central South Dakota, to western Idaho and back through Colorado to eastern New Mexico and Texas. Since then, it has been found more frequently over a much wider area, probably due to greater awareness and surveillance. Yield loss to 75% has been reported from USA.

Phillips (1994) conducted Pest risk analysis of seed-borne pest of barley, maize and sorghum from the USA and barley from Canada. Seed-borne pathogens of oats (*Avena spp.)* and rye (*Secale spp.),* including *Hordeum, Sorghum, Triticum* and *Zea spp*. have been identified by his research. Six pathogen viz. *Cercospora zeae-maydis*, maize dwarf mosaic potyvirus, wheat streak mosaic rymovirus, maize chlorotic mottle machlomovirus, *Pantoea stewartii* subsp. Stewartii, *Clavibacter michiganensis* subsp. nebraskensis found as seed borne pathogen.

Shahjahan (1993) reported that five diseases viz. leaf blight, stalk rot, mosaic, cob rot and downy mildew seriously affect the maize crop in our country.

Arvinder and Rai (1991) examined association fungi with maize seeds. They recorded *Rhizopus stolonifer*, *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, *Curvularia lunata, Bipolaris maydis* and *B. turcicum* from both external as well as internal tissues of the local maize seeds.

Brekalo *et al.* (1991) reported that the major seed-borne diseases of maize caused by *Bipolaris turcicum, Kabatiella zeae* and *Colletotrichum graminicola* are found in Western localities of Croatia. *Bipolaris zeicola* and *B. maydis* were found in the whole of Croatia.

Chatterjee *et al* (1990) reported that the major maize diseases prevalent in India are blight. These are maydis leaf blight, downy mildews, pythium stalk rot, bacterial stalk rot, common rust, charcoal-rot, brown spot and turcicum leaf blight. Moreover seed-borne diseases cause enormous losses both in storage as well as in the field.

Richardson (1990) listed 60 fungal pathogens on maize seeds. Important seed-borne diseases recorded on the crop caused by the fungal pathogens were-leaf spot/leaf blight (*Cochliobolus heterostrophus, Epicoccum sp.*), cob rot and seed rot (*Aspergillus spp., Fusarium spp., Penicillium spp.*), kernel rot (*Acremonium strictum, Botryodiplodia sp., Cladosporium spp*.),

scutellum rot (*Rhizopus spp.*), seedling blight (*Gibberella zeae*), anthracnose (*Colletotrichum graminicola*) and head smut (*Sphacelotheca reiliana*).

Zumma and Scott (1990) reported that maize kernels infected by *Fusarium* moniliforme. *Aspergillus flavus* were frequently observed in the apical section of the cobs.

Jacqua (1989) recorded *Bipolaris* leaf blight caused by *Bipolaris maydis* and *B. turcicum* as the principal seed-borne foliar disease of maize in Guatemala.

Pandurangegowda (1987) surveyed main maize growing areas of the Southern Karnataka and reported that 10 to 100 percent disease incidence on most of the hybrids and composites. Survey conducted by AICRP on maize by Arabhavi center revealed that disease severity was moderate to severe and distributed over all of the maize growing areas of Northern Karnataka.

Suryanarayana (1987) listed some important seed-borne diseases of maize. The diseases are seedling blight (*Fusarium moniliforme, F. graminearum*), leaf blight (*Bipolaris turcicum, B. maydis*), leaf spot (*B. carbonum, Diplodia macrospora*), stalk and ear rot (*Diplodia maydis, Cephalosporium* *acremonium*), late wilt (*Cephalosporium maydis*) and downy mildew (*Sclerospora philippisensis*).

Bradbury (1986) reported that *Pantoea stewartii* subsp. *stewartii* (causes stewart's disease; bacterial wilt) is spread in China, Malaysia, Thailand, Vietnam, Italy, Poland, Romania, Yugoslavia, Canada, Mexico, USA, Costa Rica, Puerto Rico, Brazil and Peru. The bacterium is seed-borne but the seed to seedling transmission rate is very low. The bacterium overwinters in seed, soil or maize stalks.

Thind & Payak, (1985) conduct an experiment on yield loss due to *Erwinia chrysanthemi* pv. Zeae (produce bacterial stalk and top rot). This disease is most prevalent and destructive in areas with high rainfall, where plants are watered by sprinkler irrigation and on land subjected to flooding. The bacterium is artificially inoculated to maize field and assumed 92% yield loss in India. This bacterium lives saprophytically on crop residue in the soil.

Atac (1984) presented the report that the seed borne *Ustilago maydis* was widespread in Mediterranean region, particularly on Turkish maize cultivars. *Bipolaris turcicum and Curvularia lunata* were common in coastal areas of turkey.

Lee (1984) reported that *Fusarium moniliforme* was the major seed-borne pathogen of maize in Taiwan. This pathogen was also reported to cause seedling blight; seed rot and stalk rot of maize.

Mikel *et al.* (1984) repoted that Maize dwarf mosaic potyvirus (MDMPV) is seed-borne and seed transmitted. The disease is important in USA where yield losses have been reported. MDMPV is spread in maize crops by transient winged (alate) aphids. Alate behavior, consisting of many short flights with frequent probing, has been related to dispersal rather than to host finding. Strong correlations have been demonstrated between aphids numbers in traps and the incidence of MDMPV.

Simon (1984) listed the most common fungi associated with maize seeds were *Fusarium moniliforme, F. proliferatum* and *F. subglutinans* and a lesser extent, other fungi such as *Penicillium spp.*, *Aspergillus spp.*, *Rhizoctonia sp.*, *Bipolaris spp.*, *Alternaria spp.*, *Trichoderma sp.* and *F. graminearum*.

Christensen (1980) reported that *Aspergillus spp.* (minor ear rot), are a major cause of deterioration of maize stored above 15% moisture content. The disease is important because of production of the carcinogenic compound, aflatoxin, in affected grain. Contamination of maize with *Aspergillus spp.*, and the subsequent production of aflatoxin, is prevalent in the Midwestern United States during years with drought conditions. Aflatoxin produced by the fungus in kernels, either before or after harvest, is carcinogenic to a number of animal species, and can seriously affect marketing of maize grain.

Shurtleff (1980) reported that *Peronosclerospora sorghi* (causes sorghum downy mildew disease) is found in USA, Latin America, North, Central and South America, Southeast Asia, India, Israel, Italy and Africa. The fungus is seed-borne. Transmission to seedlings was found when seeds from infected plants were planted immediately after harvest. It has never been found in seeds dried to 15% moisture content and below. This is a serious disease in tropics and subtropics. Severe outbreaks have occurred in India, Israel, Mexico, Thailand, Texas and Venezuela.

Bergquist (1979) conducted an experiment for evaluation of resistance maize varieties against *Colletotrichum graminicola*, *Bipolaris turcicum and B. maydis.* The fungi were reported to be seed-borne.

Dange *et al.* (1978) reported that bacterial leaf blight and stalk rot (*Acidovorax avenae*) is seed-borne and seed to seedling transmission has been demonstrated for maize under laboratory conditions. In most cases conditions of high temperature and high relative humidity favour symptom development. The bacterium is not thought to survive well in soil or plant debris. Alternative hosts such as *Paspalum urvillei* are considered an important inoculums source of Bacterial Leaf Blight (BLB) outbreaks in maize.

Prasad & Sinha (1977) reported that *Erwinia carotovora* subsp. *carotovora* causes bacterial stalk and top rot is carried on seed, but there is no evidence for seed to seedling transmission. It can survive on maize debris and is spread by water. It causes a major disease of maize in tropical and subtropical countries. The disease is particularly severe under conditions of high temperature and humidity.

Fatima *et al*. (1974) reported that *Bipolaris maydis* was known as seed-borne in maize which caused seed rot and seedling blight. The pathogen was prevalent throughout the maize growing areas of USA.

Talukdar (1974) reported nine diseases viz. Leaf blight, Cob rot, Kernel mould, Smut, Leaf spot, Brown spot, bacterial streak, Soft rot and Mosaic of maize in Bangladesh.

Futrell and Kilgore (1969) reported a number of *Fusarium spp.* from maize seeds causing *Fusarium* root rot. The most commonly repoted *Fusarium* were *Fusarium oxysporum, F. solani, F. moniliforme and F. graminearum.*

USDA (1960) reported that a total of 112 diseases are known to occur on global basis on maize and among them more than 70 are seed-borne. Richardson (1990) reported that important seed borne diseases of maize are leaf spot, leaf blight, collar rot, kernel rot, seedling blight, anthracnose and head smut.

CHAPTER III

MATERIALS AND METHOD

3.1 Experimental site

Experiments were conducted at the Seed Health Laboratory (SHL), Department of Plant Pathology (DPP), Sher-e-Bangla Agricultural University (SAU), Dhaka. Data was collected through field survey in major maize growing areas of Bangladesh.

3.2 Source of data and sampling procedure

A systematic sampling procedure was used both baseline and nationwide survey. In Bangladesh 20 major maize growing districts were selected for conducting survey analysis on maize diseases. A pre-designed questionnaire was used to collect information on level of knowledge on diseases of maize aspects of farmers and policy level officers (Annex-II, and Annex-III). The minimum sample size was 440 as shown below:

Respondent(s)		**Sample Size**
1	Farmers	400
2	Policy level officers	20
3	Focus Group Discussion(FGD)	20
	Total	**440**

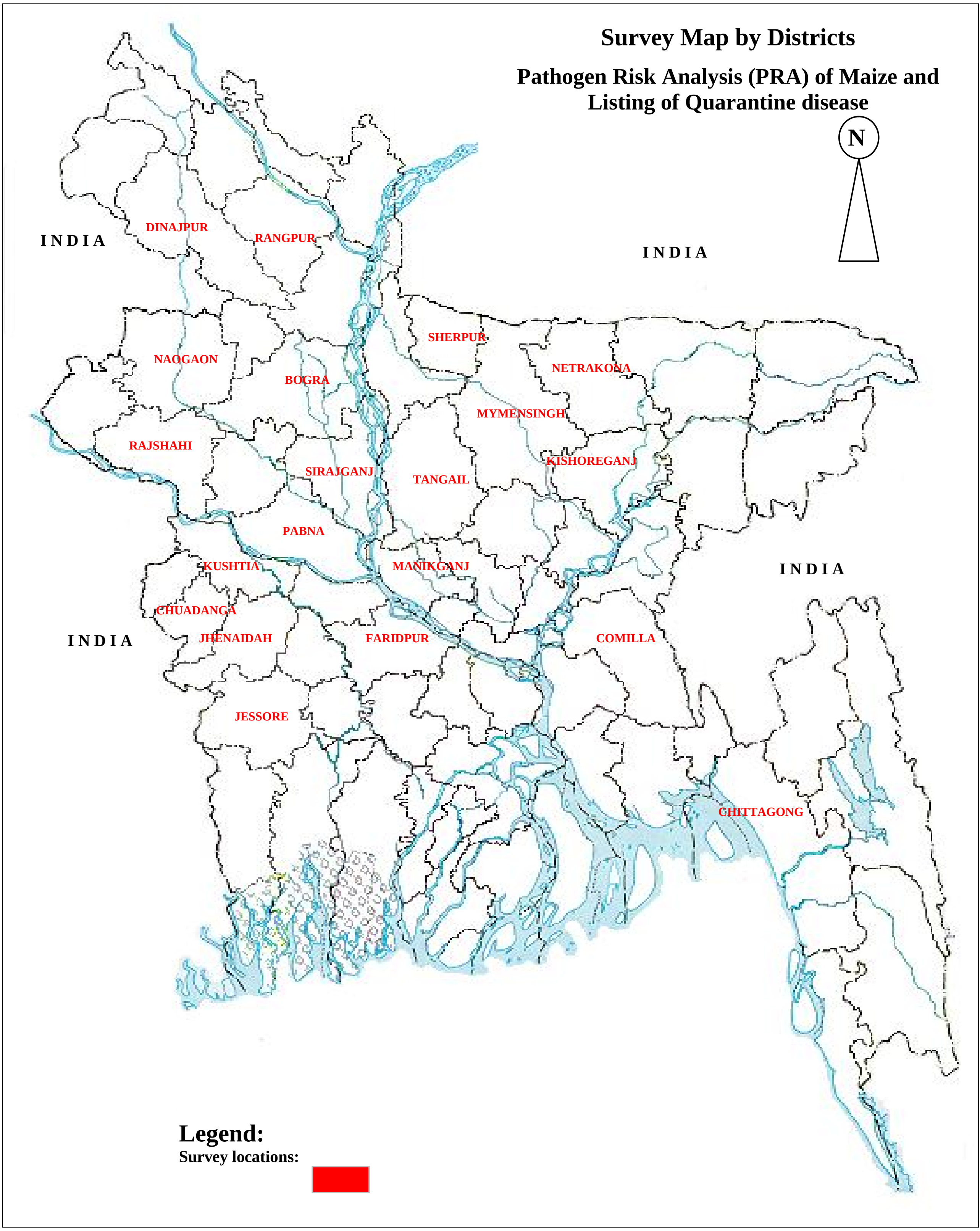
3.3 Survey on diseases of maize in selected locations of Bangladesh

The survey was conducted through a project on “Pest Risk Analysis (PRA) of Maize and listing of Quarantine Pests” was implemented by the “Quarantine Services Strengthening Programme (QSSP)” of Plant Protection Wing, DAE. A three days training program on “Pest Risk Analysis (PRA) of Maize” were arranged with 20 master’s student by Department of Plant Pathology under Sher-e-Bangla Agricultural University for conducting survey.

The survey was covered with 40 upazilla in selected 20 districts. The survey area has shown in map. The survey locations were as follows:

Sl. No.	District(s)	Sample Upazilla(s)		
		Name(s) of Sample Upazilla(s) for Survey		Numbers
1	Rangpur	Sadar	Mithapukur	2
2	Dinajpur	Sadar	Fulbari	2
3	Bogra	Sherpur	Adamdighi	2
4	Naogaon	Sadar	Patnitala	2
5	Rajshahi	Tanor	Godagari	2
6	Pabna	Sadar	Atgoria	2
7	Sirajgonj	Sadar	Ullapara	2
8	Jessore	Sador	Zikorghacha	2
9	Kushtia	Sador	Daulatpur	2
10	Jhenidah	Sadar	Harinakundu	2
11	Chuadanga	Jibon nagor	Damurhuda	2
12	Faridpur	Sadar	Nagorkanda	2
13	Tangail	Sador	Shakipur	2
14	Sherpur	Sadar	Nakla	2
15	Mymensingh	Muktagacha	Fulpur	2
16	Kishoreganj	Sadar	Kotiadi	2
17	Manikgonj	Sadar	Ghior	2
18	Netrokona	Sadar	Purbadhala	2
19	Comilla	Sadar	Burichong	2
20	Chittagong	Mirersarai	Satkania	2
	Total District 20	**Total Upazilla 40**		**40**

Survey Map:



3.3.1 Diseases of maize according to the farmer's opinion

Diseases of maize were listed according to the farmer's opinion during survey period. Survey data on "Pest Risk Analysis" was recorded through a questionnaire (Annex-II). From the questionnaire the disease part was taken in consideration. The number of respondent farmer was 400 under 20 selected districts.

3.3.2 Diseases of maize according to the policy level officer's opinion

A total of 20 Policy level officers of DAE participated as respondent one from each district of the study area. A pre-designed questionnaire was used to collect data on regarding the diseases of maize (Annex-III).

3.3.3 Diseases of maize according to focus group discussion

The Focus Group Discussion (FGD) for the "Pathogen Risk Analysis (PRA) of Maize and Listing of Quarantine Disease**"** in the target areas covering 20 districts of Bangladesh. One FGD was organized for each district/target area with 10 participants/respondents. In the FGD, picture of maize diseases was shown to the participants and their response was recorded in a pre-designed questionnaire (Annex-IV) for listing the diseases of maize. Accordingly, covering the districts/target areas under the experiment altogether 200 respondents were participated to express their opinion regarding the pests of maize and their risks. Diseases listed during focus group discussion were categorized as major and minor diseases with their causal organisms.

3.4 Laboratory Analysis

3.4.1 Collection of disease samples, identification of causal organisms and diseases of maize

3.4.1.1 Collection of disease sample

During survey period, maize disease sample was collected from farmer's field and brought to the laboratory for further analysis. Sample was collected from 3 farmer's field in each district under the study area.

3.4.1.2 Identification of causal organisms from plant sample

Maize plantation of the selected farmer's field observed carefully and symptoms of different maize diseases were recorded. To identify the pathogen, diseased plant part was collected using sterilized polythene bags and brought to the laboratory and the sample washed thoroughly under running tap water and surface sterilized with 4% NaOCl. The diseased parts were then cut into 1.0 cm long pieces. One set of pieces were placed on three layers of wet blotters equidistantly in Perspex plates and another set placed on PDA medium. Both sets were incubated for 7 days under 12/12hr. alternate cycles of near ultra violet light and darkness at 22±2^{o}C. After 8 days of incubation, the disease causal organism(s) were identified.

3.4.1.3 Identification of diseases

Four farmer's filed in each district was visited during survey period. "Maize disease: A Guide for Field Identification (4th edition, 2004)" by CEMMYT was primarily used for disease identification in field. If symptoms did not match with the guide book then those disease symptoms were collected from field and brought to the laboratory for isolation of causal organisms. After confirmation those diseases were listed.

3.4.2 Studies on prevalence of seed borne pathogens on the seeds of maize

3.4.2.1 Collection and storage of seed samples

Seeds of maize were collected from specified seed dealers and maize growers. Five seed samples (3 samples from seed dealers and 2 samples from farmers) were collected from each district. The collected seed samples were brought to the laboratory and subjected to a preliminary cleaning and then stored in paper packet in laboratory conditions for further study.

3.4.2.2 Isolation of mycoflora from the stored seeds

Fungi associated with the seeds were studied by employing the blotter techniques recommended by the International Seed Testing Association (ISTA, 1999). In all seed samples, 200 seeds were tested following Blotter Technique (ISTA, 1999).

3.4.2.3 The blotter technique

Three pieces of sterile blotting paper were placed in sterilized plastic Petri dishes (90 mm dia.) and moistened with sterilized distilled water so that a little amount of surplus water was left on the surface of paper. Ten seeds were placed in each petridish. The seeds were placed on three layered water-soaked blotters at controlled temperature of 22 ± 2°C with alternating cycle of 12h near-ultraviolet light and darkness in the incubation room of Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka. The tube lights mounted in pairs were placed at a distance of about 41 cm above the seeds. After 10-15 days of incubation, the fungi grown on the seeds were examined and the data were recorded. After incubation, fungi grow on the incubated seeds were examined under different magnification of stereo-microscope at Plant Pathology laboratory, SAU. The identification of the fungi were based on the way they grew on seed “habit character” and on the morphological characters of fruiting bodies, spores or conidia under compound microscope. The pathogens thus recorded were identified following the keys of Mathur and Kongsdal (2003). Data on % seed germination, % dead seed and % presence of pathogen were recorded.

3.4.2.4 Identification of seed borne fungi of maize

Collected seeds were incubated on blotter and disease seeds were observed under stereomicroscope. The pathogenic structures (conidia, conidiophores, mycelium, ooze) associated with microorganisms were carefully observed under compound microscope.

3.5 Preparation of a list of maize diseases

A list of commonly occurring diseases of maize in Bangladesh has been prepared based on survey report, Focus Group Discussion (FGD) and laboratory seed health testing.

3.6 Undertaking pathogen risk analysis

A systematic process of pathogen risk assessment was followed as per ISPM No 11 at the stage 3 for undertaking pest risk analysis. Three interrelated steps were followed such as: (i) pathogen categorization, (ii) assessment of the probability of introduction and spread, and (iii) assessment of potential economic consequences (including environment and biodiversity at large). Overall PRA process is shown in Annex-I.

3.6.1 Pathogen categorization

All the listed pathogens have been categorized and examines for each pathogen whether the criteria in the definition for a quarantine pathogen are satisfied. Then evaluate the pathway associated with maize for the various pests potentially associated with the pathway. The primary elements as listed here under were followed to categorize the pathogens as a quarantine pathogen. The primary elements are: identity of the pathogen, presence or absence in the PRA area, regulatory status, potential for establishment and spread in PRA area, and potential for economic consequences (including environmental consequences) in the PRA area.

CHAPTER IV

RESULT

4.1 Diseases of maize according to the farmer's opinion

During field survey, according to the farmer's opinion the diseases of maize were recorded. The level of disease incidence according to the farmer' opinion is here under (Table 1).

Table 1. Diseases of maize according to farmer's opinion

Name of Diseases	Causal organisms	Disease incidence
1. Stem rot	*Diplodia maydis*	medium
2. Leaf spot	*Cercospora zeae-maydis*	high
3. Root rot	*Gibberella avenacea*	low
4. Cob rot	*Khuskia oryzae*	high
5. Sheath blight	*Rhizoctonia solani*	medium
6. Sheath rot	*Gaeumannomyces graminis*	medium
7. Cob Sheath blight	*Rhizoctonia solani*	medium
8. Cob Sheath rot	*Gaeumannomyces graminis*	low
9. Leaf blight	*Bipolaris maydis*	high
10. Bacterial leaf blight	*Pseudomonas avenae* subsp. avenae	high
11. Maize dwarf	*Maize dwarf mosaic virus*	medium
12. Grain rot	*Diplodia maydis/ Gibberella zeae/Fusarium moniliforme/ Exserohilum turcicum*	low
13. Stored grain rot	*Penicillium sp./Aspergillus sp.*	low
14. Aspergillus ear rot	*Aspergillus niger/Aspergillus flavus*	low
15. Fusarium ear rot	*Fusarium oxysporum/Fusarium moniliforme*	low
16. Penicillium ear rot	*Penicillium oxalicum*	low
17. Stenocarpella ear rot	*Stenocarpella maydis*	low
18. Corn stunt	Corn stunt virus	low
19. Maize leaf fleck	Maize leaf fleck virus (MLFV)	low
20. Maize streak	Maize streak virus (MSV)	medium
21. Sugarcane mosaic	Sugarcane mosaic virus	medium
22. Downy mildew	*Peronosclerospora sorghi*	medium
23. Corn root-knot nematode	*Meloidogyne spp.*	low
24. Corn awl nematode	*Dolichodorus spp.*	low

4.2 Diseases of maize according to the opinion of policy level officers

During survey period, opinion of a policy level officer from each district under the study area about maize disease incidence was recorded in a questionnaire. 20 policy level officers of DAE from 20 districts opined on occurrence of maize diseases during survey period. The opinion of the policy level officers on maize disease is here under (Table 2).

Table 2. Diseases of maize according to the opinion of policy level officers

Name of Diseases	Causal organism	Disease incidence
1. Stem rot	*Diplodia maydis*	low
2. Leaf spot	*Cercospora zeae-maydis*	low
3. Root rot	*Gibberella avenacea*	low
4. Cob rot	*Khuskia oryzae*	high
5. Sheath blight	*Rhizoctonia solani*	high
6. Sheath rot	*Gaeumannomyces graminis*	medium
7. Cob sheath blight	*Rhizoctonia solani*	medium
8. Sugarcane mosaic	Sugarcane mosaic virus	medium
9. Leaf blight	*Bipolaris maydis*	high
10. Bacterial leaf blight	*Pseudomonas avenae* subsp. avenae	low
11. Maize dwarf mosaic	Maize dwarf mosaic virus	high
12. Grain rot	*Diplodia maydis*/ *Gibberella zeae*/*Fusarium moniliforme*/ *Exserohilum turcicum*	medium
13. Stored grain rot	*Penicillium sp./Aspergillus sp.*	medium
14. Aspergillus ear rot	*Aspergillus niger/Aspergillus flavus*	high
15. Fusarium ear rot	*Fusarium oxysporum/Fusarium moniliforme*	medium
16. Penicillium ear rot	*Penicillium oxalicum*	medium
17. Corn stunt	Corn stunt virus	low
18. Maize stripe	Maize stripe virus	low
19. Curvularia leaf spot	*Curvularia lunata*	high
20. Maize streak	Maize streak virus	high
21. Downey mildew	*Peronosclerospora sorghi*	low

4.3 Diseases of maize according to focus group discussion

The major findings of the FGD (Focus Group Discussion) comprising focal points are briefly mentioned in Table 3. Total 100068 hectare area was covered by maize in those targeted district.

Table 3. Diseases of maize according to focus group discussion

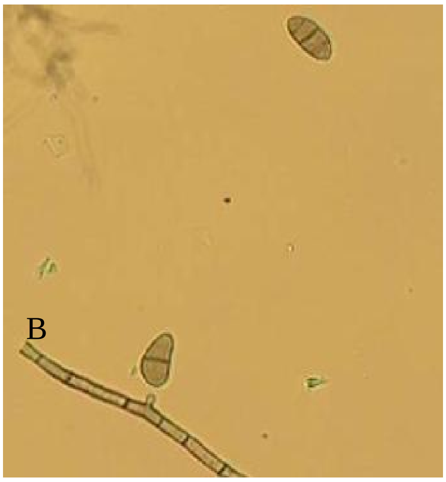
SL. No.	Name of the diseases	Causal organisms	Status
1	Leaf blight	*Pseudomonas rubrilineans*	Major
2	Sheath blight	*Rhizoctonia solani*	Major
3	Ear rot	*Aspergillus spp, Pencilliium spp.*	Major
4	Cob sheath blight	*Rhizoctonia solani*	Major
5	Stem rot	*Diplodia maydis*	Minor
6	Sheath rot	*Gaeumannomyces graminis*	Minor
7	Leaf spot	*Alternaria alternata*	Minor
8	Root rot	*Gibberella avenacea*	Minor
9	Cob rot	*Khuskia oryzae*	Minor
10	Mosaic virus	Wheat Mosaic Virus	Minor
11	Foot and root rot	*Rhizoctonia zeae*	Minor
12	Bacterial blight	*Pseudomonas avenae* subsp. avenae	Minor
13	Leaf rust	*Puccinia polysora*	Minor
14	Red rot	*Epicoccum nigrum*	Minor
15	Rust	*Physopella pallescens*	Minor
16	Aspergillus rot	*Aspergillus spp.*	Minor
17	Downy mildew	*Peronosclerospora maydis*	Minor

4.4 Identification of maize diseases during field visit

4.4.1 Curvularia leaf spot

Small necrotic or chlorotic spots with a light colored halo. Lesions are about 0.5 cm in diameter when fully developed. The disease is caused by *Curvularia lunata*. Mycelium and conidia are septate and dark colored (Plate 1).

Plate 1.



A. Maize leaf showing symptoms of curvularia leaf spot (*Curvularia lunata*), B. Conidia and mycelium of *Curvularia lunata*

4.4.2 Cob sheath blight

The disease was recognized by presence of concentric spots that cover large areas of infected cob husk and conspicuous, light brown, cottony mycelium with small, round, black sclerotia on brownish rotting ears (Plate 2).

Plate 2. Symptom of Cob Sheath Blight (*Rhizoctonia solani*)

4.4.3 Maize stripe virus

The disease was recognized by the presence of narrow parallel chlorotic stripes along the younger leaves. The chlorotic bands were dissimilar in width and were extended from the base to the tip of the leaves (Plate 3).

Plate 3. Maize stripe symptoms on maize leaf (Maize stripe virus)

4.4.4 Gray leaf spot

The disease was recognized by the elongated brown-gray necrotic spots which grown parallel to the veins. But it was observed that at primary stage those spots were small and regular (Plate 4).

Plate 4. Gray leaf spot symptoms on leaf of Maize (*Cercospora zeae-maydis*)

4.4.5 Turcicum leaf blight

The disease was recognized by slightly oval, water-soaked, small spots produced on the leaves at primary stage and then it was turned elongated, spindle-shaped necrotic lesions on leaf blade at advance stage.

Plate 5. Symptom of Turcicum leaf blight (*Helminthosporium turcicum*)

4.4.6 Sheath blight

The disease was recognized by presence of concentric spots that cover large areas of infected leaves, husks and conspicuous, light brown, cottony mycelium with small, round, black sclerotia on brownish rotting ears.

Plate 6. Symptom of Sheath Blight (*Rhizoctonia solani*)

4.4.7 Seedling blight

The disease was recognized by the presence of cottony mycelium at decayed base of young seedling which leaves were redish yellow colored. It was serious problem in maize field.

Plate 7. Symptom of Seedling blight caused by *Pythium spp*.

4.4.8 Brown spot of maize

The disease was recognized by the presence of circular and dark brown spots on mid ribs while lesions on the laminae continue as chlorotic spots. It was observed that at primary stage symptoms develop on leaf blades and consist of small chlorotic spots, arranged as alternate bands of diseased and healthy tissue.

Plate 8. Brown spot symptoms on leaf of Maize (*Physoderma maydis*)

4.4.9 Smut of maize

The disease was recognized by the presence of closed white galls (which replaced individual kernels) and fibers in infected ears. When the galls were cut with knife, black masses of spores were released from those galls. This disease was present in Manikgong, Chuadanga and Kustia (distrists) of Bangladesh.

Plate 9. Symptom of Smut of Maize (*Ustilago maydis*)

4.4.10 Ear rot of Maize

The disease was recognized by the presence of black, powdery masses of spores that cover both kernels and cob. Several species of *Aspergillus* were found in infected maize in the field. *Aspergillus niger* was the most common. This disease more or less present at major maize growing area in Bangladesh.

Plate10. Symptom of Ear rot of Maize (*Aspergillus niger*)

4.4.11 Bacterial leaf blight

The disease was recognized by the presence of several small, pale-green lesions and dry-brown conspicuous striping along with the veins. It was observed that top most leaves are more susceptible to this disease.

Plate 11. Symptom of Bacterial Blight (*Pseudomonas rubrilineans*)

4.4.12 Maize mosaic virus

The disease was recognized by dwarfing and striping along the veins. The stripes were dark yellow and finally became necrotic. It was present in all major maize growing area.

Plate 12. Symptom of Mosaic of maize (Maize mosaic virus)

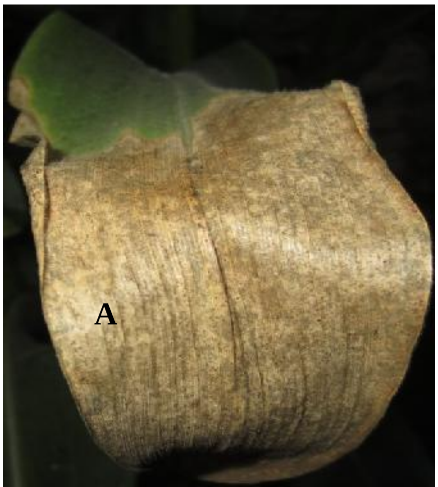
4.4.13 Anthracnose of Maize

The disease was recognized by the presence of irregular, oval-to-elongated lesions up to 15 mm long and had tan centers with reddish-brown borders and entire leaf became blighted later. But at early stage water-soaked and oval lesions were present in lower leaf. This disease was present at all major maize growing area in Bangladesh.

Plate 13. Symptom of Anthracnose of Maize (*Colletotrichum graminicola*)

4.4.14 Maydis leaf blight

The disease was recognized by the presence of rectangular and 2 to 3 cm long lesions on the leaf. It was small and diamond shaped at primary stage. At advance stage it was produced a complete burning of large areas of the leaves.



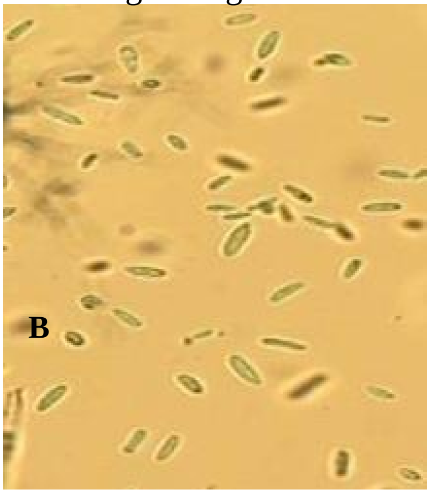


Plate 14. A. Symptom of Maydis leaf Blight (*Bipolaris maydis*), B. Conidia of *Bipolaris maydis*

4.4.15 Stalk rot

The disease was recognized by presence of water soaked, soft and dark brown lesions in lowest internodes at wilted plant and browning of phloem tissue. Internode was twisted and distorted. In infected field some plants were subjected to lodging.

Plate 15. Symptom of stalk rot of maize (*Fusarium spp.*)

4.5 Germination Percentage of Imported Maize Seed

The germination percentage of ten maize seed samples ranged from 0 – 97 (Table 4 and Plate 16). The maize variety Konok showed highest (97%) germination followed by Asha-3501 (87%) Victory seed (82%). These three maize varieties showed more than 80% germination and the rest seven varieties showed less than 80% germination. The variety Mukta 980 failed to germinate.

Table 4. Germination of maize seeds collected from seed dealers and maize growers

SL#	Variety	Country of origin/Imported from	Germination (%)	
			Germinated	Non-germinated
1	Konok Hybrid	India	97	03
2	Chamak	India	70	30
3	Victory seeds,VHM759	India	82	18
4	ASHA-3501 Hybrid	India	87	13
5	C-922	India	45	55
6	Pacific 60	Thailand	65	35
7	*Mukta 980	India	-	100
8	China seed Raza 777	China	61	39
9	Monimukta Hybrid	India	68	32
10	Shadhino-445	India	50	50

star mark (*) indicates failure to germinate

Plate  **16.**

Germination of maize seed on blotter: A. Treated imported hybrid seed, B. Untreated farmer's seed and C. Fungal association on seed surface.

4.6 Associated microorganisms in imported maize seeds

There were four fungi and one bacterium associated with the seeds of different varieties of imported maize (Table 5). Growth habitat of seed borne microorganisms in Blotter method has been shown in Plate 1. The genera of fungi were *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus spp.* and *Fusarium moniliforme* and the bacterium was *Xanthomonas sp.*. The highest incidence of *Aspergillus spp.* was found in maize variety BADC Khoi Bhutta (66%), *Penicillium spp.* in Pacific 759 (6%), *Rhizopus spp.* in RAZA-777 (41%) and *Fusarium moniliforme* in ASA 3501 (40%). The fungi found in seeds can cause seed rot, grain rot and storage rot of maize seeds. All the seed variety was collected from local market and was found in packet form. After opening the packet the seeds were found treated with fungicides. It appeared from the observation that though the seeds were treated with fungicide, the seeds were not free from infection.

Table 5. Associated microorganisms in imported maize seeds collected from different locations

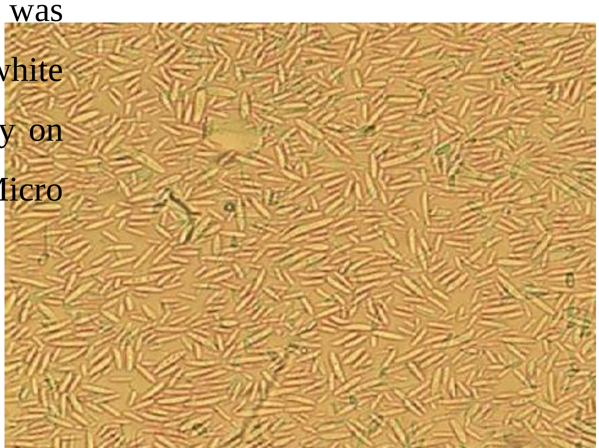
S	Variety	Country of	Associated mycroflora (%)

L#		origin/ Imported from	*Aspergillus spp.*	*Penicillium spp.*	*Rhizopus spp.*	*Fusarium moniliform*
1	Konok Hybrid	India	14	4	0	0
2	BARI Hybrid M-5	Bangladesh	55	0	6	-
3	Uttaran(BRAC)	Bangladesh	12	-	2	2
4	ASHA-3501 Hybrid	India	50	-	2	40
5	C-222	India	9		4	2
6	*Pacific 60	Thailand	-	-	-	-
7	Mukta 980	India	14	6	24	4
8	China seed Raza 777	China	11	5	41	3
9	Monimukta Hybrid	India	65	-	8	-
10	Pacific-984	India	26	-	14	4
11	Pacific-759	India	14	6	10	10
12	999	Unknown	34	4	8	4
13	Seed Tech	India	16	1	1	6
14	Horirampur/Batirampur	Unknown	27	4	-	31
15	BADC Khoi Bhutta	Bangladesh	66	2	50	-

4.7 Identification of seed borne fungi of maize seed collected from study area

4.7.1 Fusarium seed rot

The fungus *Fusarium oxysporum* was identified by the presence of white coloured mycelium grown abundantly on the surface of seeds (Plate 17A). Micro



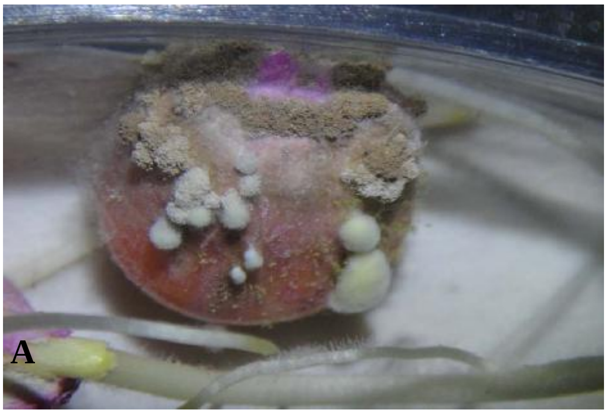
and macroconidia produced in abundance. Microconidia were oval-shaped, elliptical or reniform. Macroconidia were hyaline, thin walled, 3-5 septate and falcate to almost straight (Plate 17B).

A B

Plate 17. A. *Fusarium oxysporum* on seed, B. Macroconidia of *Fusarium oxysporum*.

4.7.2 Penicillium seed rot

Seeds of maize was investigated and found heavily infected and rotted by *Penicillium spp.* Characteristic whitish mycelial growth on seed surface (Plate 18A). Broom like conidial growth on seed surface (Plate 18B).



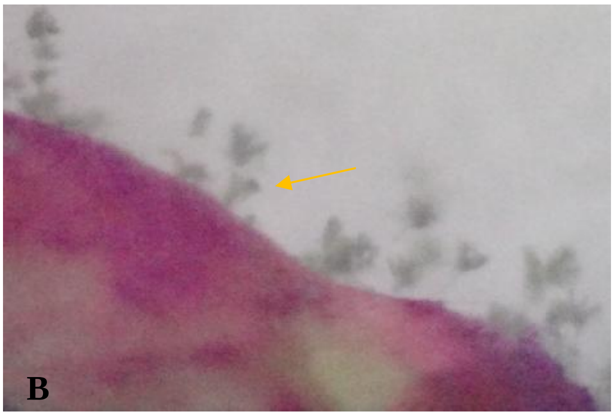


Plate 18. A. Maize seed heavily infected by *Penicillium spp.* B. Broom like growth of *Penicillium spp.* on seed surface.

4.7.3 Rhizopus rot of seed

Growth of *Rhizopus spp.* was observed on seed surface (Plate 19A). Characterized growth of sporangiospores arises from seed surface (Plate 19B).

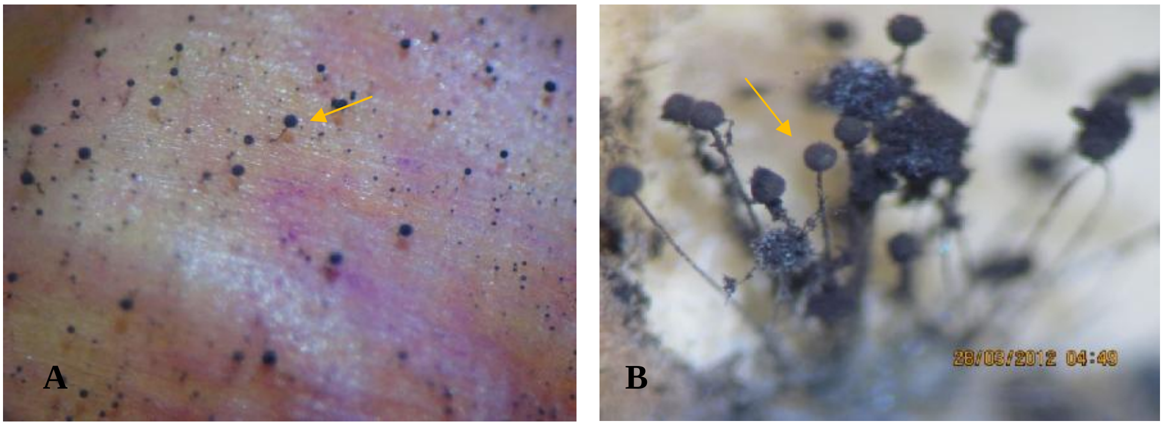


Plate 19. A. Growth of *Rhizopus spp.* on seed surface, B. Sporagiospore of *Rhizopus spp.* arises from seed surface.

4.7.4 Aspergillus seed rot

Aspergillus flavus was characterized by the presence of yellowish cream to green colored heads (Plate 20A). Conidiophores bearing the heads were conspicuously present. Conidia were globose and dark colored. *A. niger* was identified by the presence of black globose conidial heads on long erect, hyaline conidiophores (Plate 20B). Conidiophores were either alone or in groups. Conidia were globose and dark colored (Plate 20C).

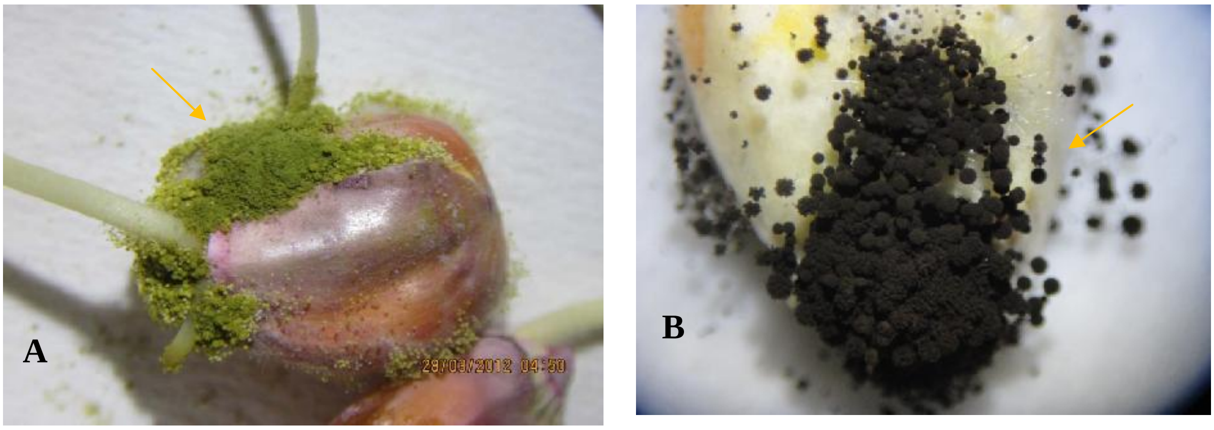


Plate 20. A. Growth of *A. flavus* on maize seed surface, B. Growth of *A. niger* on maize seed surface, C. Conidia and conidiophores of *Aspergillus spp.*

4.7.5 Bacterial seed root

Seeds were observed under stereomicroscope and found bacterial (*Xanthomonas spp.*) ooze that comes out from seed (Plate 21).

Plate 21. Bacterial ooze (*Xanthomonas spp.*) comes out from seed.

4.8 Listing of maize diseases in Bangladesh

A list of the commonly occurring diseases of maize in Bangladesh has been prepared based on survey report, Focus Group Discussion (FGD) and laboratory seed health testing. Around 30 diseases of maize with disease name, pathogenic group, causal organism and affected plant parts have been placed here under (Table 6).

Table 6. List of maize diseases in Bangladesh

Pathogenic group	Disease	SL #	Pathogen	Plant Parts affected
Fungi	Foot rot	1	*Fusarium spp.*	Root
		2	*Pythium spp.*	
		3	*Rhizoctonia solani*	
	Stalk rot	4	*Pythium aphanidermatum*	Stalk (Internode & node)
		5	*Fusarium moniliformae*	
	Leaf blight	6	*Bipolaris turcicum*	Leaf
	Brown spot	7	*Bipolaris maydis/Physoderma maydis*	Leaf
	Leaf spot	8	*Curvularia lunata*	Leaf
		9	*Cercospora sp.*	
		10	*Phyllosticta maydis*	
	Downy mildew	11	*Sclerospora philippinensis*	leaf
	Rust	12	*Puccinia polysoa*	Leaf
		13	*P. sorghi*	
	Smut	14	*Ustilago maydis*	Leaf or cob & grain
	Anthracnose	15	*Colletotrichum graminicola*	Leaf
	Cob rot/grain rot	16	*Aspergillus niger*	Cob and grain
		17	*A. parasiticus*	
		18	*A. flavus*	
		19	*A. ochraceous*	
		20	*Penicillium spp.*	
		21	*Diplodia maydis*	
		22	*Cladosporium spp.*	
		23	*Fusarium sp.*	
Bacteria	Wilt	24	*Xanthomonas spp.*	Plant
	Stalk rot	25	*Xanthomonas spp.*	Stalk
Virus	Mosaic	26	Virus	Stalk and leaf
	Dwarf	27	Virus	Leaf

	mosaic			
	Streak virus	28	Virus	leaf
Nematode	Root-lesion	29	*Tylenchorynchus zeae*	Root
	Root-knot	30	*Meloidogyne spp.*	Root

4.9 Pathogen risk analysis

Pathogen risk analysis was conducted according to the rules of ISPM No. 2. As per ISPM No.2 for risk analysis the pathogen status of importing country is taken into consideration. Bangladesh import maize from America, Australia, China and India.

4.10 Risk identification

An assessment found at least 428 potential pathogens associated with maize (Annonymous, 1998). These microorganisms were assessed for their presence in the maize exporting countries and Bangladesh, their ability to be transported with seed and bulk maize grain, and their ability to cause significant losses. The assessments for the 373 microorganisms that have been reported in the maize exporting countries are in Table 7. 16 pathogens on maize grain from the maize exporting countries which have quarantine risk to Bangladesh are in Table 8.

Table 7. Quarantine status of pathogens associated with bulk grain imports from different countries

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
BACTERIA			
Acidovorax avenae subsp. avenae	bacterial leaf blight	1,2,3,4	no
Bacillus subtilis	kernel rot; blight	1,2	no
Burkholderia andropogonis	bacterial stripe	1,2	no
Clavibacter michiganensis subsp. nebraskensis	Goss's bacterial wilt and blight	1,2,3	no
Erwinia carotovora subsp. Carotovora	bacterial stalk and top rot	1,2,3,4	yes
Erwinia chrysanthemi pv. zeae	bacterial stalk and top rot	1,2	no
Erwinia dissolvens	bacterial stalk rot	1,3,4	no
Erwinia herbicola	halo blight of corn	1,2	no
Pantoea stewartii subsp. stewartii	Stewart's bacterial wilt	1	no

Pseudomonas syringae pv. lapsa	bacterial stalk rot	1,4	no
Pseudomonas syringae pv. syringae	holcus bacterial spot	1,2,4	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Pseudomonas syringae pv. Coronafaciens	chocolate spot	1,2	no
Xanthomonas vasicola pv. holcicola	bacterial leaf spot	1,2,3	no
Xanthomonas stewartaii	wilt	3,4	yes
FUNGUS			
Absidia corymbifera		1	no
Absidia repens		1	no
Acremonium strictum	black bundle	1,2	no
Acremonium zeae	Acremonium stalk rot	1,2	no
Acrodictys erecta		1	no
Actinomucor elegans		1,2	no
Alternaria alternate	Alternaria leaf blight	1,2,3,4	no
Alternaria longissima	stalk rot	1,2,3	no
Ascochyta ischaemi	yellow leaf blight	1	no
Ascochyta maydis	Ascochyta leaf blight	1,3,4	no
Ascochyta tritici	Ascochyta leaf spot	1,4	no
Ascochyta zeicola		1,2	no
Aspergillus alliaceus		1,2	no
Aspergillus caespitosus		1	no
Aspergillus candidus		1,2	no
Aspergillus carbonarius		1,2	no
Aspergillus chevalieri		1	no
Aspergillus clavatus		1,2,3	no
Aspergillus echinulatus		1	no
Aspergillus elegans		1	no
Aspergillus equitis		1,2	no
Aspergillus flavipes		1,2	no
Aspergillus flavus		1,2,4	no
Aspergillus fumigatus		1,2	no
Aspergillus glaucus	Aspergillus ear rot; yellow Mould	1,2	no
Aspergillus hollandicus		1,2	
Aspergillus mangini		1	no
Aspergillus flavus		3,4	yes
Aspergillus nidulellus		1,2	no
Aspergillus niger	Aspergillus ear rot; black mould	1,2,3,4	yes

Aspergillus ochraceus		1,2,3	yes
Aspergillus parasiticus		1,2,3	yes
Aspergillus reptans		1,2	no
Aspergillus restrictus		1,2	no

Table 7 (cont'd)

Pathogen	**Disease**	**Present in Maize growing countries***	**Present in Bangladesh**
Aspergillus rubrobrunneus		1	no
Aspergillus stellifer		1	no
Aspergillus sulphureus		1	no
Aspergillus sydowii		1,2	no
Aspergillus tamarii		1	no
Aspergillus unguis		1	no
Aspergillus ustus		1,2	no
Aspergillus versicolor		1,2	no
Aspergillus wentii		1,2	no
Aureobasidium pullulans	brown spot	1,2	no
Aureobasidium zeae	eye spot; brown spot	1	no
Basidiobotrys pallida		1	no
Bipolaris australiensis	leaf spot	1,2	no
Bipolaris cynodontis	leaf spot	1,2	yes
Bipolaris hawaiiensis	Helminthosporium leaf spot	1,2	no
Bipolaris maydis	southern leaf blight	1,2	yes
Bipolaris sacchari		1,2	yes
Bipolaris turcicum	Leaf blight	3,4	yes
Bipolaris setariae	spot blotch	1,2	no
Bipolaris sorghicola		1,2	yes
Bipolaris sorokiniana	Helminthosporium root rot	1,2,4	no
Bipolaris urochloae	leaf spot	1,2	no
Bipolaris victoriae		1,2	no
Bipolaris zeicola	northern leaf blight	1,2	no
Blakeslea trispora		1,2	no
Botryosphaeria disrupta	ear rot	1	no
Botryosphaeria festucae	ear rot	1,2	no
Botryodiplodia theobromae	Karnel rot	4	yes
Botryosphaeria quercuum	Ear rot	1,2	no
Botryosphaeria rhodina	ear rot	1	no
Botryosphaeria zeae	gray ear rot	1	no
Botrytis cineria	Botrytis stalk rot	1,2	no
Byssochlamys nivea		1	no
Candida albicans		1	no
Candida guilliermondii		1	no
Candida intermedia		1	no

Candida krusei		1,2	no
Candida parapsilosis		1	no
Candida pseudotropicalis		1	no
Ceratocystis paradoxa	leaf spot	1,2	no
Cercospora sorghi	Gray leaf spot	1,2,4	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Cercospora zeae-maydis	gray leaf spot	1	no
Chaetomium bostrychodes		1	no
Chaetomium brasiliense		1	no
Chaetomium dolichptrichum		1	no
Chaetomium funicola		1,2	no
Chaetomium globosum		1,2	no
Chaetomium indicum		1,2,4	no
Chaetomium murorum		1	no
Chaetomium torulosum		1	no
Chrysonilia sitophilia		1,2	no
Ciccinella muscae		1,2	no
Cladosporium cladosporioides	Cladosporium rot	1,2	yes
Cladosporium herbarum	cob mould	1,2	yes
Cladosporium macrocarpum	cob mould	1,2	yes
Cladosporium tenuissimum		1,2	no
Cladosporium zeae		1	no
Colletotrichum cereal		1	no
Colletotrichum graminicola	Anthracnose	1,2	yes
Coniothyrium scirpi	leaf spot	1,2	no
Corynascus sepedonium		1,2	no
Cryptococcus laurentii		1	no
Curvularia brachyspora	leaf spot	1,2	no
Curvularia clavata	leaf spot	1,2	no
Curvularia eragrostidis	Curvularia leaf spot	1,2	no
Curvularia geniculata	Curvularia leaf spot	1,2	yes
Curvularia gudauskasii	leaf spot	1,2	no
Curvularia inaequalis	Curvularia leaf spot	1,2	no
Curvularia intermedia	Curvularia leaf spot	1,2	no
Curvularia lunata	Curvularia leaf spot	1,2	yes
Curvularia pallescens	Curvularia leaf spot; leaf spot of maize; corn leaf spot	1,2,4	no
Curvularia senegalensis	Curvularia leaf spot	1,2	no
Curvularia tuberculata	leaf spot	1,2	no
Dendrophoma zeae		1,2	no
Diaporthe phaseolorum	Seedling blight	1,2	no
Dictyochaeta fertilis	root rot	1,2	no
Dictyochora gambellii		1,2	no

Didymella exitialis	Didymella leaf spot	1,2	no
Didymium iridis		1	no
Didymosphaeria graminicola		1	no
Diplodia maydis	Diplodia ear and stalk rot	1,2,3,4	yes
Doratomyces stemonitis	Ear rot	1,2	no

Table 7 (cont'd)

Pathogen	**Disease**	**Present in Maize growing countries***	**Present in Bangladesh**
Epicoccum nigrum	red kernel; red kernel disease	1,2	no
Exserohilum monoceras	leaf blotch	1,2	no
Exserohilum pedicellatum	Helminthosporium root rot	1,2	no
Exserohilum prolatum	Exserohilum leaf spot	1,2	no
Exserohilum rostratum	Helminthosporium leaf disease	1,2	no
Exserohilum turcicum	northern leaf blight	1,2	no
Fusarium acuminatum	Root and stem rot	1,2	no
Fusarium avenaceum	Stalk and root rot	1,2,4	no
Fusarium chlamydosporum		1,2,3,4	yes
Fusarium crookwellense	stem rot	1,2	yes
Fusarium culmorum	Stalk rot	1,2	no
Fusarium episphaeria	Stalk rot	1,2	no
Fusarium equiseti	Stalk rot	1,2	no
Fusarium graminearum	Gibberella stalk rot; red ear rot; pink ear rot	1,2	yes
Fusarium merismoides	stalk rot	1,2	no
Fusarium moniliforme	Fusarium ear and stalk rot; Fusarium kernel rot	1,2,4	yes
Fusarium oxysporum	Root rot	1,2,4	yes
Fusarium pallidoroseum	Root rot	1,2	no
Fusarium poae	white cob rot; silver top	1,2,3	yes
Fusarium proliferatum	root rot	1,2	no
Fusarium roseum	Root rot	1,2,4	yes
Fusarium sacchari		1	no
Fusarium subglutinans	Fusarium stalk and ear rot	1,2,4	no
Fusarium tricinctum	root rot	1,2	no
Fusisporium cerealis		1	no
Fusarium solani	Stalk rot	1,2,3,	yes
Gaeumannomyces graminis	root rot	1,2	no
Geotrichum candidum	stalk rot	1,2,3	no
Gibberella cyanogena	root rot	1,2	no
Gibberella pulicaris	root rot	1,2	no
Gibberella zeae	Seedling blight, cob rot	3,2,4	yes

Glabrocyphella ellisiana		1	no
Gloeocercospora sorghi	zonate leaf spot	1	no
Glomerella tucumanensis		1,2	no

Table 7 (cont'd)

Pathogen	**Disease**	**Present in Maize growing countries***	**Present in Bangladesh**
Gonatobotrys simplex	Gonatobotrys seed rot	1,2	no
Gonatobotrys zeae	Gonatobotrys seed rot	1	no
Graphium penicillioides	leaf spot	1	no
Hansenula anomala		1	no
Harzia acremonioides		1,2	no
Helminthosporium ahmadii		1	no
Illosporium pallidum		1	no
Isariopsis subulata		1	no
Lasiodiplodia theobromae	black kernel rot	1,2	no
Lecanidion atratum		1,2	no
Leptosphaeria macrospora	leaf spot	1	no
Leptosphaeria maydis	leaf spot	1	no
Leptosphaeria variisepta	Leptosphaeria leaf spot	1	no
Leptosphaerulina trifolii		1,2	no
Leptothyrium zeae	leaf spot	1	no
Ligniera junci		1	no
Lophiosphaera zeicola		1	no
Lophiostoma arundinis		1	no
Macrophomina phaseolina	charcoal rot	1,2,4	no
Macrosporium maculatum		1	no
Marasmius graminum	seedling and foot rot	1,2,4	no
Marasmius sacchari	Marasmius root and stalk rot	1,2,3,4	no
Mariannaea elegans	stalk rot	1,2,3,4	no
Massarina arundinacea		1	no
Melanospora zamiae		1	no
Microascus cinereus		1	no
Microascus cirrosus		1	no
Microascus desmosporus		1	no
Microascus longirostris		1	no
Microdochium bolleyi	Microdochium root rot	1,2	no
Microdochium nivale	Microdochium root rot	1,2	no
Monascus purpureus	silage mold	1	no
Monascus ruber	silage mold	1	no
Mucor circinelloides		1,2	no
Mucor fragilis	seedling rot	1,2,3,4	no

Mucor heimalis		1	no
Mucor mucedo		1	no
Mucor plumbeus		1,2,3,4	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Mucor racemosus		1,2,3,4	no
Mycosphaerella zeae	leaf blight	1,3,4	no
Myrothecium cinctum	root rot	1,2	no
Myrothecium gramineum	shuck rot	1	no
Myrothecium verrucaria	root rot	1,2,4	no
Nigrospora oryzae	Nigrospora ear rot	1,2	no
Nigrospora sphaerica	stalk rot	1,2	no
Olpitrichum macrosporum		1,2	no
Olpitrichum tenellum		1	no
Ophiliosphaerella herpotricha		1	no
Paraphaeosphaeria michotii	leaf spot	1,2	no
Penicillium aurantiogriseum		1,2	no
Penicillium brevicompactum		1,2	no
Penicillium canescens		1,2	no
Penicillium chrysogenum		1,2,3,4	yes
Penicillium citrinum		1,2,4	no
Penicillium clarviforne		1,3	no
Penicillium crustosum		1,2	no
Penicillium expansum	Penicillium ear rot	1,2,3	yes
Penicillium felludanum		1	no
Penicillium funiculosum		1,2	no
Penicillium glabrum		1	yes
Penicillium granulatum		1	no
Penicillium grisefulvum		1	no
Penicillium herquei		1	no
Penicillium implicatum		1	no
Penicillium janthinellum		1,4	no
Penicillium oxalicum		1	no
Penicillium puberulum		1	no
Penicillium purpurogenum		1	no
Penicillium roquefortii		1	no
Penicillium rugulosum		1	no
Penicillium sclerotiorum		1,4	no
Penicillium thomii		1	no
Penicillium variabile		1	no
Penicillium verrucosum		1	no
Penicillium viridicatum		1	no
Penicillium waksmanii		1	no

Perichaena vermicularis		1,2	no
Periconia circinata	root rot	1,2	no
Periconia macrospinosa		1,2	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Perisporium zeae		1	no
Peronosclerospora sorghi	sorghum downy mildew	1,3,4	no
Phaeocytostroma ambiguum	Phaeocytosporella stalk infection	1,2	no
Phaeosphaeria eustoma	Phaeosphaeria leaf spot	1,2	no
Phaeosphaeria herpotricha	Phaeosphaeria leaf spot	1,2	no
Phaeotrichoconis crotalariae		1,2	no
Phoma americana	root rot	1	no
Phoma terrestris	pink root; stalk rot	1,2,3,4	no
Phoma zeicola	root rot	1	no
Phomopsis sp.	Phomopsis seed rot	1,2	no
Phycomyces nitens		1,2	no
Phyllosticta maydis	yellow leaf blight	1,3	yes
Phyllosticta zeae	Phyllosticta leaf spot	1	no
Phymatotrichopsis omnivora	root rot	1	no
Physalospora abdita		1	no
Physarum pusillum	slime mould	1,2	no
Physoderma maydis	brown spot of maize	1,2,4	yes
Physopella pallescens	leaf rust	1	no
Phytophthora cactorum	root rot	1,2	no
Phytophthora drechsleri	root rot	1,2	no
Phytophthora nicotianae	root rot	1,2	no
Pithoascus intermedius		1	no
Pithoascus schumachrei		1	no
Pithomyces maydicus	ear rot	1	no
Pleospora straminis		1	no
Podospora minor		1	no
Polyschema olivacea		1	no
Puccinia polysora	Rust of maize	1,2,3,4	yes
Puccinia sorghi	common maize rust	1,2,3,4	yes
Pyricularia grisea	white leaf spot	1,2	no
Pyronema omphalodes		1,2	no
Pythium acanthicum	root rot	1	yes
Pythium adhaerens	root rot	1	no
Pythium angustatum	root rot	1	yes
Pythium aphanidermatum	Pythium stalk rot	1,2,4	yes
Pythium arrhenomanes	root rot	1,2	no
Pythium graminicola	root rot	1,2,4	yes

Pythium irregulare	seedling blight, damping off	1,2	yes
Pythium myriotylum	root rot	1,2	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Pythium paroecandrum	root rot	1	no
Pythium pulchrum	root rot	1,2	no
Pythium rostratum	root rot	1,2	yes
Pythium splendens	root rot	1,2	no
Pythium sylvaticum	Seed rot	1	no
Pythium ultimum	root rot	1,2	no
Ramulispora sorghi	brown leaf spot	1,2	no
Rhizoctonia solani	Rhizoctonia foot & root rot	1,2,3,4	yes
Rhizoctonia zeae	sclerotial rot	1,2,3,4	no
Rhizopus arrhizus	Rhizopus ear rot	1,2	no
Rhizopus microsporus	Rhizopus ear rot	1	no
Rhizopus microsporus	Rhizopus ear rot	1	no
Rhizopus stolonifer	Rhizopus ear rot	1,2,3,4	no
Rhopographus zeae	stalk rot	1	no
Sclerophthora macrospora	crazy top	1,2	no
Sclerospora graminicola	Graminicola downy mildew; green ear	1,4	yes
Sclerotinia sclerotiorum	Sclerotinia stalk rot	1,2,3,4	no
Sclerotium rolfsii	Sclerotium ear rot	1,2	no
Scopulariopsis brevicaulis	ear rot	1,2,3,4	no
Scopulariopsis brumptii	ear rot	1,2,3,4	no
Septoria zeae	leaf spot	1	no
Septoria zeicola	leaf spot	1,2	no
Septoria zeina	leaf spot	1	no
Sphaerella paulula		1	no
Sporidesmium folliculatum		1	no
Sporisorium holci-sorghi	head smut	1,2,3,4	no
Stachybotrys zeae		1	no
Stauronema cruciferum		1	no
Stenocarpella macrospora	Diplodia ear and stalk rot	1,2,3	no
Stenocarpella maydis	Diplodia ear and stalk rot	1,2,3	no
Sterile white basidiomycete	SWB root rot	1	no
Stictis radiata		1	no
Stictis stellata		1,2	no
Syncephalastrum racemosum		1,2	no
Talaromyces luteus		1	no
Talaromyces stipitatus		1	no

Thamnidium elegans		1	no
Trichoderma koningii		1,2	no
Trichoderma viride	Trichoderma ear rot	1,2,4	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Trichothecium roseum	pink mould	1,2	no
Tritirachium oryzae		1,4	no
Tubeufia cylindrothecia		1	no
Typhula phacorrhiza	snow mould	1	no
Ulocladium lanuginosum		1	no
Ustilaginoidea virens	false smut	1,3,4	no
Ustilago zeae	smut	1,2,3,4	yes
Verticillium tenerum		1,2	no
Wolfiporia cocos	wood rot	1	no
NEMATODES			
Belonolaimus longicaudatus	sting nematode	1,2	no
Criconema mutabile	ring nematode	1,2	no
Ditylenchus dipsaci	bulb and stem nematode	1,2,4	no
Dolichodorus heterocephalus	awl nematode	1	no
Filenchus exiguus		1,2	no
Helicotylenchus multicinctus	spiral nematode	1,2,3,4	no
Helicotylenchus multicinctus	spiral nematode	1,2	no
Helicotylenchus pseudorobustus	spiral nematode	1,2	no
Heterodera avenae	cereal cyst nematode	1,2	no
Heterodera zeae	corn cyst nematode	1	no
Hoplolaimus columbus	lance nematode	1	no
Hoplolaimus galeatus	lance nematode	1	no
Longidorus breviannulatus	needle nematode	1	no
Macroposthonia ornata	ring nematode	1,2	no
Meloidogyne arenaria	root-knot nematode	1,2	no
Meloidogyne chitwoodi	root-knot nematode	1	no
Meloidogyne incognita	root-knot nematode	1,2,3,4	yes
Meloidogyne javanica	root-knot nematode	1,2,3,4	yes
Nacobbus dorsalis		1	no
Paratrichodorus christiei	stubby-root nematode	1	no
Pratylenchus brachyurus	root lesion nematode	1,2	no
Pratylenchus crenatus	root lesion nematode	1,2	no
Pratylenchus hexincisus	root lesion nematode	1,2	no
Pratylenchus neglectus	root lesion nematode	1,2	no
Pratylenchus penetrans	root lesion nematode	1,2	no
Pratylenchus scribneri	root lesion nematode	1	no
Pratylenchus thornei	root lesion nematode	1,2	no
Pratylenchus zeae	root lesion nematode	1,2	no

Quinisulcius acutus	stubby-root nematode	1	no
Radopholus similis	burrowing nematode	1,2	no
Rotylenchulus parvus	reniform nematode	1,2	no

Table 7 (cont'd)

Pathogen	Disease	Present in Maize growing countries*	Present in Bangladesh
Tylenchorhynchus dubius	stunt nematode	1,2	yes
Xiphinema americanum	dagger nematode	1,2	no
PHYTOPLASMA			
Maize bushy stunt phytoplasma	maize bush stunt	1,3,4	no
Spiroplasma kunkelii	corn stunt	1	no
VIRUSES			
Brome mosaic bromovirus (BMV)	brome mosaic	1,2	no
Cucumber mosaic cucumovirus (CMV)	cucumber mosaic	1,2,3	no
High Plains virus	High Plains disorder	1	no
Johnsongrass mosaic potyvirus (JGMV)	Johnson grass mosaic	1,2	no
Maize chlorotic dwarf waikavirus (MCDV)	maize chlorotic dwarf	1,3,4	no
Maize chlorotic mottle machlomovirus (MCMV)	maize chlorotic mottle	1,3,4	no
Maize dwarf mosaic potyvirus (MDMV)	maize dwarf mosaic	1,3,4	yes
Maize mosaic nucleorhabdovirus	maize mosaic	1	yes
Maize rayado fino marafivirus (MRFV)	maize rayado fino	1	no
Maize stripe tenuivirus (MSpV) maize	maize stripe	1,2,4	no
Maize white line mosaic satellivirus	maize white line mosaic	1	no
Maize white line mosaic virus (MWLMV)	maize white line mosaic	1,3	no
Wheat streak mosaic rymovirus (WSMV)	wheat streak mosaic	1,4	yes
Wheat striate virus (WStMV)	wheat striate	1,4	no
Barley yellow dwarf luteovirus (BSMV)	barley yellow dwarf	1,2	no

*NOTE: America=1, Australia=2, China=3, India=4

Table 8. Qualitative analysis of the relative risk to Bangladesh of 16 quarantine pathogens on maize grain from the maize exporting countries

Pathogen (hosts)	Disease Introduction Risks	Economic Damage Risks	Disease Management costs	Overall Risk
Peronosclerospora sorghi(downy mildew of maize, sorghum)	very high	high	high	very high
Maize dwarf mosaic potyvirus	extremely high	medium	medium to high	high
High Plains virus (maize, wheat)	high	high	low to medium	medium to high
Wheat streak mosaic rymovirus (WSMV) (maize, wheat)	very high	high	low to medium	medium to high
Sclerospora graminicola (maize, sorghum, pearl millet and many grasses)	medium to high	high	medium	medium to high
Phymatotrichopsis omnivore (Texas root rot of cotton and other dicotyledonous plants)	medium	high	medium	medium
Maize chlorotic mottle machlomovirus (maize)	very high low to	medium	low	medium
Cercospora zeae-maydis (gray leaf spot of maize)	high	low to medium	low to medium	medium
Pantoea stewartii subsp. stewartii (Stewart's wilt of sweet corn)	medium to high	medium	low to medium	medium
Clavibacter michiganensis subsp. nebraskensis (Goss's bacterial wilt of maize)	high	low	low to medium	medium
Heterodera zeae (maize cyst nematode)	low	low	low	low
Ustilaginoidea virens (false smut of maize)	low to medium	low	extremely low	low
Dolichodorus heterocephalus (awl nematode)	very low	very low	very low	very low
Hoplolaimus columbus (lance nematode)	very low	very low	very low	very low
Longidorus breviannulatus (needle nematode)	very low	very low	very low	very low
Pratylenchus scribneri (root lesion nematode)	very low	very low	very low	very low

CHAPTER V

DISCUSSION

The changing global scenario is compelling policymakers to adhere to the regulations and obligations set by the World Trade Organization (WTO) (Pierce and Nicolaidis, 2009). To satisfy the prerequisite the WTO for maize trade, it is necessary to conduct pathogen risk analysis of maize in Bangladesh. Principles of plant quarantine as related to international trade were constituted as ISPM-1 in 1995 (FAO). In terms of international trade in seed for planting, the probability of introducing (entry and establishment) of new pathogen to a new area as a result of seed transmission is high (Pataky and Ikin, 2003). Pest risk analysis (PRA) provides the rational phytosanitary measures for a specified PRA area. It evaluates scientific evidence to determine whether an organism is a pest. If so, the analysis evaluates the probability of introduction and spread of the pest and the magnitude of potential economic consequences in a defined area, using biological or other scientific and economic evidence. If the risk is deemed unacceptable, the analysis may continue by suggesting management options that can reduce the risk to an acceptable level. Subsequently, pest risk management options may be used to establish phytosanitary regulations (ISPM No. 2, 2007). “Pest Risk Analysis (PRA), in particular pathogen of Maize and listing of Quarantine pathogen was undertaken to identify the diseases of maize in major maize growing regions of Bangladesh. Under the present study surveys was conducted in 40 Upazilla under 20 districts considering 2 Upazilla from each of the districts. Surveys were conducted to find out the present status of the disease(s) of maize in the field through questionnaires, interviews and FGD. The two categories of respondents namely, Farmers and Policy Level Officers were interviewed and Focus Group Discussion (FGD) were conducted to gather information on diseases of maize, asses their risks and listing of quarantine diseases through pre-tested questionnaires. Diseased sample collected by field survey were also examined in the laboratory to resolve doubtful symptoms of different diseases. The data collected by field survey were analyzed through a computer based software SPSS.

The survey of field diseases of maize revealed that all stages of maize crops were attacked by the diseases. In this study altogether 30 maize diseases of which 23 fungal, 2 bacterial, 3 viral and 2 nemic diseases have been identified as maize diseases of Bangladesh. The association of fungal pathogen in maize was found from seedling stage to mature plant. In field condition foot rot, stalk rot, leaf spot, leaf blight, brown spot, downy mildew, rust, smut, cob sheath blight, anthracnose and cob rot are major fungal pathogenic diseases of maize. Among these fungal diseases foot rot (*Fusarium spp*), leaf spot (*Curvularia lunata*), leaf blight (*Bipolaris turcicum*), cob sheath blight (*Rhizoctonia spp.*) and cob rot (*Aspergillus flavus*) is observed more frequent in maize field while stalk rot (*Pythium aphnidermatum*), brown spot (*Bipolaris maydis*), downy mildew (*Sclerospora philippinensis)*, rust (*Puccinia polysoa*), smut (*Ustilago maydis*) and anthracnose (*Colletotrichum graminicola*) found irregularly distributed in maize field. In case of bacterial disease bacterial stalk rot (*Erwinia carotovora* f. sp. zeae) was found more than that of bacterial wilt (*Erwinia carotovora* f. sp. zeae) disease. Maize mosaic virus (MMV), Maize dwarf mosaic virus (MDMV) and Maize streak virus (MSV) were found more frequent in maize field. In some cases around70% of the maize plant in field was found damaged by Maize dwarf mosaic virus (MDMV). The nemic disease root-lesion (*Tylenchorynchus zeae*) and root-knot (*Meloidogyne spp.*) was found in very minor cases.

Diseases of maize in Bangladesh were studied and reported by few workers in recent years. Talukdar (1974) reported nine diseases viz. leaf blight, cob blight, kernel mould, smut, leaf spot, brown spot, bacterial streak, soft rot and mosaic of maize in Bangladesh. Shahjahan (1993) reported that five diseases viz. leaf blight, stalk rot, mosaic, cob rot and downy mildew seriously affect the maize crop in our country. Bari and Alam (2004) reported 28 diseases of maize. Among them 21 are caused by fungi, 2 by bacteria, 3 by virus/mycoplasma and 2 are caused by nematode. Alam *et al* (2003) reported seven diseases viz. seed rot and seedling blight, leaf blight, downy mildew, stalk rot, cob and grain rot, storage rot and mosaic commonly occurred in maize growing period and in storage.

In germination test, the seed sample showed significant rate of germination because every seed sample were treated seed. Six seed borne pathogens viz. *Aspergillus flavus, A. niger, Penicillium spp., Rhizopus spp.* and *Fusarium moniliforme* and *Xanthomonas sp.* have been detected through seed health testing even though tested seed samples were treated with fungicide. The fungi found in seeds are seed rot, grain rot and storage rot. Fakir (2001) reported that *Aspergillus spp., Penicillium spp., Rhizopus spp.* and *Fusarium moniliforme* and *Xanthomonas sp.* are seed borne pathogen of maize causes seed rot, kernel mould and storage rot. Bari and Alam (2004) identified *Aspergillus spp.* and *Fusarium spp.* as seed-borne pathogen. Appropriate measures based on the effectiveness in reducing the probability of introduction of the pest were chosen on basis of the principles of plant quarantine as related to international trade (ISPM No. 1). Phytosanitary measures shown to be cost-effective and feasible, principle of "minimal impact, reassessment of previous requirements, principle of equivalence, principle of non-discrimination. The options for measures to be applied within the importing country such as careful surveillance to try and detect the entry of the pest as early as possible, eradication programs to eliminate any foci of infestation and/or containment action to limit spread and prohibition of certain variety of hybrid Maize or seeds or plants. Quarantine certificates and other strict compliance measures should be taken. Devitalization of maize seed by grinding would be an effective strategy to prevent entry and establishment of the viral diseases (Annonymous, 1998). This strategy alone would not be fully effective for management of quarantine bacteria and fungi associated with maize grain. Heat treatment is the only viable option for minimizing risk of entry of quarantine pathogens (Anonymous, 1998). This treatment should be done either at the port of entry to Bangladesh or off shore. If it is delayed until the seed reaches the feed-lot, the possibility of grain spillage or dust discharge during handling and transport of grain presents a high risk to Bangladesh maize farming. If an incursion occurs, containment and eradication would be difficult if not impossible.

The assessment found at least 428 potential pathogens associated with maize worldwide. These microorganisms were assessed for their presence in the maize exporting countries and Bangladesh, their ability to be transported with seed and bulk maize grain, and their ability to cause significant losses. The assessments for the 373 microorganisms that have been reported in the maize exporting countries. Of these pathogenic organisms, 55 were excluded as they have not been recorded in the maize exporting countries. A further 202 were excluded because they either occur in Bangladesh, or are unlikely to enter Bangladesh in seed and bulk maize, while 106 were not examined further as there was insufficient information available to form a judgement. Of the 65 pathogens that occur in the maize exporting countries and not in Bangladesh and can occur in the pathway, 49 were excluded as they are not reported to cause significant economic losses. Sixteen pathogens were identified that are present in the maize exporting countries, can occur in the pathway, are not present in Bangladesh, and are capable of causing significant economic damage. Among the 106 pathogens with insufficient data for judgement there are several pathogens that have important pathogenic races. The status of their races in Bangladesh is unknown, and these pathogens have not been examined further in this review. If further studies show that some races in the maize exporting countries do not occur in Bangladesh, then one or more of these pathogens may need to be considered for quarantine management. In addition, there are many quarantine pathogens of other crops potentially present in admixtures likely to be in imported maize grain. Risk analyses have not been done on the 106 pathogens with insufficient data for judgement or quarantine pathogens of other crops potentially present in admixtures, as their risks would be managed by treatments to control the major maize pathogens. However, if untreated bulk maize of maize exporting countries origin, containing admixtures of other crops, were moved into agricultural areas of Bangladesh, there is significant risk that these other pathogens could be introduced.

Pathogen risk analysis shows the sixteen organisms viz. *Phymatotrichopsis omnivore*, *Cercospora zeae-maydis*, Maize dwarf mosaic potyvirus, High Plains virus, Wheat streak mosaic rymovirus, *Sclerospora graminicola*, *Phymatotrichopsis omnivore*, Maize chlorotic mottle machlomovirus, *Pantoea stewartii* subsp. *Stewartii*, *Clavibacter michiganensis* subsp. *nebraskensis*, *Heterodera zeae*, *Ustilaginoidea virens*, *Dolichodorus heterocephalus*, *Hoplolaimus Columbus*, *Longidorus breviannulatus* and *Pratylenchus scribneri* identified as potential quarantine pathogens, ranked on their likelihood of entering and causing loss in Bangladesh.

Ten of these pathogens viz. *Phymatotrichopsis omnivore*, *Cercospora zeae-maydis*, Maize dwarf mosaic potyvirus, High Plains virus, Wheat streak mosaic rymovirus, *Sclerospora graminicola*, *Phymatotrichopsis omnivore*, Maize chlorotic mottle machlomovirus, *Pantoea stewartii* subsp. *Stewartii*, *Clavibacter michiganensis* subsp. *nebraskensis* have a higher overall risk. Some have the capacity to cause serious losses on commodities of substantially higher value than maize. Some of these high risk pathogens have relatively wide host ranges, extending to sorghum, wheat and naturalised grasses such as Johnson grass. In Bangladesh there are many situations where feedlots and crops of maize and wheat are in close proximity to each other. It is useful to compare the 10 highest risk pathogens with the work of Phillips (1994). This study lists six of these pathogens as quarantine pathogens of concern but he did not include High Plains virus, *Sclerospora graminicola* and *Phymatotrichopsis omnivora*. Since this study, High Plains virus has been shown to be seed-borne, which justifies its present inclusion. The scope of the study did not cover pathogens that are not seed-borne. *S. graminicola* and *P. omnivora* are trash and soil-borne, and therefore could be present as contaminants in bulk maize.

Peronospora sorghi has been reported from USA, Latin America, North, Central and South America, Southeast Asia, India, Israel, Italy and Africa (Shurtleff, 1980). The fungus is seed-borne (Richardson, 1990). Transmission to seedlings was found when seeds from infected plants were planted immediately after harvest. It has never been found in seeds dried to 15% moisture content and below. This is a serious disease in the tropics and subtropics. Severe outbreaks have occurred in India, Israel, Mexico, Thailand, Texas and Venezuela (Frederiksen & Renfro, 1977). Worldwide, there appears to be at least two strains of *S. sorghi*, one is the sorghum-maize strain and the other is the maize-strain. The sorghum –maize strain infects both sorghum and maize. The maize strain is present in Northwestern India (Rajasthan) and Thailand (Bonde, 1982). The fungus has been recorded on Sorghum and Zea in the USA (Farr *et al.,* 1989).

Maize dwarf mosaic potyvirus (MDMPV) is seed-borne and seed transmitted (Mikel *et al.*, 1984). The disease is important in USA where yield losses have been reported (Gregory & Ayers, 1982). MDMPV is spread in maize crops by transient winged (alate) aphids (Vangessel, 1993). Alate behavior, consisting of many short flights with frequent probing, has been related to dispersal rather than to host finding. Strong correlations have been demonstrated between aphids numbers in traps and the incidence of MDMPV (Vangessel, 1993).

High Plain Virus (HPV) was first found in wheat and maize plants from Texas and Idaho, and in 1994 the disease was observed in Kansas and Colorado. By the end of 1995, HPV had been confirmed in maize and wheat samples from nearly 100 countries in an area extending from the Texas panhandle to eastern Nebraska, to central South Dakota, to western Idaho and back through Colorado to eastern New Mexico and Texas (Jensen *et al.,* 1996). Since then, it has been found more frequently over a much wider area, probably due to greater awareness and surveillance (Marcon *et al.,* 1997). Yield loss to 75% has been reported from USA (Jensen *et al.,* 1996). Systemic spread of the virus appears to be important in the severity of the disease and potential crop yield loss. Seed transmission of the virus has been demonstrated in sweetcorn (Foster *et al.,* 1996). The vector of this virus is the wheat curl mite, *Aceria tosichella* (Seifers *et al.,* 1997).

Hosts include barley, maize, oat, rye and wheat, as well as the grasses *Bromus secallinus*, *Setaria glauca* and *Setaria viridis* (Seifers *et al.,* 1998). Importation of the disease through seed poses a serious economic threat to both the maize and wheat farming in Bangladesh. HPV (High Plain Virus) is a devastating virus in susceptible maize genotypes.

Pantoea stewartii subsp. *stewartii* (Stewart's wilt of sweet corn) spreads in China, Malaysia, Thailand, Vietnam, Italy, Poland, Romania,Yugoslavia, Canada,Mexico, USA, Costa Rica, Puerto Rico, Brazil, and Peru (Bradbury, 1986). It was first report of *P. stewartii* subsp. *stewartii* isolated from diseased maize in Argentina (Orio *et al.,* 2012).The bacterium is seed-borne (Richardson, 1990) but the seed to seedling transmission rate is very low (Block, *et al.,* 1994). The bacterium overwinters in seed, soil or maize stalks. However, the main means of overwintering is in the corn flea beetle, *Chaetocnema pulicaria* Melsh. (Munkvold, *et al.,* 1996). Variability occurs in pathogenicity (Braun, 1982). Outbreaks of the disease in 1990 and 1992 caused substantial losses to the maize seed industry in Iowa. In 1995, the disease caused heavy losses in Illinios (Pataky *et al.,* 1996). *Tripsacum dactyloides, Zea mays* and *Zea mexicana,* are the natural hosts of this pathogen (Bradbury, 1986).

Ustilaginoidea virens (false smut) distributed worldwide in rice growing regions. The fungus has been reported to be seedborne in maize (Richardson, 1990). A minor disease that is favoured by hot wet weather (Sharma & Verma, 1979). This fungus has been recorded on Oryza and Zea in the USA (Farr *et al.*, 1989). False sumt is generally a minor disease of rice, but epidemics of the disease have been reported in India, Burma, Peru, and the Philippines. The fungus also infects *Digitaria adscendens, Panicum trypheron*, and wild *Oryza* spp. (Webster & Gunnell, 1992).

The pathogen *Clavibacter michiganensis* subsp. *nebraskensis* is confined to USA and is *seed-borne* (Richardson, 1990). Seed-borne inoculum is thought to be of minor significance in the epidemiology of the pathogen in areas where the pathogen is present, as the transmission rate in seed appears to be low. This may explain why the pathogen has not become widespread in the USA and the world (Biddle *et al*. 1990). The bacterium can overwinter in maize crop residues, which are the most important inoculum source (Smidt & Vidaver, 1986). Within fields, the main source of inoculum is plant debris, with the pathogen possibly being dispersed by wind and rain. Seed transmission may spread the disease over large areas. Losses are generally minor, but may be severe in individual fields (Wysong *et al.* 1973). Losses as great as 50% attributable to this disease have been mitigated in field maize in recent years through the use of resistant germ plasm (Smidt & Vidaver, 1986). Variation in pathogenicity (Schuster *et al.* 1972) and the occurrence of different strains has also been reported (Vidaver *et al.*, 1981). Maize is the only natural host of this pathogen (Bradbury, 1986).

Dolichodorus heterocephalus is an ectoparasite and normally inhabiting wet locations such as swamps, marshes and the edges of lakes and streams. This nematode has been recorded principally in the USA, where it is mainly confined to the eastern States, particularly Florida also recorded in South Africa. Although *Dolichodorus heterocephalus* can be devastating where it occurs, outbreaks are localised and it does not seem to be common or widespread enough to make it a pest of major importance. Field damage has been noted on celery, sweetcorn and water chestnut (Orton Williams, 1974b). Severe damage can result from relatively small populations (Shurtleff, 1980). Cyst nematode (*Heterodera zeae*) has been reported on maize in Egypt (Kheir *et al*., 1989), India (Bajaj & Gupta, 1994), Pakistan (Maqbool, 1981), Thailand (Chinnasri *et al*., 1995), and Maryland (Sardanelli *et al*., 1981) and Virginia (Eisenback *et al*., 1993) in the USA. Races of the nematode have been reported (Bajaj & Gupta, 1994).

Hoplolaimus columbus is root parasite exhibiting both ecto and endoparasitic habits. This nematode is an important parasite of soybean and cotton in Georgia and South Carolina in the USA (Fassuliotis, 1976). Weeds and cover crops serve as overwintering hosts (Fassuliotis, 1976). Wide host range, including maize (Lewis & Smith, 1976).

Longidorus breviannulatus is an ectoparasite. It can be a devastating pathogen of maize. Occurs mainly in temperate regions around the world. *Longidorus breviannulatus* is known to attack maize in Delaware, Illinois and Iowa in the USA. Maize, grasses, potato, celery, grape, lettuce, and many other plants are hosts of this nematode (Shurtleff, 1980).

Pratylenchus scribneri is widespread in the USA. Also recorded in Africa, Bulgaria, Egypt, India, Israel, Japan, Mexico, Netherlands, Nigeria, Sweden and Turkey. Wide host range including apple, barley, cowpea, fescue, lucerne, maize, onion, peach, potato, roses, strawberry, sorghum, soybean, Sudan grass, sugarcane, sweet potato, tobacco, tomato, water melon and white clover. Maize is reported to be a good host of this nematode (Loof, 1985).

Maize chlorotic mottle machlomovirus was first reported in maize from Peru (Brunt *et al.*, 1996). Spreads in Argentina, Mexico, Peru and the USA (Kansas, Nebraska and Hawaii) (Brunt *et al.*, 1996). Maize is the natural host. Transmitted in a non-persistent manner by *Diabrotica* spp. and thrips, but the vectors are not known to move the virus over long distance (Jensen, 1985). Seed to seedling transmission at low level (Jensen *et al.,* 1991). Seed transmission makes MCMV a threat to the maize industry in Bangladesh.

Wheat streak mosaic rymovirus was first reported in wheat from the USA (Brunt *et al.*, 1996). Spreads in Canada, Jordan, Romania, and the USA (Brunt *et al.*, 1996). Maize, wheat and several grasses are the natural hosts. WSMV is *seed-borne* and transmitted naturally at a low level (Hill *et al.*, 1974). The virus is also transmitted by the wheat curl mite *Eriphoyes tulipae* (Nault *et al*., 1967). After overwintering on winter wheat the mites leave this crop as it matures and are blown by the wind to spring wheat, barley and maize. WSMV causes severe mosaic of winter wheat and is a minor pathogen of sweetcorn, although severe outbreaks have been reported from Idaho (Finley, 1957). Infected maize provides an over summering host for wheat in midwest USA (Gardner, 1981). MCMV is part of viral complex associated with lethal necrosis (Uyemoto, 1983). It can also cause the aberrant ratio mutation effect (Brakke & Samson, 1981). Importation of the disease through seed poses a threat to both the maize and wheat industries in Bangladesh. WSMV is an important disease of winter wheat. Maize is seldom seriously affected, but may play a role in harbouring both the virus and its mite vector.

Phymatotrichopsis omnivora, a minor pathogen of maize but serious on cotton and many other dicotyledons, was regarded as having a lower potential for establishment because it would be soil or trash- borne only. If an incursion did occur, however, and it became established, this pathogen would be extremely difficult to manage.

Cercospora zeae-maydis is a serious disease on maize in humid areas. However, it is regarded as less of an overall risk than some of the other fungal, bacterial and viral pathogens because it is likely to be only trash-borne and to be pathogenic only on maize.

Throughout the PRA process, information were gathered and analyzed as required to reach recommendations and conclusions. Scientific publications as well as technical information such as data from surveys and interceptions were relevant. All the diseases listed are not found every year because their occurrence is influenced by environmental factors (temperature, humidity, soil moisture), previous cropping histories, crop location within the state, and availability of insect vectors (Zitter, 2009). As the analysis progresses, information gaps may be identified necessitating further enquiries or research.

CHAPTER VI

SUMMARY AND CONCLUSION

The demand of hybrid maize seed in local market is increasing day by day. Most of the seeds are used for planting purposes. To meet the growing demand of the farmer, the importer has to import a great quantity of maize from overseas countries. So, the opportunity for a new pathogen to get entry into the importing country is high enough. The newly introduced pathogen may cause catastrophic losses to crops and those might be most dangerous for the host country because it is escaped from their natural enemies in the native country. A survey was conducted in 40 upzilla in selected 20 districts named Rangpur, Dinajpur, Bogra, Noagaon, Rajshahi, Pabna, Sirajgonj, Jessore ,Kushtia, Jhenidah, Chuadanga, Faridpur, Tangail, Sherpur , Mymensingh, Kishorgonj, Netrokona, Comilla, Manikgonj and Chittagong under the Rangpur, Rajshahi, Khulna, Barisal, Dhaka and Chittagong divisions for gathering knowledge about field diseases of maize. Four farmer’s fields were visited in each upazilla during survey period and diseased maize plant parts were matched with the guide book for field identification. The major diseases identified during field survey is seedling blight, stalk rot, brown spot, grey leaf spot, smut, ear rot, anthracnose, sheath blight, maydis leaf blight, bacterial leaf blight, mosaic virus and leaf stripe virus. If symptom does not match then diseased plant parts were carried to laboratory for confirmation of diseases. Farmer's opinion about maize diseases were recorded and the major diseases of maize opined by the farmer is stem rot, leaf spot, root rot, cob rot, sheath blight, sheath rot, cob sheath blight, cob sheath rot, leaf blight, bacterial leaf blight, maize dwarf mosaic virus and grain rot. From each district under the study area opinion of the policy level officers of DAE were taken into consideration in listing maize diseases of Bangladesh. A focus group discussion was carried out in each district of the study area. The major diseases of maize come out from focus group discussion is leaf blight, sheath blight, ear rot, cob sheath blight, downy mildew, bacterial blight, foot and root rot and mosaic virus.

All the hybrid variety of maize was attacked by quarantine pathogen. Imported hybrid maize seed sample viz. Pacific 60, NK-40, 900M, 900M gold, Pinacle hybrid, Uttaron 900M, Konok hybrid, ASHA-3501 hybrid, C-222, Mukta 980, China seed Raza 777, Pacific 984, Pacific 759, Seedtech, BADC khoi bhutta were brought to seed health laboratory and seed borne pathogens were observed using blotter method. It is found in the study that all the variety was infected by seed borne pathogen. The genera of seed fungi detected from seeds are *Aspergillus spp., Penicillium spp., Rhizopus spp.* and *Fusarium moniliforme* and the bacterium was *Xanthomonas sp.*. A list of 30 maize diseases were prepared considering the report field survey, focus group discussion and laboratory analysis. The diseases of maize in Bangladesh are Fusarium rot, Pythium rot, Rhizoctonia rot, Pythium stalk rot, Fusarium stalk rot, leaf blight, brown spot, Curvularia leaf spot, Cercospora leaf spot, Phyllosticta leaf spot, downy mildew, rust, smut, anthracnose, cob rot, grain rot, bacterial wilt, bacterial stalk rot, maize mosaic, maize dwarf mosaic, maize leaf streak, root-lesion and root knot. The study found at least 428 potential pathogens associated with maize. These microorganisms were assessed for their presence in the maize exporting countries and Bangladesh, their ability to be transported with seed and bulk maize grain, and their ability to cause significant losses. 16 pathogens on maize grain from the maize exporting countries which have quarantine risk to Bangladesh agriculture. The pathogens are *Peronosclerospora sorghi*(downy mildew of maize, sorghum), Maize dwarf mosaic potyvirus, High Plains virus (maize, wheat), Wheat streak mosaic rymovirus (WSMV) (maize, wheat), *Sclerospora graminicola* (maize, sorghum, pearl millet and many grasses), *Phymatotrichopsis omnivore* (Texas root rot of cotton and other dicotyledonous plants), Maize chlorotic mottle machlomovirus (maize), *Cercospora zeae-maydis* (gray leaf spot of maize), *Pantoea stewartii* subsp. stewartii (Stewart's wilt of sweet corn), *Clavibacter michiganensis* subsp. nebraskensis (Goss's bacterial wilt of maize), *Heterodera zeae* (maize cyst nematode), *Ustilaginoidea virens* (false smut of maize), *Dolichodorus heterocephalus* (awl nematode), *Hoplolaimus columbus* (lance nematode), *Longidorus breviannulatus* (needle nematode) and *Pratylenchus scribneri* (root lesion nematode).

Ten of these pathogens viz. *Peronosclerospora sorghi*, Maize dwarf mosaic potyvirus, High Plains virus, Wheat streak mosaic rymovirus (WSMV), *Sclerospora graminicola*, *Phymatotrichopsis omnivore*, Maize chlorotic mottle machlomovirus, *Cercospora zeae-maydis*, *Pantoea stewartii* subsp. stewartii and Clavibacter *michiganensis* subsp. nebraskensis have a higher overall risk. The options for measures to be applied within the importing country such as careful surveillance to try and detect the entry of the pest as early as possible, eradication programs to eliminate any foci of infestation and/or containment action to limit spread and prohibition of certain variety of hybrid Maize or seeds or plants. Quarantine certificates and other strict compliance measures should be taken. Heat treatment is the only viable option for minimizing risk of entry of quarantine pathogens. This treatment should be done either at the port of entry to Bangladesh or off shore.

CHAPTER VII

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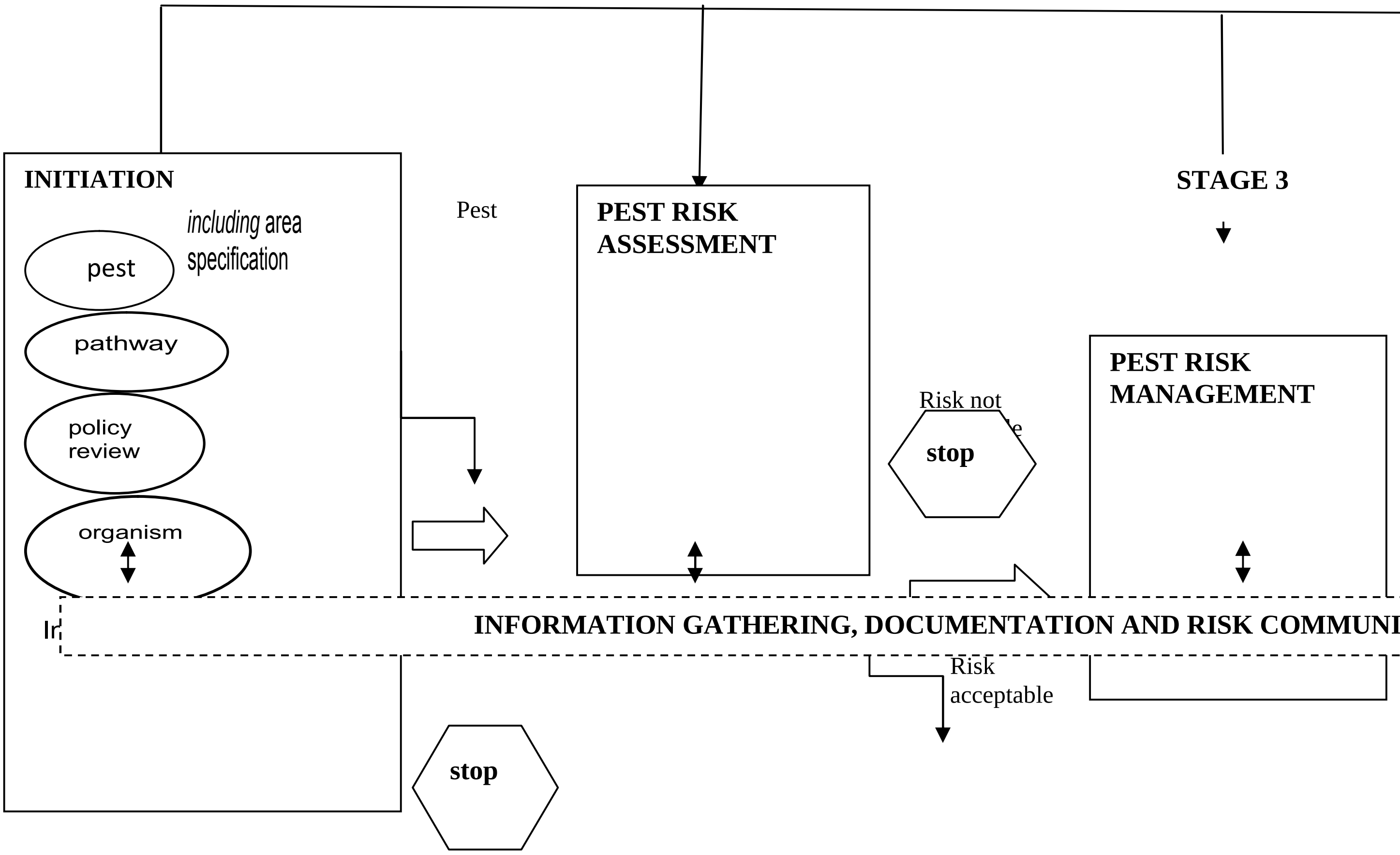
Annex -I

PEST RISK ANALYSIS FLOW CHART



Annex -II

Sher-e-Bangla Agricultural University

Department of Plant Pathology

Questionnaire for **Farmers** on Conducting Pest Risk Analysis (PRA) of Maize and listing of Quarantine Pest

Serial				

Cell Phone											

Name of Respondent:…………………… Village:……………………………..… Agri Block ……………….

Upazila…………………….…………. District: …………………………..… Education: ……..…………….

Age-------- Sex-------- Profession …………………………..…….

1. Land Use Pattern by Maize

Land Use Pattern(s)	**Area (decimal)**
1. Total land owned	
2. Cultivable land under total land owned	
3. Land cultivated by Maize	
4. How long cultivating maize	

2. Cultivation of Maize by Variety in Rabi and Kharif Season

Name of Variety Used	Area (Decimal)		Time of Planting		Time of harvesting		Yield (ton/ acre)	
	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif
1. Local Variety								
2. BARI HYV Variety								
3. BARI Hybrid variety								
4. Imported Hybrid variety								
5. Other Variety(if any)								

3. Sources of purchasing seeds

Sources	Amount of seeds used per bigha	Expiry of date checked	Germination and Quality tested by you (√)	
			Yes	No
1. From Seed Dealer				
2. From Pesticide Dealer				
3. From BADC				
4. Directly from Importer				
5. From Agril. Extension Dept.				
6. From Research Station				
7. Farmers seed: put (Tick mark) (1) Own seed (2) Neighbor seed (3) Local market seed				
8. Other sources(if any)				

* 1 bigha =33 decimals

4. Cost involved for pest management

Total Maize cultivated Land	Rabi pests control cost/ bigha (Taka)			Kharif pests control cost/ bigha (Taka)			Other pest control cost /bigha (TK
	Insects	Diseases	Weeds	Insects	Diseases	Weeds	
Total cost							

5. Insects infestation in Maize field (please put √)

Name of Insects pest	Incidence of insect pest (Y/N)	Stage of infestation of maize crop			Incidence/severity		
		Seedling	Vegetative	Reproductive	High	Moderate	Low
1. Termites							
2. Cutworm							
3. Corn borer							
4. Com leaf aphid							
5. Fall Armyworm							
6. Grasshoppers							
7. African pink borer							
8. African maize stem borer							
9. Corn stunt leafhopper							
10. European corn borer							
11. Diabrotica beetle and rootworms							
12. Maize bill bug and billbug grub							
13. Spider mites							
14. Southwestern maize borer							
15. Sugarcane borer							
16. Spotted sorghum stem borer							
17. White grub							
18. Wireworm							
19. Others (if any)							

6. Disease infestation in Maize field (please put √)

Name of Diseases	Incidence of diseases (Y/N)	Infestation stage of maize crop			Incidence/severity		
		Seedling	Vegetative	Reproductive	High	Moderate	Low
1. Stem rot							
2. Leaf spot							
3. Root rot							
4. Cob rot							
5. Grain rot							
6. Downy mildew							
7. Leaf Virus							
8. Aspergillus ear rot							
9. Fusarium ear rot							
10. Penicillium ear rot							
11. Stenocarpella ear rot							
12. Corn stunt							
13. Maize streak virus							
14. Sugarcane mosaic virus							
15. Field Corn Nematode							
16. Store grain Rot							
17. Others (if any)							

7. Weeds Infestation in Maize field crops (please put √)

Name of Weeds	Incidence of weeds (Y/N)	Infestation stage of maize crop			Incidence/severity		
		Seedling	Vegetative	Reproductive	High	Moderate	Low
1. Broadleaf							
2. Sedge							
3. Aquatic weeds							
4. Grass							
5. Others (if any)							

8. Is there any relationship among insect, disease and weed pest infestations in the maize field?

Yes = 1, No=2]

9. **If yes, what is the relationship among insect, disease and weed incidence in maize field?**

9.1 Insect population high when weed incidence is:

1. high, 2. medium, 3. low and 4. don't know

9.2 Disease incidence high when weed incidence is:

1. high, 2. medium, 3. low and 4. don't know

9.3 Disease incidence high when incidence of insect vector is:

1. high, 2. medium, 3. low and 4. don't know

10. When the pest infestations become high in the maize field? (please put √)

Pests	Season	
	Rabi	Kharif
1. Insect		
2. Disease		
3. Weed		

11. Pests infestation in Stored Grain Maize (please put √)

Insect pests/ Diseases	Incidence of pests (Y/N)	Extent of Damage			Types of container used for storing maize grains					
		High	Medium	Low	Poly bag	Jute bag	Bamboo dhole	Tin	Earthen container	Plastic container
A. Insect pests										
1. Corn earworm										
2. Ear maggot										
3. Grain borers										
4. Grain weevils										
5. Indian meal moth										
6. Angoumois grain moth										
7. Seedcorn maggot										
8. Rats and birds										
9. Others (if any)										
B. Diseases										
10. Cob rot										
11. Grain rot										
12. Aspergillus ear rot										
13. Fusarium ear rot										
14. Penicillium ear rot										
15. Stenocarpella ear rot										
16. Corn stunt										
17. Store grain Rot										
18. Others (if any)										

12. Whether any control measures taken against pests in your store maize?
[Yes=1, No=2]

If yes please tell

13. What preventive/curative measures are taken against these stored pests?

a. Preventive (name):
…………………………………………………………………………………….

b. Curative (name): ……………………………………………………………………………………...

14. What are the Major Diseases of maize in your area? Put no. upto 10 according to severity

High → Low

Seed rot =**1**
Stem rot =**2**
Leaf spots =**3**
Root rot =**4**
Cob rot =**5**
Grain rot =**6**

Downy mildew =**7**
Leaf Virus =**8**
Aspergillus ear rot =**9**
Fusarium ear rot =**10**
Penicillium ear rot =**11**
Stenocarpella ear rot =**12**

Corn stunt =**13**
Maize streak virus =**14**
Sugarcane mosaic virus =**15**
Field Corn Nematode =**16**
Store grain Rot =**17**
Other =**18**

15. What are the Major Insects of maize in your area? Put number upto 10 according to severity

High → Low

Cutworm =**1**
Corn borer = **2**
Com leaf aphid = **3**
Termites = **4**
Fall Armyworm = **5**
Grasshoppers = **6**
African pink borer = **7**

African maize stem borer = **8**
Corn stunt leafhoppers = **9**
European maize borer = **10**
Diabrotica beetles and rootworms =**11**
Maize billbugs and billbug grubs =**12**
Spider mites =**13**

Southwestern maize borer = **14**
Sugarcane borer =**15**
Spotted sorghum stem borer =**16**
White grubs =**17**
Wireworms =**18**
Others (if any) =**19**

16. What are the Major Weeds of maize in your area? Put no. according to severity

High → Low

Broadleaf =**1;** Sedge =**2;** Aquatic weeds =**3;** Grass = **4;** Others (if any) =**5**

17. When Termites attacks the maize plants?
[At tillering stage = 1, seedling = 2, elongation = 3, others (please specify) = 4]

18. Whether any control measures taken against the pests of maize in the field?
[Yes = 1, No=2]

19. If yes, what control measure is used against the pests in maize field?
[Preventive=1, Curative=2, Both=3]

20. How do you control pests in the maize field? Put numbers

A: Insects:

High → Low

B: Diseases:

High → Low

C. Weeds:

High → Low

[Through pesticides = 1, use resistant variety = 2, use imported hybrid maize = 3, seed treatment method = 4, cultural practices and control measures = 5, barriers to dispersion = 6, IPM method = 7, others (please specify) = 8]

21. What curative measures are taken against these diseases, insects & weeds in maize field?

Pests	Dose/bigha	Frequency (No.)	Measure is effective	
			Yes	No
1. Insect				
2. Disease				
3. Weed				
4. Other pest (if any)				

22. **From where You receive Assistance and Services in controlling the pests and diseases of maize?**

A:							Insects:

High → Low

B:							Diseases:

High → Low

C.							Weeds:

High → Low

[From DAE= 1, From Research =2, From Dealers =3, from Ngo=4, from neighbors=5, Others=6]

23. **Put your suggestions for better management of Insect and disease of maize.**

A. Insect Management

1.……………………………………………………………………………………………………

2.……………………………………………………………………………………………………

3.……………………………………………………………………………………………………

4.……………………………………………………………………………………………………

5.……………………………………………………………………………………………………

B. Disease Management

1.……………………………………………………………………………………………………

2.……………………………………………………………………………………………………

3.……………………………………………………………………………………………………

4.……………………………………………………………………………………………………

5.……………………………………………………………………………………………………

----------------------------------- Signature of Surveyor

--------------------------------------- Signature of Supervisor

----------------------------------- Name of Surveyor

--------------------------------------- Name of Supervisor:

Date: / /2012

Date: / /2012

Annex-III

Sher-e-Bangla Agricultural University

Department of Plant Pathology

Checklist for FGD on Conducting Pest Risk Analysis (PRA) of Maize and listing of Quarantine pest for policy level officers

Serial				

Cell Phone											

Respondent Name: …………………………………………… Designation: …………………………..………..

Upazila : ……………….………………………………… District ……………………………………..……

1. Position:
[Deputy Director =1, District Training Officer=2, CPS=3, PPS=4, Researcher=5, Scientist of BARI =6, BADC seed officials =7, Other (please specify) = 8]

2. What are the major and minor diseases of Maize in your area? Put numbers into 8 blank cells

2.1 Major								
2.2 Minor								

Seed rot =1
Stem rot =2
Leaf spots=3
Root rot =4
Aspergillus ear rot =5
Penicillium ear rot =6
Corn stunt =7
Sugarcane mosaic virus =8
Grain rot =9
Cob rot =10
Fusarium ear rot =11
Stenocarpella ear rot =12
Maize streak virus =13
Field Corn Nematode =14
Downy mildew= 15
Leaf Virus =16
Store grain Rot=17
Other =18

3. What are the major and minor insect pests commonly attack in Maize crops of your area? Put nos. into 8 blank cells

3.1 Major								
3.2 Minor								

Termites =1
Corn borer = 2
Grasshoppers =3
Corn leaf aphid =8
African pink borer =9
African maize stem borer =10
Spider mites =15
Southwestern maize borer =16
Sugarcane borer =17

Cutworms =4	Corn stunt leafhoppers =11	Wireworms =18,
Fall Armyworm =5	European maize borer =12	Others (if any) =19
Spotted sorghum stem borer =6	Diabrotica beetles and rootworms=13	
White grubs =7	Maize billbugs and billbug grubs =14	

4. What are the major and minor weeds attack in Maize crops as per information received?
Put number into 5 blank cells

4.1	Major						
4.2	Minor						

[Broadleaf weed=1, Sedge=2, Aquatic weeds=3, Grass=4, Other (if any) =5]

5. Was there any insect pest infestation or disease infection occurred that were not seen previously? 1=Yes, 2= No

6. **If yes, please mention the name of variety, source of seed used and stage of attack?**

Pests	Occurred in maize variety	Sources of seeds	Stages of attacks
6.1 Insects			
6.2 Diseases			

7. **What is the major and minor store grain pests attack in stored Maize as per information received? Put nos. into 6 blank cells**

7.1 Major						
7.2 Minor						

Corn earworm =1, Ear maggot =2, Grain borers =3 , Grain weevils =4, Indian meal moth =5, Angoumois grain moth =6, Seedcorn maggot =7, Seedcorn maggot =8, Others (if any)=9

8. **Do you know the pests of Maize in neighboring countries?** [Yes = 1, No = 2],
If yes, please tell name

8.1 Insects: ………………. ………………..
…………………………………………………………

8.2 Diseases: ………………. ……………………
…………………………………………………….

8.3 Weeds : ………………… ………………….
…………………….………………………………….

8.4 Others (if any) ………………………….
…………………………………………………………….

9. **Do you think that quarantine pest of maize are coming from neighboring countries in Bangladesh ?**
[Yes = 1, No = 2],

If yes, please tell name

9.1 Insects: ………………. ………………..
…………………………………………………………

9.2 Diseases: ………………. ……………………
…………………………………………………….

9.3 Weeds : ………………… ………………….
…………………….………………………………….

9.4 Others (if any) ………………………….
…………………………………………………………….

10. **Is there any relationship among insect, disease and weed infestations in the maize field?**
[Yes=1, No=2]

11. **If yes, what is the relationship among insect, disease and weed incidence in maize field?**

11.1 Insect population high when weed incidence is:

1. high, 2. medium, 3. low, 4. don't know

11.2 Disease incidence high when weed incidence is:

1. high, 2. medium, 3. low, 4. don't know

11.3 Disease incidence high when incidence of insect vector is:

1. high, 2. medium, 3. low, 4. don't know

12. In which the pest infestations become high in the maize field? Please put (√) tick

Pests	Season	
	Rabi	Kharif
12.1 Insect		
12.2 Disease		
12.3 Weed		

13. Is there any influence of weather factors (temperature, rainfall and rainfall) on the population of insects, diseases and weeds in maize field? [Yes = 1, No = 2]

14. If yes, what type of influence of weather factors is observed on the population of insects, diseases and weeds in maize field? [**Put tick (√) mark in the blank cells]**

Pests	Influence of weather factors								
	Temperature			Relative humidity			Rainfall		
	High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
1. Insect									
2. Disease									
3. Weed									

15. Do you think that imported hybrid varieties are the sources of coming Quarantine pests in our country? [Yes = 1, No = 2]

16. Do you take any preventive measures for intercepting from new coming quarantine pests in your area? [Yes = 1, No = 2]

If yes, Please Specify--

17. Do you think the preventive measures taken are effective? [Yes = 1, No = 2]

If No, Please Specify--

18. What are the major risks/threat of coming new quarantine pests in our country? (Put √)

1. Introduction of new insects/diseases/weeds,
2. New biotypes of pests (Insects/pathogen),
3. Increase intensity of crop damage,
4. Others--------

19. Have you taken any steps or supervised or monitored the quarantine pests of maize in the field? [Yes = 1, No = 2]

If yes, how ………………………………………………………..……………………………

…………………………………………….…………………………………………...

20. Do you think the existing facilities of quarantine service are sufficient to cope with the diseases and pest control of Maize in our country? [Yes = 1, No = 2]

If not, please give your suggestions for improvement of control of quarantine pests in our country

1………………………………………………………………………………………………

2…………………………………………………………………………………..………….

3…………………………………………………………………………………………………….

4…………………………………………………………………………………………………….

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Signature of Surveyor	Signature of Supervisor
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Name of Surveyor	Name of Supervisor:
Date: / /2012	Date: / /2012

Annex-IV

Sher-e-Bangla Agricultural University

Department of Plant Pathology

Checklist for FGD on Conducting Pest Risk Analysis (PRA) of Maize and listing of Quarantine Pest

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Location of FGD:

Name …………….. ………………………….. Designation ……………………………..

Village:………………………..…………… Ward ………………………………….

Upazila:……………………………………………. District:…………………………..……

1. **How much Area covered by Maize in this Area?**

2. **What are the sources of seeds used by the farmers?**

3. **What are the varieties of maize used by the Farmers?**

4. **Are there any Insect, diseases, weeds and other pests outbreaks in the maize field?**
(Put √) Yes =1 or No =2, if yes

   i. **What type of Insect of Maize is usually seen in your area? (Put √)**
      a. Major:
      b. Minor:

   ii. **What type of disease is seen in Maize in your area? (Put √)**
      a. Major:
      b. Minor:

   iii. **What type of weed of Maize is usually seen in your area? (Put √)**
      a. Major:
      b. Minor:

   iv. **At what stages of maize usually pest and disease attacks?**
      a. Stage of insect pests attacks
      b. Stage of disease pests attacks

vi. **Was there any occurrence of pest/ diseases attacks that could not identify?**

5. **Is there any relationship among the incidence/present of insect, disease and weeds in the maize field?** (Put √) Yes =1 or No =2, if yes

6. **What is the relationship among insect, disease and weed incidence in maize field? (Put √)**

6.1 Insect population high when weed incidence is high / medium / low
6.2 Disease incidence high when weed incidence is high / medium / low
6.3 Disease incidence high when incidence of insect vector is high/medium / low

7. **What might be the sources of diseases?**

8. **What might be the sources of Insects?**

9. **What might be the sources of weeds?**

10. **Whether insects, diseases and weeds spread from field to field? (Put √) Yes =1 or No =2, if yes, how**

10.1 Insect: (Through weeds/ seeds / indigenous / others)

10.2 Disease: (Through weeds/ seeds / indigenous / others)

10.3 Weed: (Through weeds/ seeds / indigenous / others)

11. **Whether diseases / insects/weeds cause yield loss in maize field? (Put √) Yes =1 or No =2, if yes how much?**

11.1 Insect: Severe (%) / moderate (%) / low (%) / no damage (%)
11.2 Disease: Severe (%) / moderate (%) / low (%) / no damage (%)
11.3 Weeds: Severe (%) / moderate (%) / low (%) / no damage (%)

12. **What steps are usually taken as control measures in case of high level of pest infestation?**

13. **What preventive measures may be taken against these diseases, insects and weeds in the maize field? [Use of pests free seeds, pesticides, resistant variety and others------]**

14. **Whether the pests (insects/diseases) attack the maize grains in storage? (Put √) Yes =1/No =2, if yes,**

14.1 Insects (name):

14.2 Diseases (name):

15. **What preventive/curative measures may be taken against these stored pests?**

15.1 Preventive (name):

15.2 Curative (name):

16. **Whether the used control measures by the growers effective? (Put √) Yes =1 or No =2, if yes**

Name the method(s): a. ………………………..………………………..………………………..
b. ……………………….………………………..………………………..
c. ………………………………………………..……………………..
d. ……………………….………………………..………………………..
e. ……………………….………………………..………………………..

17. How can we improve the control measures of quarantine pests for maize?

18. Suggestions for pest risk analysis and listing of quarantine pests?