AN INVESTIGATION ON THE DISEASES OF TUBEROSE (*POLYANTHES TUBEROSA*)

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Reg. No. 2003-08-1258

A THESIS

Submitted
To
Bangabandhu Sheikh Mujibur Rahman Agricultural University
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE

DEPARTMENT OF PLANT PATHOLOGY

SUMMER 2005

AN INVESTIGATION ON THE DISEASES OF TUBEROSE
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THESIS ABSTRACT

AN INVESTIGATION ON THE DISEASES OF TUBEROSE (*POLYANTHES TUBEROSA*)

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An investigation was conducted at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) farm, Gazipur to record the diseases of tuberose (*Polyanthes tuberosa*) by studying the symptoms in the field. The pathogens associated with the disease were isolated and identified. Pathogenicity test was conducted to confirm the causal agents of the diseases observed. Under natural conditions, three fungal diseases namely stem rot, blossom blight and *Alternaria* spot were found to attack tuberose plants grown at the experimental farm of BSMRAU. They were caused by *Sclerotium rolfsii, Fusarium equiseti* and *Alternaria polyanthi*, respectively. One bacterial disease known as bacterial leaf blight was recorded from the tuberose field. The causal bacterium was identified as *Xanthomonas campestris*. A nemic disease root-knot was also recorded and the pathogen was identified as *Meloidogyne sp.*. Among the diseases leaf blight was found to be highest in prevalence causing 100 % plants infection. It was followed by blossom blight (44 %), leaf spot (36 %), root-knot (5 %) and stem rot (4 %). The percent disease index (PDI)

of leaf blight caused by *Xanthomonas campestris* was found to increase with the decrease of temperature and relative humidity. Highest PDI value of the disease was observed at around 15 ^{0}C temperature and about 54 % relative humidity during the month of February and no incidence was recorded during July when both temperature (29 ^{0}C) and humidity (70 %) were higher.

ACKNOWLEDGEMENT

The author would like to convey his deepest sense of gratitude and immense indebtedness to Dr. Md. Abu Ashraf Khan, Major Professor and Chairman of the Advisory Committee, Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), for his adept guidance, suggestions, encouragement and generous assistance throughout the whole period of research and preparation of the manuscript.

The author also wishes to express his sincere appreciation and indebtedness to the members of the Advisory Committee, Dr. Md. Ismail Hossain Mian, Professor, Department of Plant Pathology and Dr. Nasreen Sultana, Associate professor, Department of Horticulture of BSMRAU for their suggestions, constructive criticism and comments in preparing the thesis.

The author is also grateful to Dr. Md. Abdul Mannan Akanda and Dr. Md. Khurshed Alam Bhuiyan, Professor, Department of Plant Pathology, BSMRAU, for their valuable suggestions and constructive criticism.

Special thanks to Md. Belal Hossain, Md. Jahan Al Mahomud, Alaul Hossain Md. Sharif and Moshiur Rahman for their help in the preparation of this manuscript.

The author feels pleasure to remember the scientific staffs of Plant Pathology Department, BSMRAU, for their valuable and sincere help in carrying out the research work.

Last but not the least, the author express his special appreciation and indebtedness to his father and mother whose sacrifice, inspiration, encouragement and continuous blessing paved the way to his higher education. He is also grateful to his brothers, sister who continuously inspired for his success and completion of the study

Summer 2005 **The Author**

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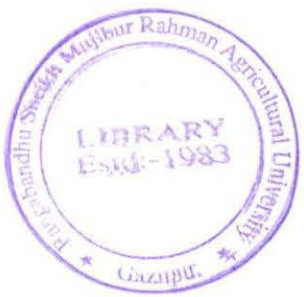

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CHAPTER I

INTRODUCTION

Tuberose (*Polyanthes tuberosa* L.; family: Amaryllidaceae) is a bulbous ornamental flowering plant of tropical and subtropical areas. In India and Bangladesh the flower is known by the local name: Rajanigandha (Mallavarapu 1995, Ahmed 1995). The flowers are commercially cultivated for cut and loose flower trade. The flower remains fresh for pretty long time and stands long distance transportation and fills a useful place in the flower markets (Desai 1957). It is used for artistic garland, floral ornaments, bouquets and buttonholes. The long flower spikes are excellent as cut-flowers for table decoration which arranged in bowles and vases (Bose and Yadav 1998). It is also important for the extraction of its highly valued natural oil which is one of the most expensive raw materials of high-grade perfumes (Mallavarapu 1995).

Though it is believed that the origin of the flower is Mexico, at present it is cultivated in Bangladesh for its commercial value. But the most important factor that affects its cultivation drastically and jeopardizes the whole enterprise is the disease problem (Das 1961, Rangaswamy *et al.* 1970, Jayaraman *et al.* 1975, Roy 1984).

Tuberose is attacked by many diseases. Foot and tuber rot caused by *Sclerotium rolfsii* is first reported by Das (1961). The disease is also known as wilt and stem rot (Rangaswamy *et al.* 1970, Dutta 1975). The disease has been found to be severe during warmer months and rainy season. Dutta (1975) observed that the fungus initially attacks roots and later advances to the tubers and petioles to cause rotting. Blossom blight of tuberose caused by *Fusarium equiseti* has been reported by Roy (1984). Light brown lesions develop on petals, which soon darken and result in the

drying up of the tissue. The blighted blossoms drop off from the plant. Infection also noticed on the flower stalk resulting in its collapse. Tzeng and Tung (1998) report blossom blight on tuberose (*Polyanthes tuberosa*) in Taiwan. A leaf spot disease caused by *Alternaria polyanthi* is also prevalent in the tuberose (Mariappan *et al.* 1977). The disease manifests as brown spots with faint concentric rings on midrib and rarely on the margin of the leaf. Infected leaves and peduncles become necrotic and dry up. Flower bud rot disease caused by *Erwinia* sp. appears on young flower buds and results in dry rotting of the bud (Maity 1999).

Chang and Chen (1999) report a virus, during a survey for ornamental plant virus diseases in 1994. Foliar mild mosaic symptom has been noticed on most tuberose plants in Taiwan. A virus isolated from infected plants is subsequently identified as a newly recognized potyvirus, designated as tuberose mild mosaic virus (TMMV), based on particle morphology, aphid transmissibility and the cylindrical inclusions it induces in infected cells. Tuberose is the only host identified so far for TMMV in the inoculation experiment. Chen *et al.* (1998) also report tuberose mild mosaic potyvirus (TMMV) causes mild mosaic symptoms on leaves and flower stems of tuberose (*Polyanthes tuberosa*).

Ganguly *et al.* (1993) report the association of *Meloidogyne incognita* on *Polyanthes tuberosa* in New Delhi. This is the first report of a disease complex involving *Meloidogyne*. The association of *Meloidogyne incognita* with tuberose, causes yellowing, drying of leaves and retarded growth. The emergence of panicles is suppressed resulting in an absolute loss of flower (Jayaraman *et al.* 1975). However,

there is no systematic research works on the disease problem of tuberose in Bangladesh.

Considering the above facts the present piece of research has been undertaken to record the occurrence of diseases of tuberose grown under natural field condition.

CHAPTER II

REVIEW OF LITERATURE

Stem rot

Foot and tuber rot caused by *Sclerotium rolfsii* Sacc. was first reported on tuberose by Das (1961) from Kolkata, India. The disease symptom was characterized by the appearance of prominent mycelial masses on leaf surface at or near the soil level. Soon the infected spots lost green color due to rotting which extended and covered the whole leaf. The infected leaves fell on the soil surface and become detached from the plant. More or less round sclerotia, brown in color, were formed on and around the infected leaves. As a result the infected plants became weak and sent out few or none of the flowering shoots in case of severe damage.

Rangaswamy *et al.* (1970) reported the wilt and stem rot disease from tuberose growing regions in India. The disease was found to be severe during warmer months and rainy season. In the field, the disease appeared in patches and initial symptoms were dropping of polar of the leaves followed by yellowing and drying of the plant. Under moist conditions, a characteristic fan-shaped mycelium strand of the fungus appeared at the base of the infected plant. Later, brown mustard-like round sclerotia developed on the mycelial growth.

Dutta (1975) observed that the fungus initially attacked roots and later advanced to the tubers and petioles to cause rotting.

Blossom blight

Blossom blight of tuberose caused by *Fusarium equiseti* (Corda) Sacc. first reported by Roy (1984) from Karbi Anglong of Assam, India. The disease was prevalent during October-December months. Light brown lesions developed on petals, which soon darken and resulted in the drying up of the tissue. The blighted blossoms dropped off from the plant. Infection also noticed on the flower stalk resulting in its collapse. Under humid conditions, flower tips also became brown on which brown spore mass developed.

A leaf spot disease of tuberose (*Polyanthes tuberosa*) in Taiwan was reported by Tzeng and Tung (1998). The causal agent was isolated and identified as *Fusarium equiseti*. The initial symptoms of this disease were water-soaked spots which turned into elliptical or round, brown spots. *Fusarium equiseti* could infect leaves, stems and flowers. The pathogen grew rapidly on PDA plates; the colonies reaching 8.8 cm in diameter after 5 days at 25 °C. Sickle-shaped macroconidia from monophialides, with a foot-shaped basal cell, were 3-5 septate and 22.5-45 X 2.5-5 µm in size. Microconidia were oval and produced from aerial mycelium. Chlamydospores were formed in chains or clumps. The optimum temperature for mycelial growth ranged from 20-30 °C. The temperature range for conidial germination was between 20 and 35 °C, and the optimum temperature was 30 °C. The optimum temperature for disease development was 25 to 35 °C, and the symptoms were observed in 2 days. However, no symptoms were observed at 15-20 °C, seven days after inoculation. The disease was found both on double and single petal tuberose varieties.

Alternaria leaf spot

A leaf spot disease caused by *Alternaria polyanthi* (Mariappan *et al.* 1977) also prevalent on the tuberose during August-December in India. The disease manifested as brown spots with faint concentric rings on midrib and rarely on the margin of the leaf. Infected leaves and peduncles became necrotic and dried up. Occasionally peduncle might also be infected showing circular to oval spots which measured 10-30 mm in length and 4-5 mm in diameter. The pathogen was a weak parasite and leaf injury was needed for causing infection.

Leaf blight

Chang and Chen (1999) reported the leaf blight disease of tuberose caused by *Xanthomonas campestris* from Taiwan. The lesion started from the tip of the leaf. Initially, water-soaked stripes developed, the infected leaf showed withering at the tip. Gradually, it enlarged and spread downward along the margins parallel to the veins and extended laterally to the healthy regions. Eventually, a large portion of the leaf blade was affected. The edges of the lesions were usually irregular or wavy. The leaf turned yellow or dirty white and withered.

Root-knot

Jayaraman *et al.* (1975) reported the association of *Meloidogyne incognita* with the tuberose caused root-knot disease. The plant showed yellowing, drying up of leaves and retarded growth. In severely affected plants the emergence of panicles was

suppressed resulting in an absolute loss of flower. Emergence of side shoot from bulbs was also affected and the numbers were conspicuously become less.

Sundara and Vadivelu (1989) reported that the *Meloidogyne arenaria* caused the maximum reduction in length and weight of shoot and root of *Polyanthes tuberosa* cv. Single whorl, followed by *Meloidogyne incognita* and *Meloidogyne javanica* in pot experiments in which 500 nematodes were inoculated per plant. Damage to the plants was directly correlated with the initial inoculum level.

Ganguly *et al.* (1993) reported the association of *Meloidogyne incognita* on *Polyanthes tuberosa* in New Delhi.

An experiment was carried out by Mohanty and Das (1996) to study the pathogenicity of *Meloidogyne incognita* on tuberose (*Polyanthes tuberosa*) cv. Single. They investigated the inoculum levels of 0, 10, 100, 1000, 5000, 10,000 and 20,000 nematodes under semi sterile pot culture conditions. There was a progressive reduction in plant height, root length and number of leaves with the increased inoculum levels over the control. The maximum number of juveniles recorded was 2972/pot with 10 nematodes and the minimum was 603/pot with 20,000 nematodes. The rate of reproduction was maximum in plants inoculated with 10 nematodes (305.6) and minimum (0.26) in plants treated with 20,000 nematodes/pot.

CHAPTER III

MATERIALS AND METHODS

Investigation was carried out to record the occurrence of diseases of tuberose grown under natural conditions.

Growing of tuberose

The crop was grown at the experimental farm of Banghabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during 2003-2004 crop season. The experimental site was located at 24.09 N latitude and 90.26 E longitude with an elevation of 8.4 meter from sea level. The soil of the experimental field belongs to the agroecological zone, Madhupur tract (AEZ-28) with pH 5.8 to 6.5 (Haider *et al.* 1991).

During the experimentation fortnightly average temperature and relative humidity were obtained from the weather station of BSMRAU, Gazipur.

The soil of the experimental plot was well prepared by ploughing followed by laddering. Cowdung (30 tons/ha) and fertilizer (80 kg N, 60 kg P and 40 kg K/ha) were applied as recommended by Singh *et al.* (1976) and Nambisan and Krishnan (1983).

The bulbs of a single petal tuberose variety were planted at a distance of 25 cm X 50 cm in two 10 m X 1 m plots on October 3, 2003. Intercultural operations and irrigation were applied whenever required. There were 240 plants in two plots.

Observation of the plants

The plants in each plot were checked regularly to observe appearance of any disease symptoms from seedling to flower harvesting stage at 15 days interval. Just after appearance of any disease the infected plants were tagged and observed regularly. The characteristics of visible symptoms, further development of the symptoms, kinds of symptoms and the spread of the individual symptom were recorded with the progress of time. The severity of the diseases was indexed following a standard scale of 0-5 and expressed as percent disease index (PDI) as described by Dater and Mayee (1981) and Mian (1995). PDI was worked out by the following formula:

$$PDI = \frac{\Sigma \text{ (Individual scale X No. of plants under that scale)}}{\text{Total no. of plants X Highest degree of severity}} \text{ X } 100$$

The disease severity was correlated with prevalent atmospheric temperature and relative humidity (Suhag *et al.* 1985). The correlation co-efficient 'r' between disease severity and weather conditions was calculated by using the following formula given by Suhag *et al.* (1985).

$$r = \frac{N \Sigma XY - \Sigma(X)(\Sigma Y)}{NX^2 (\Sigma X)^2 (N\Sigma Y^2 - \Sigma Y)^2}$$

N = Number of observations

X = Disease incidence

Y = Meteorological parameter

Σ = Summation

Regression analysis was also performed to find out the effect of weather on PDI. The weather data were collected from BSMRAU Weather Station to relate the fortnightly disease prevalence and its development. The percent disease index was calculated only for bacterial disease. For each of the diseases, initial symptom(s) and its development were carefully observed, noted and photographed for further illustration.

Isolation and identification of fungal pathogens

The fungal pathogens associated with diseased specimens were isolated following standard tissue culture methods (Dasgupta 1981, Agostini and Timmer 1992). Plant specimens (leaf, stem and flower) with infected and healthy areas were collected from the field and carried to the laboratory in polyethylene bags. The specimens were cut into small pieces (5 mm). The pieces were surface sterilized by dipping in 1% NaOCl for three minutes followed by washing with sterilized water for 2-3 times. Sterilized plant pieces were plated on petridishes (90 mm diameter) containing solidified potato dextrose agar (PDA) medium. The plates were incubated at 25 ± 1^0C temperature in an incubator. After appearance of characteristic growth of the fungus, hypal tips were transferred aseptically to petridishes containing solidified PDA. The inoculated PDA plates were incubated to allow the fungus to grow and sporulate. The isolated fungi were purified following single spore culture method for sporulating fungi and hyphal tip method in case of non sporulating fungi. After purification, the fungi were identified based on colony characters, hyphal and spore morphology and measurement.

Pathogenicity of *F. equiseti* and *S. rolfsii* isolated from infected plants

Two fungal pathogens namely *Fusarium equiseti* and *Sclerotium rolfsii* associated the tuberose plants showing blossom blight and foot and tuber rot diseases.

(a) *Fusarium equiseti*

Fusarium equiseti was grown on PDA and the spores were collected from seven days old culture by scraping. The spores were suspended in sterilized water to prepare inoculum suspension (Mian 1995). The spores were thoroughly mixed using a magnetic stirrer and passed through two layers of cheese cloth to remove mycelium fragments and pieces of PDA if any. The spore concentration was adjusted to about 5 X 10^3 spores/ml. Two weeks old plants of tuberose grown in a net-house were inoculated with the spore suspension following foliar spray method with a micro sprayer. About 0.1 ml of Tween-20 was added to the spore suspension before spraying the inoculum suspension to prohibit the spores from clumping. Five plants were inoculated with the spore suspension. Another five plants were sprayed with sterilized plain water which served as control.

For pathogenicity test, inoculation was done on April 15, 2004. The average temperature of the nethouse was 28 ± 5^0C. After inoculation, plants were placed under a polyethylene bag and water was sprayed inside periodically to maintain high humidity up to 28 hours. The plants were inspected every day for two weeks to observe the appearance of the symptoms. The type of the symptoms appeared on the leaves were noted and photographs were taken for further illustration. The leaves showing symptoms after inoculation were collected and carried to the laboratory for reisolation and identification of the pathogen to confirm either the disease was caused

by the inoculated pathogen or not. The symptoms observed in the field under natural conditions and those developed on inoculated plants were noted and compared.

(b) *Sclerotium rolfsii*

Tuberose plants were grown in the nethouse. Two weeks old plants were inoculated with the sclerotia of *S. rolfsii* produced on PDA plates. Ten sclerotia were placed around each plant and covered with sandy loam soil a depth of 1 cm. The plants were watered carefully to maintain suitable moisture content for the growth of the plants and the fungus. Five plants were inoculated with the pathogens and another five plants were inoculated with plain water. The inoculated plants were checked every day to observe the appearance of the symptoms of foot and tuber rot and continued for 30 days. The type of the symptom appeared on plants were noted and photographed for further illustration. The plants showing symptoms after inoculation were collected and carried to the laboratory for isolation and identification of the pathogen to confirm either the disease was caused by the inoculated pathogen or not.

Isolation of bacteria from diseased specimens

Bacteria were isolated from diseased leaves by "streak-plate" method as described by Schaad (1988). Blight infected tuberose leaves were collected, cut into small pieces (5 mm) and surface sterilized with 1% NaOCl solution for three minutes. Sterilized leaf was washed with sterilized water for three times to removed the residue of hypochlorite. After rinsing, the leaf was placed in sterilized petridish containing five milliliter sterile water and crushed it with a sterile scalpel. The petridish was kept

undisturbed for several minutes so that bacteria associated with leaf were released in the water.

Potato semi-synthetic agar (PSA) medium was prepared. The medium was prepared by dissolving decoction of 300 g potato slices, $Na_2HPO_4.12H_2O$ 2 g, $Ca(NO_3)_2.4H_2O$ 0.5 g, peptone 5 g, sucrose 15 g and agar 15 g into distilled water to make the volume 1000 ml. After thoroughly mixing, pH of the mixture was adjusted to 7.2 using 0.1N NaOH or 0.1N HCl. After mixing thoroughly the medium was cooked for dissolving agar and poured into an Erlenmeyer flask and autoclaved at 121C under 1.1 kg/cm^2 pressure for 20 minutes. The medium was poured into petridishes (90 mm) at 20 ml/dish. After solidification, one loopful of the bacterial suspension (water containing bacteria) was streaked on the PSA medium in each plate. The plates were incubated at 30 C for 48 hr. The colonies of bacteria grew on the plates were re-streaked on separate PSA plate to get the pure culture.

Preservation of bacteria

Individual virulent colonies were selected based on colony character (Schaad 1988) and preserved in 10 ml of sterilized distilled water in test tubes. To preserve in sterile water, 3-5 loopfull of colonies grown on PSA medium were suspended in sterilized distilled water in screw capped test tubes and preserved at room temperature (25 ± 1 ^0C). New suspension of virulent colony was prepared every month by sub-culturing on PSA medium and preserved. The test tubes were labeled with name and date.

Pathogenicity test for bacterial pathogen

The bacterial colony recultured in PSA medium was used for pathogenicity test. The tuberose plants of two weeks old grown in a net-house were inoculated by wound spraying method (Schaad 1988). The blades of the young leaves were punctured with a sharp needle in several locations. The inoculum suspension (10^9cfu) was sprayed with a automizer. In case of control only sterilized water was sprayed. The plants were inspected for two weeks to observe the blighting symptoms. Seven different isolates collected from various blighted plants were used in the experiment. Three plants each as a replicate were inoculated for individual isolates.

Identification bacterial isolate

The isolated bacteria were identified following rapid extraction Thin Layer Chromatography (TLC) using lipid profile and by physiological and biochemical tests.

(a) Rapid extraction thin layer chromatography (TLC)

Rapid extraction TLC was conducted for lipid profiles following the procedures described by Khan and Matsuyama (1999). Each culture was grown on a slant of King's B agar medium at 30 C for 3 days. The medium was prepared by dissolving peptone 20.0 g, K_2HPO_4 1.5 g, $MgSO_4.7H_2O$ 1.5 g and agar 15.0 g in 1000 ml of 1% glycerol solution. Before autoclaving pH of the medium was adjusted to 7.2. One loopful of bacterial cells was placed in a small glass vial and 0.2 ml of chloroform-methanol-0.3% NaCl solution (2: 1: 0.4, v/v/v) was added and the vial was capped

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tightly. Bacterial cells were missed gently and kept for 15 min. at room temperature for extraction of lipid.

About 10 µl of lipid extract was spotted on the origin of pre-collected silica gel TLC plate and was completely dried by a hair drier. Sample spots were placed 1.5 cm apart from each other maintaining a distance of 1.0 cm from the bottom edge of the plate. The plate was developed with chloroform-methanol-0.2% calcium chloride (55: 35: 8, v/v/v) solvent system in a rectangular glass tank for 1hr. The development process was conducted in an incubator at 25 C without interruption. After development, the TLC plate was dried well. The lipid spots on the TLC plates were detected by spraying ninhydrin followed by heating the plate at 100 C for 10 min. in an oven. The chromatograms of isolates were recorded by photographs or hand drawing.

(b) Physiological and Biochemical Tests

A series of physiological and biochemical tests for the isolates were conducted following standard methods to identify the isolated bacteria.

(i) Gram stain reaction

The alternative non-staining KOH solubility test was conducted to determine the gram stain reaction for its quickness and easiness. On a glass slide, a loopful of bacteria from a well-grown colony was mixed with a drop of 3% KOH aqueous solution. When the wire loop was raised a few centimeters from the glass slide, if strands of viscid material are seen the bacterium was denoted as Gram-negative (Schaad 1988).

(ii) Oxidase test

Fresh (24 hrs old) cultures on nutrient agar supplemented with 1% glucose were used as inoculum. A small loopful of bacterial cells was rubbed on a filter paper impregnated with freshly prepared 1% (w/v) aqueous tetramethyl-p-phenylenediamine dihydrochloride solution. The stain was rated oxidase-positive if a purple color developed within 10 seconds, delayed positive if coloration develops after 10 seconds and negative if color develops after 60 seconds (Kovacs 1956).

(iii) Catalase test

Young cultures (18-24 hrs old) and 3% hydrogen peroxide (H_2O_2) was used to observe production of gas bubbles which indicated positive reaction. The reaction was observed by mixing a loopful of cells with a drop of H_2O_2 on a regular glass slide (Schaad 1988).

(iv) Color and slime formation/ Mucoid growth in YDC

The best medium for observation of colony color and mucoid growth was YDC (Schaad and White 1974). It consists of yeast extract 10 g, dextrose 20 g, calcium carbonate 20 g and agar 15 g in 1000 ml distilled water. The medium was then autoclaved for 20 min. at 121 C. The autoclaved medium then dispensed in the petridishes by swirling. The bacterial cells suspension was streaked on the solidified medium in the plates. The plates were incubated at 30 C for 48 hrs. The yellow colony color and mucoid growth indicated as positive reaction.

(v) Urease production

A modified YS (yeast salt) broth was used in this test. The medium was prepared by adding $NH_4H_2PO_4$ 0.5 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 5.0 g, yeast extract 1.0 g and cresol red 0.016 g with 1000 ml of distilled water. The medium was then autoclaved. Filter sterilized urea solution was added to the medium to give it 2% concentration. Five milliliter of the medium was then dispensed into each sterile test-tube and were incubated with cells of 24 hr culture, and incubated at 30 C. An increasing in alkalinity indicated by a magenta red color was the evidence of the urease activity (Schaad 1988).

(vi) Esculin hydrolysis

The broth for esculin hydrolysis was prepared by dissolving peptone 10g, NaCl 5 g, esculin1g and ferric citrate 0.5 g in 1000 ml of distilled water (Dye 1962). The pH of the broth was adjusted as 7.2. After dispensing 5 ml of broth in each test-tube, sterilized for 20 min. at 121 C in autoclave then cooled immediately. Stab inoculation was done using cells from 24 hr culture. Development of dark brown color after shaker inoculation for up to 28 days was regarded as an indication of utilization of esculin.

(vii) Gelatin liquefaction

The medium for gelatin liquefaction test contained beef extract 3.0 g, peptone 5.0 g, gelatin 120.0 g and distilled water 1000 ml. The medium was produced by dissolving the ingredients by steam heating. After dispensing 5 ml of medium in test-tube they were sterilized for 20 min. at 121 C in an autoclave and then cooled immediately without slanting. Stab inoculation was done using cells from 24 hr nutrient agar slant

and incubated at 30 C for several days. After 3, 7, 14 and 21 days, tubes were placed at 4 C for 30 min. prior to record results. The readily flows of the medium when tubes were gently tipped was considered the gelatin was hydrolyzed. It was denoted as positive reaction for the test (Schaad 1988).

(viii) Protein digestion

The skim milk solution was prepared by dissolving 100 g of reconstituted powdered milk contained 0.004 % bromocresol purple (w/v) in 1000 ml of distilled water (Sahaad and White 1974). The milk solution of about 5 ml was dispensed in each test-tube and sterilized for 20 min. at 121 C in an autoclave then cooled immediately. Stab inoculation was done using cells from 24 hr culture and incubated at 27 C. Clearing after 3, 5 or 7 days indicates digestion of casein and denoted as positive in protein digestion test.

(ix) Hypersensitivity reaction

Approximately 10^9 colony forming unites (cfu)/ml water of freshly cultured bacteria were injected into the intercellular space of a leaf of tobacco cv. "Burley" with a 25 gauge needle and syringe. Complete collapse of the tissue after 24 hr was recorded as positive (Klement and Goodman 1967).

(x) Utilization of carbon

Ayers medium was used in carbon utilization test. The medium contained $NH_4H_2PO_4$ 1.0 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.2 g, bromothymol blue (1.6% alcohol solution) 1ml, agar 12 g dissolved in 1000 ml of distilled water (Ayers *et al.* 1919). The pH was maintained at 7.0 and autoclaved at 121 C for 20 min. Filter sterilized 0.1% carbon solution was added and dispensed on sterile petridishes. The plates were spot

inoculated with a drop of bacterial suspension and incubated at 30 C. Growth of the bacteria was observed periodically at 3, 7, 14 and 21 days. The minimal medium without any addition of carbon source was used as control. The growth of the bacteria denoted as utilization of carbon.

Identification of nematode from the root of tuberose plant

The galled roots of the tuberose were collected by uprooting the plants. The roots were then rinsed in running tap water to make the roots dirt free. Then the roots were soaked with blotting paper to remove the excess water.

The stain solution was prepared by adding 5 ml of 1% cotton-blue per 100 ml of lactophenol (Mian 1995). The galled roots were immersed in boiling stain for about 1-2 minutes then rinsed in tap water and immersed in clear lactophenol solution for destining until maximum contrast between root tissues and nematodes was obtained.

The stained roots were mounted on a glass slide and covered with another glass slide and pressed the mount between fingers to flat them. The mount was then examined under a compound microscope to observe the nematode in the root. The nematode was identified studying perennial pattern under a compound microscope.

CHAPTER IV

RESULTS AND DISCUSSION

Identification of fungal and bacterial diseases of tuberose

Under natural conditions, three fungal diseases namely stem rot, blossom blight and *Alternaria* spot were found to attack tuberose plants grown at the experimental farm of BSMRAU. They were caused *by S. rolfsii, F. equiseti* and *A. polyanthi*, respectively. One bacterial disease known as bacterial leaf blight was recorded from the tuberose field. The causal bacterium was identified as *Xanthomonas campestris*. The diseases were identified based on symptomatology and confirmed through inoculation under control conditions. Symptoms of the diseases along with their causal agents were summarized in Table 1. Brief description of the individual diseases along with their causal agents were described below.

(i) Stem rot

In the field, the disease appeared in patches. The leaves lost green color due to rotting of tissues. The infected leaves dropped on the soil surface and detached from the plant followed by yellowing and drying of the plant (Table 1 and Fig. 1). On inoculation foot and tuber rot symptoms were developed (Fig. 2). Under moist conditions, characteristic fan-shaped mycelial strands of the causal fungus appeared at the base of the infected plant at or near the soil surfaces. Later, brown mustard-like round sclerotia developed on the mycelial growth. The causal fungus was identified as *S. rolfsii* (Fig. 3).

Table 1. Diseases of tuberose identified from the experimental field along with major symptoms and causal pathogens

Disease	Major symptoms	Causal pathogen
Stem rot	The leaves lost green color due to rotting. Infected leaves dropped followed by yellowing and drying of the plant. Under moist conditions, mycelial strands of the fungus appeared at the base of the infected plant. Later, brown mustard-like round sclerotia developed on the mycelial growth.	*Sclrotium rolfsii*
Blossom blight	Initially water-soaked spots on petals, which soon darken and resulted in the drying up of the tissue. The blighted blossoms dropped off from the plant. Infection also noticed on the flower stalk resulting in its collapse. Under humid conditions, flower tips also became brown on which brown spore mass developed.	*Fusarium equiseti*
Alternaria leaf spot	The disease appeared as brown spots with faint concentric rings on midrib and rarely on the margin of the leaf. Infected leaves and peduncles became necrotic and dried up. Occasionally peduncle may also be infected showing circular to oval spots which measured 10-30 mm in length and 4-5 mm in diameter. Infected leaves and peduncles became necrotic and dried up.	*Alternaria polyanthi*
Bacterial leaf blight	The lesion started from the tip of the leaf. Initially, water-soaked stripes developed, the infected leaf showed withering at the tip. Gradually, it enlarged and spread downward along the margins parallel to the veins and extended laterally to the healthy regions. Eventually, a large portion of the leaf blade was affected. The edges of the lesions were usually irregular or wavy. The leaf turned yellow or dirty white and withered.	*Xanthomonas campestris*

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Fig.1. Symptoms of stem rot disease of tuberose as recorded from experimental field.

Fig. 2. Development of foot and tuber rot symptoms on tuberose inoculated with *Sclerotium rolfsii*.

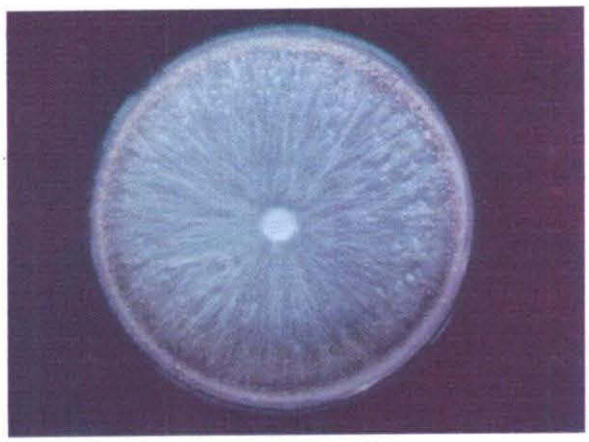

Fig. 3. Colony of *Sclerotium rolfsii* with cotony mycelium and sclerotia on PDA plate.

(ii) Blossom blight

The initial symptoms of blossom rot were water-soaked spots on petals, which soon became darken and resulted in the drying up of the tissue. The blighted blossoms dropped off from the plant. Infection also noticed on the flower stalk resulting in its collapse. Under humid conditions, flower tips also became brown on which brown spore mass developed. The symptom also found on the infected leaves, stems (Table 1 and Fig. 4). The causal fungus of blossom rot was identified as *F. equiseti* (Fig. 5)

(iii) *Alternaria* leaf spot

Alternaria leaf spot disease manifested as brown spots with faint concentric rings on midrib and rarely on the margin of the leaf. Infected leaves and peduncles became necrotic and dried up. Occasionally peduncle may also be infected showing circular to oval spots which measure 10-30 mm in length and 4-5 mm in diameter. Infected leaves and peduncles became necrotic and dried up (Table 1 and Fig. 6). The causal fungus was identified as *A. polyanthi* (Fig. 7).

(iv) Bacterial leaf blight

Only one bacterial disease viz. leaf blight disease was found to occur in tuberose field. The lesion started from the tip of the leaf. Initially, water-soaked stripes developed then the infected leaf showed withering at the tip. Gradually, it enlarged and spread downward along the margins parallel to the veins and extended laterally to the healthy regions. The edges of the lesions were usually irregular or wavy. The leaf turned yellow or dirty white and withered (Table 1 and Fig. 8). The causal bacterium was identified as *Xanthomonas campestris.*

Fig . 4. Blossom blight (*F. equiseti*) symptoms of tuberose appeared in the experimental field under natural conditions.

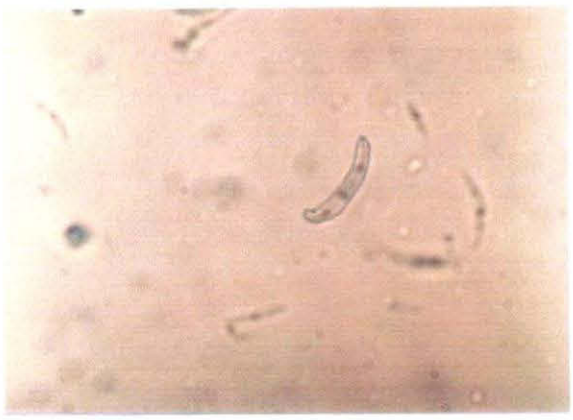

Fig. 5. Conidia of *Fusarium equiseti* developed on PDA culture after isolation from blossom rot infected tissues of tuberose

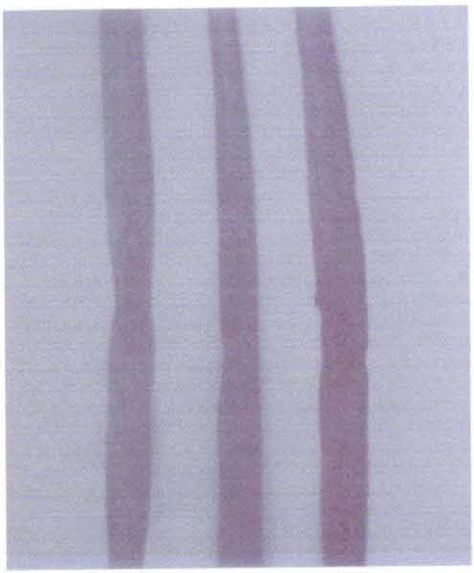

Fig. 6. *Alternaria* leaf spot (*A. polyanthi*) symptoms of tuberose developed under natural condition.

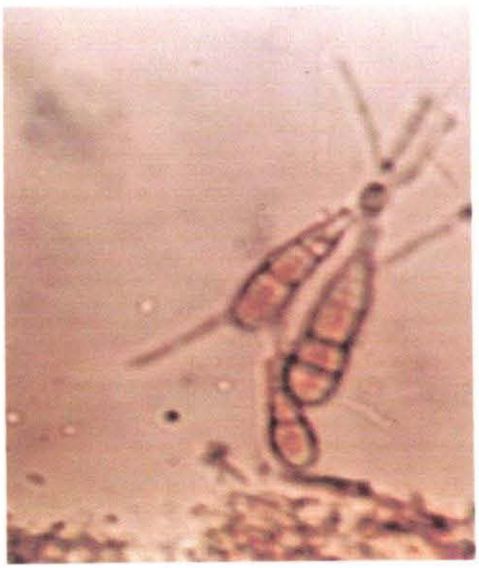

Fig. 7. Conidia of *Alternaria polyanthi* developed on PDA plate after isolation from tuberose

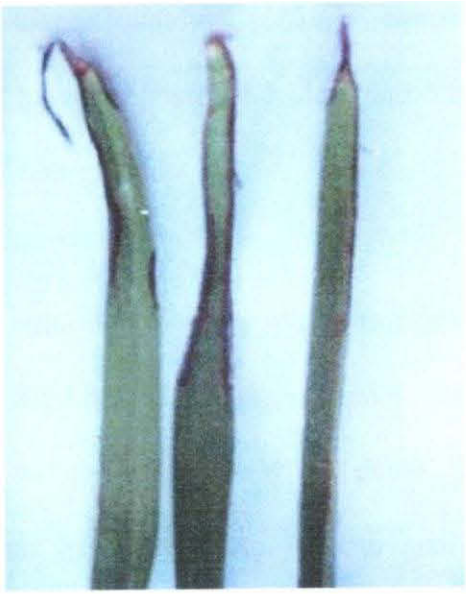

Fig. 8. Leaf blight symptoms (*Xanthomonas* sp.) recorded on tuberose grown under natural condition in the field

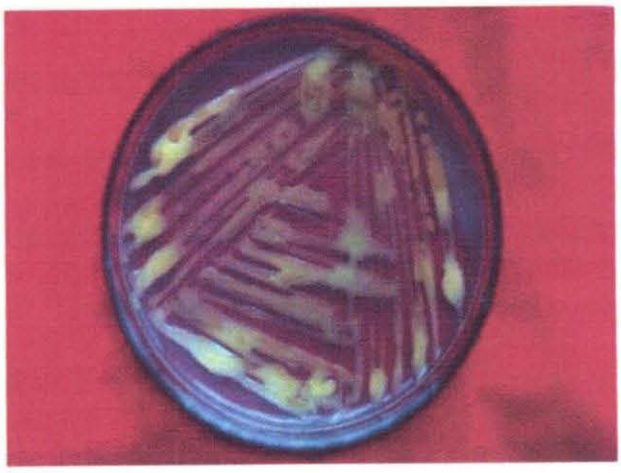

Fig. 9. Culture of a bacterial isolate (isolate-1) isolated from blighted leaf of tuberose

Identification of three fungal and one bacterial pathogen

A total of three fungal pathogens namely *S. rolfsii, F. equiseti* and *A. polyanthi* were isolated from the infected tuberose plants and identified based on colony characters, morphology of hyphae and spores.

(i) *Sclerotium rolfsii*

The pathogen *S. rolfsii* was identified by observing growth characters, hyphae, mycelia and sclerotia described by Das (1961). Colonies from infected plant materials or on agar medium grow out rapidly, were white, dense, and branched with clamp connection at each septation. In a week or so, white hyphal knots appeared on aerial mycelia. These bodies known as sclerotia gradually deepen in color and become brown, more or less globose varying in sizes from 0.5 to 2 mm in diameter. Internally sclerotia were differentiated into a thin brown outer rind and a thick inner cream color cortex. Organism was identified as *Sclerotium rolfsii* Sacc (Fig. 3). The same results also reported by Das (1961), and Rangaswamy *et al.* (1970).

(ii) *Fusarium equiseti*

To identify *F. equiseti* descriptions of Tzeng and Tung (1998) were followed. *Fusarium equiseti* was identified by observing growth characters, conidiophore and conidial shape and size. *Fusarium equiseti* developed as thick cottony white colony on PDA medium. Fulcate or sickle shaped, septate, hyaline macro-conidia were abundant in number while single shaped micro-conidia were less in number. The length of the macro-conidia varied from 22-45 µm with average was 33 µm while the width range from 2-5 µm with the average was 3 µm. The coniodiophores were short hyaline and septate (Fig. 5).

(iii) *Alternaria polyanthi*

Alternaria polyanthi was identified following the characteristics described by Ellis (1971) and Dube (1994). Muriform conidia developed on short dark colored conidiophore in chain. The conidiophores were broader than the vegetative hyphae and was a sympodula, bearing geniculations marked with scars of detached conidia (Fig. 7). The length of the conidia was measured which ranged from 30-50 µm with an average of 39.20 µm while the width range from 10-15 µm with an average 11 µm. The conidia were dark brown, typically obclavate, and short beak.

(iv) *Xanthomonas campestris*

A total of seven bacterial isolates were isolated from the leaf blight infected tuberose plant. All the isolates produced yellow colony color on PSA medium (Fig. 9). On inoculation all isolated produced characteristic symptoms of bacterial leaf blight. The symptom appeared after 5-7 days of inoculation and the leaves were blighted (Fig. 10). The development of the blight symptoms proved that all the isolates were pathogenic.

The bacterial isolates were preliminary identified by lipid profile using TLC and the results of the TLC were confirmed by physiological and biochemical tests.

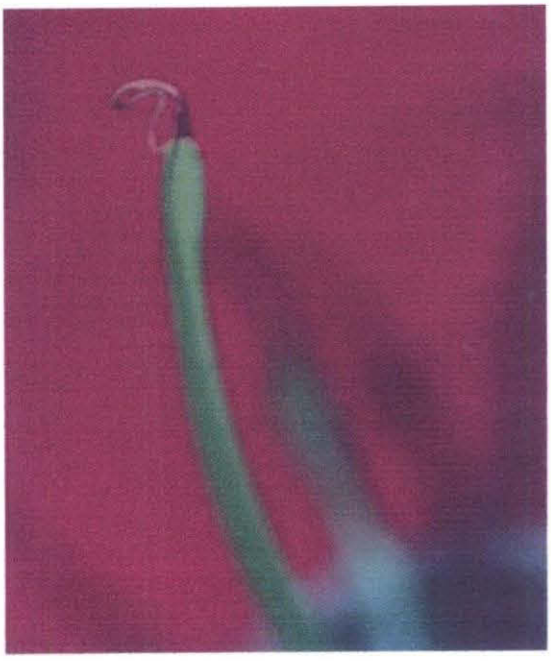

Fig.10. Development of leaf blight symptom on tuberose leaf inoculated with an isolate.

Thin layer chromatography: The TLC chromatograms of bacterial isolates are given in Fig. 11. The chromatographic profiles of lipids from all the seven yellow colony isolates used in TLC were found identical. A common spots at Rf 0.75 was found in all isolates. Similar types of chromatographic profiles were reported earlier for the species of the genus *Xanthomonas* (Matsuyama *et al.* 1993). According to Schaad (1988), the yellow color colony of plant pathogenic bacteria were observed in genus *Xanthomonas.* The results of TLC suggested that the yellow isolates were member of the genus *Xanthomonas.*

Gram staining reaction: All the seven yellow colony color isolates, isolated from diseased leaves were gram negative (Table 2).

Oxidase test: All the yellow isolates were oxidase negative. Did not produce deep purple color on the filter paper within ten seconds after the addition of tetramethyl-p-phenylenediamine hydrochloride, proved to be negative for all isolate (Table 2).

Catalase test: The isolates were catalase positive (Table 2). One loopful of bacterial cell when mixed with a drop of H_2O_2 produced gas bubbles which was an indication of positive reaction.

Mucoid growth in YDC: All seven isolates when cultured in YDC medium showed distinct yellow colony color and mucoid growth which indicated positive reaction (Table 2).

Urease test; An increase in alkalinity indicated by magenta red color was the evidence of urease activity. But all the bacterial isolates showed no urease activity (Table 2).

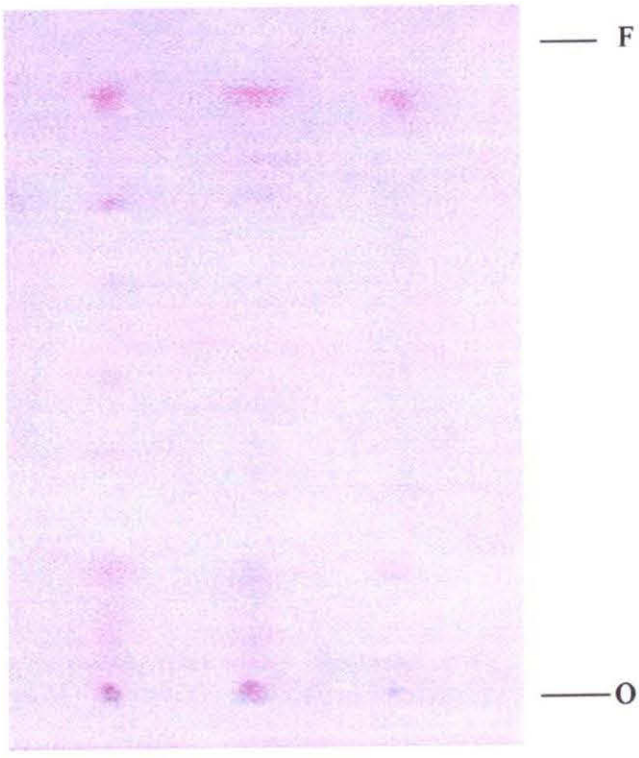

Fig.11. TLC chromatograms of lipids from bacterial isolates isolated from tuberose leaves [F = Front line of solvent; O = Origin]

Table 2. Physiological and biochemical tests for isolates isolated from diseased leaves of tuberose

Test	Present isolate (n=7)	*Xanthomonas campestris* *
Gram stain test	-	-
Oxidase test	-	-
Catalase test	+	+
Mucoid growth in YDC	+	+
Urease test	-	-
Esculin hydrolysis	+	+
Gelatin liquefaction test	+	+
Protein digestion	+	+
Tobacco HR test	Chlorosis	

+positive; -negative; HR-hypersensitivity reaction; n = Number of isolates

* Results from Burgey's Mannual of systematic bacteriology (Krieg and Holt 1984)

Gelatin liquefaction test: All the seven yellow colored isolates were positive in gelatin liquefaction test. The readily flows of the medium when tubes were gently tipped was considered the gelatin was hydrolyzed (Table 2).

Protein digestion: All the isolates digested protein. Clearing of the medium after inoculation indicated the digestion of casein (Table 2).

Tobacco hypersensitivity reaction (HR) test: The isolates did not show hypersensitivity reaction. But produced chlorosis symptoms on tobacco leaves (Table 2).

Utilization of carbon: All the yellow isolates utilized cellobiose, mannose, milibiose, maltose, arabinose, glucose, saccharose, trehalose, mannitol and β-alanine as a sole source of carbon. But these isolates did not utilized lactose, sorbitol, inositol tartaric acid and benzoic acid as their carbon source (Table 3).

The results of TLC chromatographs, and physiological and biochemical tests-gram stain test, oxidase test, catalase test, esculin hydrolysis, gelatin liquefaction, urease production, protein digestion, utilization of carbon demonstrated that the bacterial isolates isolated from leaf blight symptoms on tuberose were *Xanthomonas campestris*.

Table 3. Utilization of different carbon source by the isolates isolated from diseased leaves of tuberose

Carbon source	Present isolate N=7	*Xanthomonas campestris* *
Cellobiose	+	+
Mannose	+	+
Sorbitol	-	-
Maltose	+	+
Lactose	-	-
Glucose	+	+
Saccharose	+	+
Trehalose	+	+
Mannitol	+	+
Inositol	-	-
Melibiose	+	+
Arabinose	+	+
B-Alanine	+	+
Tartaric acid	-	-
Benzoic acid	-	-
Glutamic acid	+	+
Acetic acid	+	+
Control	-	

+positive; -negative; n = Number of isolates.

* Results from Burgey's Mannual of systematic bacteriology (Krieg and Holt 1984)

Identification of root-knot disease of tuberose

Characteristic symptoms of root-knot disease were found to be associated with tuberose plants. There were two types of symptoms, the above ground and under ground.

The above ground symptoms were yellowing and drying up of leaves and retarded growth. In severely affected plants the emergence of panicles was suppressed resulting in an absolute loss of flower. Emergence of side shoot from bulbs was also affected and the production of flowers was severely reduced.

Infected roots swelled at the point of invasion and developed into the typical root-knot or galls which were two folds larger in diameter than the healthy root. Infected roots remained smaller (Fig. 12). The causal nematode was identified as a species of *Meloidogyne* sp. (Fig. 13).

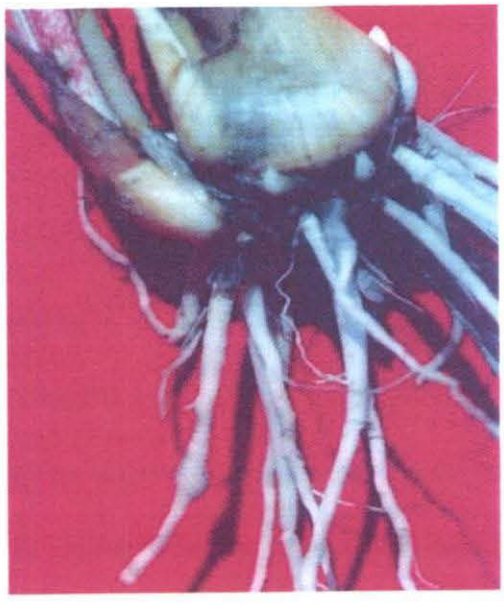

Fig. 12. Root-knot (*Meloidogyne* sp.) symptoms of tuberose recorded from the experimental field

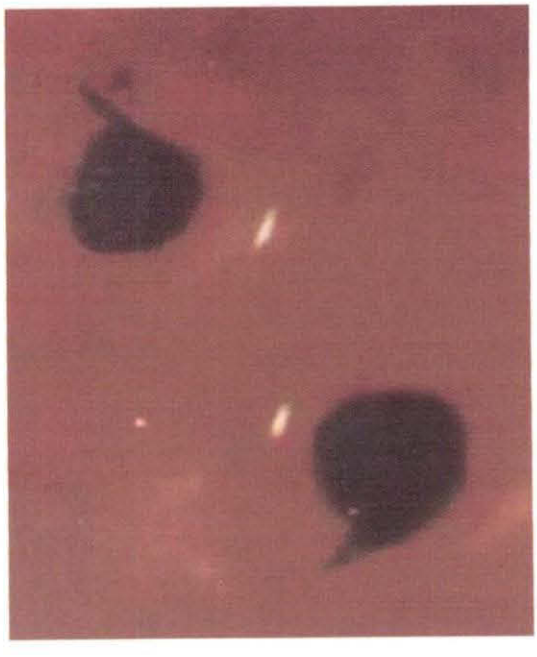

Fig.13. Mature female of *Meloidogyne* sp. found in root gall of tuberose

Incidence of tuberose diseases in experimental field

The severity of the tuberose diseases in the field in different growth stages of plant was summarized in Table 4. The results reveled that the stem rot caused by *S. rolfsii*, and leaf spot caused by *A. polyanthi* appeared in the field at the early stage of plant growth. Blossom blight caused by *F. equiseti* appeared at flowering stage. The bacterial leaf blight caused by *X. campestris* was found to occur at early stage. The root-knot caused by *Meloidogyne* sp. was recorded in later stage of plant growth.

Among the diseases of tuberose, the highest numbers of plants were infected with leaf blight which was followed by blossom blight, leaf spot, root-knot and stem rot. The percent plant infected by different diseases showing in Table 4.

The diseases identified in the present investigation have been reported to occur on tuberose in different tuberose growing area of the world (Rangaswamy *et al.* 1970, Dutta 1975, Roy 1984, Tzeng and Tung 1998 and Mariappan *et al.* 1977). Moreover, systematic research on tuberose diseases has not yet been done in Bangladesh since tuberose as a crop in Bangladesh has been newly introduced. The diseases of tuberose so far identified in the present study may be the first record in Bangladesh. Further study at different agro-climatic zones is necessary for proper recording of tuberose diseases and their causal agents.

Table 4. Incidence of different diseases of tuberose in the experimental field condition of BSMRAU

Disease	Causal agent	(%) plant infected	Remarks
Leaf blight	*Xanthomonas campestris*	100	Prevalent at early stages of plant growth
Blossom blight	*Fusarium equiseti*	44	Appeared at flowering stage
Leaf spot	*Alternaria polyanthi*	36	Appeared early stage of plant growth
Root-knot	*Meloidogyne* sp.	5	Appeared at later stage of plant growth
Stem rot	*Sclerotium rolfsii*	4	Appeared at early stage of plant growth

Severity of leaf blight of tuberose in relation to temperature and humidity

The result as presented in the Table 5 suggested that there was a trend of increase of percent disease index (PDI) with the decrease of temperature. The increased PDI was also observed during low relative humidity. Highest PDI of the disease was recorded during the month of February followed by January and March while no disease incidence was recorded in July (Table 5).

Correlation co-efficient 'r' between PDI and temperature had negative and highly significant. There was also negative and highly significant correlation between PDI and relative humidity. The regression equation obtained were $Y = 20.00 - 0.58X$ and $Y = 37.32 - 50X$ for temperature and relative humidity, respectively (Fig 14 and Fig 15).

Table 5. Fortnightly percent disease index, mean temperature, relative humidity during Jan. 1 to July 30, 2004

Period (fortnight)	Percent disease index (PDI)	Mean temperature ^{0}C	Relative humidity (%)
Jan.1-Jan. 15	8.0	15.30	67.86
Jan. 16-Jan. 30	10.0	18.30	58.10
Jan.31-Feb. 14	11.5	14.96	54.30
Feb. 15-Feb. 29	10.0	23.06	56.65
March 1-March 15	9.0	23.61	55.16
March 16-March 31	7.0	24.69	61.36
April 1-April 15	5.0	27.24	67.65
April 16-April 30	4.0	28.11	68.73
May 1-May15	4.0	31.48	58.13
May 16-May30	3.8	29.68	65.10
May 31-June 15	2.8	29.89	65.90
June 16-June 30	0.8	28.77	71.26
July 1-July 15	0.0	29.66	72.83
July 16-July 31	0.0	29.14	71.10

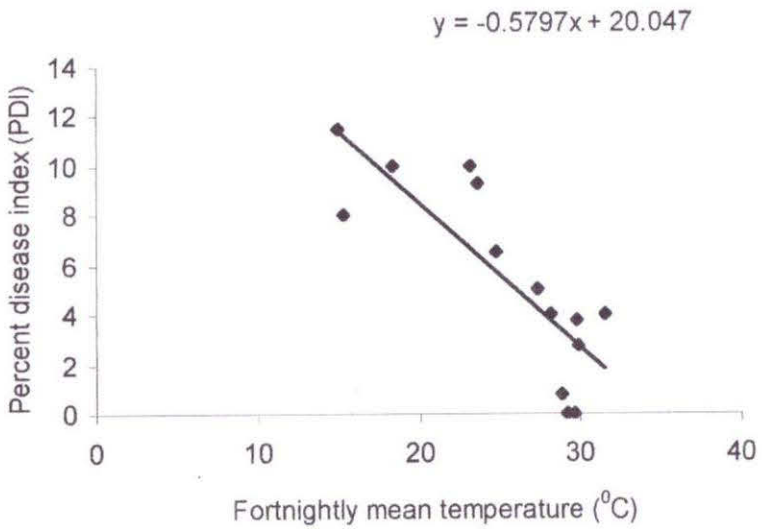

Fig.14. Relationship between severity of leaf blight of tuberose and temperature

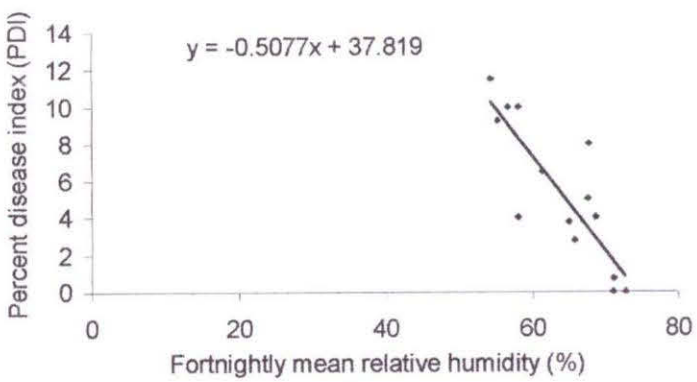

Fig.15. Relationship between severity of leaf blight of tuberose and humidity (%)

CHAPTER V

SUMMARY AND CONCLUSION

Investigation of tuberose diseases was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) farm during 2003-2004 seasons. The experimental plots were inspected regularly to record initiation of any disease symptoms. The diseases were identified by studying the symptoms observed in the field preliminarily. Fungal pathogens were identified by isolating the pathogen, purifying them and studying different characteristics of the pathogens under compound microscope followed by pathogenicity test. The bacterial pathogen was identified by lipid profiling and studying several physiological and biochemical characteristics followed by pathogenicity test. The nematode was identified by morphological study.

There were three different fungal diseases; one bacterial disease and one nemic disease of tuberose (*Polyanthes tuberosa*) were identified with their causal agents. The identified fungal diseases were stem rot caused by *Sclerotium rolfsii*, blossom blight caused by *Fusarium equiseti*, and leaf spot caused by *Alternaria polyanthi*. In case of stem rot, initially the leaves lost green color due to rotting and dropped followed by yellowing. In case of blossom blight, water soaked spots appeared on the petals which soon darken and dried up. In A*lternaria* leaf spot, brown spots appeared with faint concentric rings on the midrib and rarely on the margin of the leaf. The identified bacterial disease was leaf blight. Water soaked spots first developed at the tip of the leaf which gradually enlarged and spread downward along the margin. The causal agent was identified as *Xanthomonas campestris*. All the seven isolates were identical

in lipid profiling. Bacteriological characters distinguishing the isolates were, gram staining reaction, oxidase test, catalase test, esculin hydrolysis, protein hydrolysis, gelatin liquefaction, urease production and carbon source utilization. The nematode disease was identified as root-knot. Infected plants showed yellowing, drying up of the leaves and retarded growth. Infected roots swelled at the point of invasion and typical root-knots or galls were formed. The causal agent was identified as *Meloidogyne* sp.

All the diseases except blossom blight and root-knot were found to occur in early stages of plant growth. The blossom blight was found to occur in flowering stage and root-knot was in later stage of plant growth. The leaf blight found to occur severely during early stage of plant growth when temperature and relative humidity remained relatively low. The prevalence of leaf blight disease was found very high 100 % plants were infected followed by blossom blight (44 % plant infected), leaf spot (36 % plant infected), root-knot (5 % plant infected) and stem rot (4 % plant infected). The percent disease index (PDI) of leaf blight was found to be increased with the decreasing of temperature and relative humidity. Highest PDI value (11.5 %) of the disease was observed at around 15 ^{0}C and about 54 % relative humidity. The results of the present investigation demonstrate the need of careful investigation of disease problem of tuberose at different agro ecological zone of the country.

CONCLUSION

There were three fungal diseases viz., stem rot caused by *Sclerotium rolfsii*, blossom blight caused by *Fusarim equiseti*, and *Alternaria* leaf spot caused by *Alternaria polyanthi* were recorded.

One bacterial disease viz., leaf blight caused by *Xanthomonas campestris* was recorded from the experimental plots under field conditions

One nemic disease-root-knot caused by *Meloidogyne* sp. was also recorded from the experimental plots under field conditions.

CHAPTER VI

LITERATURE CITED

Agostini, J. P. and Timmer, L. W. 1992. Selective isolation procedure for the differentiation of two strains of *Colletotrichum gloeosporioides* from citrus. Plant Dis. 76: 1176-1178.

Ahmed, K. U. 1995. Flowers, Fruits and Vegetables. Call Quick Printing Press. 149/A. Air Port Road. Dhaka. 118 p.

Ayers, S. H., Rupp, P. and Johnson, W. T. 1919. A study on the alkali-forming bacteria in milk. U. S. Dept. Agric. Bull. 782 p.

Bose, T. K. and Yadav, P. L. 1998. Commercial Flowers. Naya Prokash. 206 Bidhan Sarani, Kolkata, India. 540 p.

Chang, C. A. and Chen, C. C. 1999. Current status of tuberose virus research and the development of its indexing techniques. Plant Pathol. Bull. 8: 89-94.

Chen, C. C., Chiang, F. L. and Chang, C. A. 1998. Distribution of tuberose mild mosaic potyvirus in tuberose (*Polyanthes tuberosa* L.). Plant Protec. Bull. Taipei. 40: 199-207.

Das, A. C. 1961. Disease of Rajanigandha caused by *Sclerotium rolfsii*. Sci. Cult. 27: 549-550.

Dasai, B. L. 1957. Tuberose. Indian Farming. 7: 10.

Dasgupta, B. 1981. Sporulation and relative virulence among isolates of *Colletotrium capsici* causing anthracnose in betelvine. Indian Phytopathol. 32: 196-199.

Dater, V. V. and Mayee, C. D. 1981. Epidemiology of early blight of tomato caused by *Alternaria solani*. Indian Phytopathl. 35: 434-437.

Dube, H. C. 1994. An introduction to fungi. Vikas Publishing House PVT LTD., Janpura, New Delhi, India. 412-418 pp.

Dutta, A. K. 1975. Sclerotium wilt of *Polyanthes* and *Caladium* and thier control. Sci. Cult. 41: 424.

Dye, D. W. 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. NZ. J. Sci. 5: 393-416.

Ellis, M. B. 1971. Dematiaceous Hypomycetes. Commonwealth Agric. Bureau. England. 608 p.

Ganguly, S., Misra, R. L. and Mishra, S. D. 1993. New disease complex of tuberose (*Polyanthes tuberosa*) involving root-knot nematode, *Meloidogyne incognita* and a mite species. Curr. Nematol. 4: 113-114.

Haider, J. Marumoto, K. and Azad, A. K. 1991. Estimation of microbial biomass carbon and nitrogen in Bangladesh Soil. Sci. Plant Nutr. 37: 591-599.

Jayaraman, V., Rajendran, G. and Muthukrishnan, T. S. 1975. Occurrence of root knot nematodes in *Polyanthes tuberosa* L. Tamil Nadu. Indian J. Nematol. 5:101-102.

Khan, A. A. and Matsuyama, N. 1999. A rapid extraction TLC method for differentiation of *Burkholderia spp. Ralstonia solanacearum, Herbaspirillum rubrisubalbioans* and *Pseudomonas syringae* pathovars. J. Fac. Agr. Kyushu Univ. 44: 49-58.

Klement, A. and Goodman, R. 1967. The hypersensitive reaction to infection by bacterial plant pathogens. Ann. Rev. Phytopathol. 5: 17-44.

Kovacs, N. 1956. Identification of *Pseudomonas pyocyana* by the oxidase reaction. Nature. 178-703 p.

Krieg, N. R. and Holt, J. G. Eds. 1984. Burgey's manual of systematic bacteriology. Vol. I Williams and Wilkins, London. 141-177 pp.

Maity, R. G. 1999. Tuberose. Floriculture and landscaping. Eds: T. K. Bose, R. G. Maity, R. S. Dhua and P. Das. Naya Prokash, Kolkatta, India. 505 p.

Mallavarapu, G. R. 1995. Essential oils from some aeromatic ornamental plants. Advance in Horticulture 12: 1046. Ornamental Plants. Eds: K. L. Chadha and S. K. Bhattacharjee. 1995. Malhotra Publishing House, New Delhi, India.

Mariappan, V., Kochubaba, T. and Kandaswamy, T. K. 1977. A new leaf spot disease of tuberose caused by a new species of *Alternaria.* Curr. Sci. 46: 311.

Matsuyama, N. Mian, I. H. Akanda, A. M. and Furuya, N. 1993. Comparative studies on thin-layer chromatograms of lipids from various phytopathogenic bacteria. Ann. Phytopathol. Soc. Japan. 59: 528-532.

Mian, I. H. 1995. Methods in Plant Pathology. IPSA-JICA Project. 135 p.

Mohanty, L. P. and Das, S. N. 1996. Pathogenicity of root knot nematode (*Meloidogyne incognita*) on tuberose. Orissa J. Hort. 24: 51-54.

Nambisan, K. M. P. and Krishnan, B. M. 1983. Better cultural practices for high yield of tuberose in South India. Indian Hort. 28: 17- 20.

Rangaswamy, G., Seshadri, V. S. and Chennamma, L. K. A. 1970. Fungi of South India. 193 p.

Roy, A. K. 1984. Two new *Fusaria* diseases of Karbi Anglong, Assam, India, Curr. Sci. 53: 1303-04.

Schaad, N. W. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. APS press. 164 p.

Schaad, N. W. and White, R. L. 1974. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology. 64: 876-880.

Singh, R. S. Motial, V. S. and Singh, L. B. 1976. Effects of nitrogen, phosphorus and potash fertilizer on tuberose (*Polyanthes tuberosa*). Indian J. Hort., 33: 289-294.

Suhag, T. V., Singh, R. and Malik, Y. S. 1985. Epidemiology of pod and leaf blight of radish seed crop caused by *Alternaria alternata*. Indian Phytopathol. 38: 148-149.

Sundara, B. R. and Vadivelu, S. 1989. Pathogenicity of *Meloidogyne* sp. to tuberose (*Polyanthes tuberosa* L.). Indian J. Nematol. 18: 146-148.

Tzeng, S. L. and Tung, B. K. 1998. The occurrence of leaf spot disease of tuberose. Plant Protec. Bull. Taipei. 40: 145-152.