FUNGAL PATHOGENS ASSOCIATED WITH ROSELLE (*Hibiscus sabdariffa*) IN PENANG, AND CONTROLS

By

TOUBA ESLAMINEJAD

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DEDICATION

This piece of work is dedicated to my parents, especially my mom who supports me in all my life and encourages me to fulfil my dream.

Thank you and I love you

TOUBA

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LIST OF ABBREVIATIONS

µg	micro gram
µm	micro metre (10^{-3}mm)
ALGR	Average Linear Growth Rates
ANOVA	Analysis Of Variance
BCA	Biological Control Agents
CLA	Carnation Leaf-piece Agar
cm	centimetre
CMA	Corn Meal Agar
CZA	Czepak's Agar
Dai	Days after inoculation
Diam	Diameter
DOA	Department Of Agriculture
DS	Disease Severity
f. sp.	Forma specialis
f. spp.	formae speciales
g	Gram
GUP	General Use Pesticide
ha	Hectare
h	Hour
KSKP	Karanj Seed Kernel Powder
LCB	Lactophenol Cotton Blue
MA	Malt Agar
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
min	Minute
mL	Millilitre
mm^2	Millimetre squared
N	Normality
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
NLP	Neem Leaf Powder
NSKP	Neem Seed Kernel Powder
NSO	Neem Seed Oil
°C	degree centigrade
OA	Oat Agar
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
psi	pound per square inch
RCBD	Randomized Complete Block Design
s	Second
SEM	Scanning Electronic Microscopy
sp.	Species
SPSS	Statically Package for the Social Science
U.S	United State
U.S.A	United State of American
USM	Universiti Sains Malaysia

U.S.S.R	Union of Soviet Socialist Republics
var.	Variety
VJA	Vegetable Juice Agar
W	Watt
WA	Water Agar
WAM	Waksman Agar Medium

KULAT PATOGEN YANG DIKAITKAN DENGAN ROSELLE (*Hibiscus sabdariffa*) DI PULAU PINANG, DAN PENGAWALANNYA

ABSTRAK

Objektif utama kajian ini adalah untuk mengenal pasti serta mencirikan patogen kulat yang dikaitkan dengan penyakit Roselle di Pulau Pinang, serta mengkaji pengawalannya. Sejumlah 200 isolat kulat telah diperolehi daripada 90 sampel tisu Roselle yang mempunyai simptom. Kulat yang diasingkan dikenal pasti berdasarkan ciri kultur dan morfologi serta kepatogenannya. Patogen kulat yang paling banyak diasingkan adalah *Phoma exigua* (wujud dalam 45 % daripada sampel yang dikutip), diikuti dengan *Fusarium nygamai* (25 %), *Rhizoctonia solani* (19 %) dan *F. camptoceras* (11 %). Ujian kepatogenan menunjukkan bahawa *P. exigua*, *F. nygamai*, *F. camptoceras* dan *R. solani* boleh menjangkiti anak benih yang terluka dan tidak terluka berdasarkan darjah keterukan yang berbeza, seperti yang dinyatakan dalam keterukan penyakit (DS). *R. solani* merupakan kulat patogen yang paling patogenik menjangkiti kedua-dua anak benih yang terluka dan tidak terluka, diikuti oleh *P. exigua* yang patogenik terhadap anak benih yang terluka. *F. nygamai* kurang patogenik, manakala yang paling kurang patogenik adalah *F. camptoceras*, yang hanya menjangkiti anak benih yang tidak terluka. Potensi *Trichoderma viride* sebagai agen kawalan biologi dinilai secara *in vitro* terhadap *P. exigua*, *F. nygamai* dan *R. solani* menggunakan teknik dua-kultur. Perencat meruap dan perencat tidak meruap bagi *Trichoderma* juga dinilai bagi tujuan tersebut. *T. viride* telah menunjukkan kesan perencatan yang baik ke atas patogen yang diuji dalam eksperimen dua-kultur. Perencatan maksimum berlaku terhadap *F. nygamai* dengan pengurangan 78.80 % dalam pertumbuhan radius miselium. Ketiga-tiga patogen, *P. exigua, F. nygamai* dan *R. solani* didapati rentan terhadap perencat meruap yang dihasilkan oleh *T. viride*, menyebabkan perencatan kira-

kira 68 % bagi setiap patogen. Apabila metabolit tidak meruap *T. viride* diuji terhadap patogen, perencatan maksimum berlaku terhadap *R. solani* (perencatan pertumbuhan miselium 73.95 %), diikuti dengan *P. exigua* (perencatan 37.17 %). Namun demikian, kesan perencatan metabolit tidak meruap ke atas *F. nygamai* adalah minimum. Ekstrak mentah sembilan tumbuhan iaitu, peria, buah pelaga, cili, ketumbar, bawang, bawang putih, halia, lengkuas dan biji sawi disediakan dengan menggunakan air panas dan air sejuk dan diuji kesan antikulat masing-masing. Sediaan berformula, AFX juga diuji aktiviti antikulatnya terhadap tiga patogen Roselle menggunakan 'teknik makanan beracun'. Kesemua ekstrak tumbuhan yang dikaji menunjukkan aktiviti antikulat yang signifikan pada tiga kepekatan (10, 20 dan 30 % daripada ekstrak mentah) secara *in vitro***.** Ekstrak air sejuk bagi peria, bawang putih dan biji sawi menunjukkan aktiviti antikulat yang baik terhadap ketiga-tiga kulat yang diuji. Dalam kes ekstrak air panas, ekstrak peria, bawang putih dan halia menunjukkan aktiviti antikulat. Daripada dua kaedah pengekstrakan ini, secara umumnya, ekstrak air sejuk lebih berkesan jika dibandingkan dengan ekstrak air panas dalam mengawal patogen Roselle. Melawan *P. exigua,* ekstrak air sejuk bawang putih, lengkuas, biji sawi, ketumbar dan peria berjaya merencat pertumbuhan miselium patogen sepenuhnya (100 %). Perencatan seratus peratus pertumbuhan miselium *F. nygamai* dicapai dengan ekstrak air sejuk daripada peria dan bawang putih. Melawan *R. solani*, ekstrak air sejuk bawang putih secara efektif menghasilkan 100 % perencatan. Sehubungan itu, ekstrak bawang putih didapati paling berkesan untuk mengawal ketiga-tiga *P. exigua, F. nygamai* dan *R. solani* secara *in vitro*. Tiada diantara ekstrak air panas tumbuhan yang dapat menghasilkan 100 % perencatan dalam pertumbuhan miselium patogen. AFX, suatu ekstrak tumbuhan yang dirumus, secara signifikan dapat mengurangkan pertumbuhan kesemua patogen pada kepekatan 10 dan 20 %. Penilaian racun kulat komersial iaitu, Thiram dan Benomyl dalam dua kepekatan (10 dan 20 %) mengesahkan bahawa kedua-duanya efektif dalam merencatkan pertumbuhan patogen kulat.

FUNGAL PATHOGENS ASSOCIATED WITH ROSELLE (*Hibiscus sabdariffa*) IN PENANG, AND CONTROLS

ABSTRACT

The main objective of this study was to identify and characterize fungal pathogens associated with Roselle diseases in Penang, and to study their control. A total of 200 fungal isolates were obtained from 90 samples of symptomatic Roselle tissues. The isolates were identified based on cultural and morphological characteristics, as well as their pathogenicity. The fungal pathogen most frequently isolated was *Phoma exigua* (present in 45 % of the samples collected), followed by *Fusarium nygamai* (25 %), *Rhizoctonia solani* (19 %) and *F. camptoceras* (11 %). Pathogenicity tests showed that *P. exigua, F. nygamai, F. camptoceras* and *R. solani* were able to infect both wounded and unwounded seedlings with different degrees of severity as indicated by the Disease Severity (DS). *R. solani* was the most pathogenic fungus affecting both wounded and unwounded Roselle seedlings, followed by *P. exigua* that was highly pathogenic on wounded seedlings. *F. nygamai* was less pathogenic while the least pathogenic fungus, *F. camptoceras,* infected only the unwounded seedlings but, surprisingly, not the wounded plants. The potential of *Trichoderma viride* as a bio control agent was evaluated *in vitro* against *P. exigua, F. nygamai* and *R. solani* using the dual culture technique. Volatile and non-volatile inhibitors of *Trichoderma* were also evaluated for this purpose. *T. viride* was shown to have a marked inhibitory effect on the tested pathogens in the dual culture experiments. Maximum inhibition occurred against *F. nygamai,* with 78.80 % reduction in mycelial radial growth. The three pathogens, *P. exigua, F. nygamai* and *R. solani* were also found to be susceptible to the volatile inhibitors produced by *T. viride,* giving rise to growth inhibition of about 68 % in each

case. When *T. viride* non-volatile metabolites were tested against the pathogens, maximum inhibition occurred against *R. solani* (73.95 % mycelial growth inhibition), followed by *P. exigua* (37.17 % inhibition). The inhibitory effect of the non-volatile metabolites on *F. nygamai* was, however, minimal. Crude extracts of nine plants, *viz.* bitter gourd, cardamom, chilli, coriander, onion, garlic, ginger, galangale and brassica were made using cold water and hot water and tested for their anti-fungal effects. A formulated preparation (AFX) was also examined for its antifungal activity against the three Roselle pathogens using the 'poisoned agar technique'. All nine plant crude extracts studied showed significant antifungal activity at three concentrations (10, 20 and 30 % of the crude extract) *in vitro.* The cold water extracts of bitter gourd, garlic, and brassica exhibited good antifungal activity against all three tested fungi. In the case of the hot water extracts, bitter gourd, garlic and ginger showed antifungal activity. Of the two extraction methods, cold water extraction was generally more effective than hot water extraction in controlling the Roselle pathogens. Against *P. exigua*, the 30% of cold water extracts of garlic, galangale, brassica, coriander and bitter gourd achieved total (100 %) inhibition of pathogen mycelial growth. Total inhibition (100 %) of *F. nygamai* mycelial growth was similarly achieved with the cold water extracts of bitter gourd and garlic. Against *R. solani*, the cold water extract of garlic was effective in resulting 100 % inhibition. Therefore, garlic extract was found to be the most effective in controlling all three *P. exigua F. nygamai* and *R. solani in vitro*. None of the hot water extracts of plant succeeded in achieving 100 % inhibition of the pathogen mycelial growth. AFX, a formulated plant extract significantly reduced the growth of all the pathogens at 10 and 20 % concentrations. Evaluation of the commercial fungicides i.e., Thiram and Benomyl, at two concentrations (10 and 20 %) confirmed that both fungicides were effective in inhibiting the mycelial growth of the fungal pathogens.

CHAPTER ONE

INTRODUCTION

1.1 Roselle

Roselle, or Jamaica sorrel (*Hibiscus sabdariffa*) belongs to the Malvaceae family of the Hibiscus section Furcaria (Verdcourt, 2003). As it is resistant to short periods of drought, Roselle can be cultivated throughout the tropics and subtropics during hot and rainy seasons (McClintock & Tahir, 2004). It is a popular vegetable in many tropical regions, including Indonesia, India, West Africa (Tindal, 1986) and the North- Eastern and middle belt regions of Nigeria (Akanya *et al.*, 1997). The plant thrives in a wide range of soil conditions. It grows satisfactorily in relatively infertile soils, but for commercial cultivation, a soil rich in organic materials and essential nutrients is essential (Tindal, 1986). Roselle is an annual tropical plant cultivated for its leaves, seeds, stems and calyces (Halimatul *et al.*, 2007; Lawton, 2004). The dried calyces are used to prepare tea, syrup, jams and jellies and as beverages (Fasoyiro *et al.*, 2005). Roselle tea is used to control high blood pressure and its leaves are used as a source of mucilage in medicine and cosmetics (McClintock & Tahir, 2004). Its root decoction has laxative properties; it increases the flow of urine and relieves fevers (McClintock & Tahir, 2004). The Roselle plant is also a rich source of fibre known as Roselle Hemp, which has been used in the paper industry in the United States and Asia (Lawton, 2004; McClintock & Tahir, 2004). Roselle is a popular health drink among Malaysians who consume it for its high vitamin C and anthocyanin contents (Bajaj, 1993). This plant was initially introduced in Malaysia in the early 1990s. The Department of Agriculture first promoted commercial planting of Roselle in Terengganu in the 1993, and other states soon followed suit (Halimatul *et al.,* 2007).

1.2 Roselle Diseases

There are various diseases that attack Roselle in the field as well as the harvested crop in storage. Roselle is susceptible to a number of plant pathogens in the field. Mildew is an important disease that destroys the leaves, especially late in the year (Rolfs, 1929). Some of the diseases of Roselle are root rots and stale rots caused by *Phythophtora parasitica*, root rots caused by *Botrytis cinerea*, leaf fleck caused by *Phoma sabdariffa*, blackleg, stalk base and root rots caused by *Macrophomina phaseolina*, root and seed rots caused by *Rhizoctonia solani*, seed and stem rots caused by *Sclerotium rolfsii*, leaf spot caused by *Cercospora hibisci*, and powdery mildew caused by *Oidium abelmoschii* (McClintock & Tahir, 2004). In addition, vascular wilt can also result from infection by *Fusarium oxysporum* derived from plant debris and infected soil (Amusa, 2004). In Malaysia, *Fusarium oxysporum* has been reported to cause Fusarium wilt (Ooi & Salleh, 1999).

Among the fungal pathogens of Roselle, *Phoma,* a Coelomycete (Alexopoulos *et al.*, 1996), is known to cause leaf and stem spots in plants (Aveskamp *et al.*, 2008; Zhang *et al.*, 2009). Approximately 50 % of *Phoma* species described by Boerema *et al.* (2004) are recognised as phytopathogenic, including some that have been assigned quarantine status (Aveskamp *et al.*, 2010). Although most *Phoma* species are present as saprobes in the soil, many of them become pathogenic when a suitable host is encountered (Aveskamp *et al.*, 2008).

Another common fungus pathogenic to Roselle is *Fusarium* which is widely distributed throughout the world (Alastruey-Izquierdo *et al.*, 2008; Levic *et al.*, 2009). The genus contains species that exist in both pathogenic and non-pathogenic forms (Alastruey-Izquierdo *et al.*, 2008; Rodrigues & Menezes, 2005). The pathogenic forms cause diseases in a number of economically important crops such as banana, cotton, legumes, maize, rice and wheat (Summerell *et al.*, 2003). According to Alastruey-Izquierdo *et al*. (2008), the most common pathogenic species are *F. solani* and *F. oxysporum.* Previous research has found that *F. oxysporum* and *Phyllosticta hibiscini* are associated with the Roselle plant in Malaysia and Nigeria respectively (Amusa, 2004; Amusa *et al.*, 2001; Amusa *et al.*, 2005; Ooi & Salleh, 1999; Ooi *et al.*, 1999).

Roselle is also susceptible to *Rhizoctonia*, which are ubiquitous soil inhabitants (Sneh *et al.*, 1996). Many isolates of *Rhizoctonia* are saprophytic and some of them are mycorrhizal with orchids while others can act as bio-control agents (Sneh *et al.*, 1996). The genus *Rhizoctonia* is among the most important plant pathogens that can cause serious damage to various plant species (Aye *et al.*, 2008). Many *Rhizoctonia* species are able to thrive in wide range of environmental conditions, their propagules surviving as a thick-walled hyphae and sclerotia associated with plant debris in the soil (Aye *et al.*, 2008). Important diseases caused by *Rhizoctonia* spp. include damping-off and root rots caused by *R. solani.* These are among the most common and destructive diseases which infect young seedlings of many plant species in forest nurseries around the world (Camporota & Perrin, 1998). Rhizoctonia root rots are one of the most common soil-borne diseases of soybeans in the world. Black scurf disease caused by *R. solani* is globally present in the majority potato-producing areas (Johnson & Leach, 2003) while web blight caused by *R. solani* is a well-known disease of cowpea in Brazil (Nechet *et al.*, 2006).

1.3 Scope of the Study

Roselle has drawn the attention of food, beverage and pharmaceutical manufacturers because of its commercial potential as a natural food and colouring agent that can replace some synthetic products (Morton, 1987; Qi *et al.*, 2005). Due to its global demand, cultivation of Roselle is on the increase (McClintock & Tahir, 2004). Being a relatively new crop in Malaysia, the diseases of Roselle in this country are not well-documented. Most of the research on Roselle in Malaysia has so far concentrated on its antioxidant activity (Halimatul *et al.*, 2007; Mohd-Esa *et al.*, 2010; Tee *et al.*, 2002), health benefits (Hirunpanicha *et al.*, 2006) and nutritional value (Halimatul *et al.*, 2007). There is hence a gap in knowledge related to its agronomic aspects in the Malaysian environment, notwithstanding the fact that pest and disease problems are among the important factors that limit its commercial cultivation.

To address the relative lack of information on the impact of diseases on Roselle cultivation in Malaysia, this study was undertaken to investigate the potential threat that diseases pose to Roselle growing. The study was also designed to screen a range of fungicides and plant extracts to evaluate their usefulness in the chemical and biological control of Roselle fungal diseases.

1.4 Objectives

To identify pathogens associated with Roselle diseases based on their morphological and cultural characteristics.

To determine the pathogenicity of four fungi infecting Roselle seedlings, namely *Phoma exigua*, *Fusarium nygamai*, *Fusarium camptoceras* and *Rhizoctonia solani*.

To study the control of the pathogens using biological agents, plant extracts and commercial fungicides *in vitro*.

1.5 Thesis Layout

Chapter One provides the background to this study, and the rationale for the research. The objectives and details of the thesis organization are also discussed here. Chapter Two provides an overview of the Roselle plant with particular reference to its diseases, the causative fungal pathogens and the methods of control. Chapter Three describes the laboratory materials and methods used in this study. Chapter Four presents the results of the investigations carried out to identify the fungal pathogens, to test their pathogenicity and to control their infection on Roselle plants. The results are discussed in relation to the methodologies adopted and the results that were obtained in this study. Chapter Five states the conclusions drawn from this study and suggests possible directions for future research.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Hibiscus sabdariffa*

According to Anderson (2006), there are about 300 tropical and sub-tropical *Hibiscus* species. They have a wide variety of uses as fibres, foods, medicines, and ornaments. Fibres can be obtained from many of the species, notably *H. cannabinus*, *H. sabdariffa*, *H. surattensis, H. planifolius, H. flococcus* and *H. macrophyllus* (Anderson, 2006). The leaves from the *H. lunarifolius, H. rostellatus*, *H. squamosus, H. tiliaceus, H. cannabinus* and *H. sabdariffa* are consumed as green vegetables in West Africa and the swollen calyces are eaten raw in salads (Anderson, 2006). The dried calyces of *H. sabdariffa* are used in making an infusion beverage known as “zobo” in Nigeria while the leaves of Roselle are used as medicines (Akpan, 2000). Several species of *Hibiscus* are used as ornamental plants, such as *H. rosa- sinensis* cultivated in China, India, Japan and the Pacific Islands (Anderson, 2006).

Roselle is an annual plant which takes about six months to mature. The major morphological characteristics of Roselle are illustrated in Figure 2.1. The leaves of Roselle plants are divided into three to five lobes and they are arranged on the stem alternately. Each calyx lobe of the Roselle flower has a prominent central rib and two marginal ribs; these characteristics place the plant in the section of Furcaria (Douglas Wilson, 1993). Flowers are white to pale yellow in colour, with fleshy and soft calyces. The mature fruits are bright red (Halimatul *et al.*, 2007; Morton, 1987; Qi *et al.*, 2005).

Figure 2.1: Morphology of Roselle plant. (A) Leaves; (B) pink or yellow flower; (C) Red fresh calyces; (D) Fruit; (E) Dark brown seeds. [Source; (A, E) McClintock and Tahir (2004)].

Roselle is known by different names in different countries (Table 2.1). Roselle is locally known as 'Mesta' or 'Meshta' on the Indian Subcontinent (Halimatul *et al.*, 2007; Udayasekhara Rao, 1996). It is known as Roselle (English), L'oiselle (French), Jamaica (Spanish), Karkade (Arabic), Bissap (Wolof) and Dâ (Bambara) among other names (Arthney & Dennis, 1991; Rice *et al.*, 1990).

Table 2.1: Names of Roselle in different regions

Regions	Vernacular names	Source
Caribbean	Sorrel	Halimatul *et al.*, (2007)
Egypt, Saudi Arabia, and Sudan	Karkade	Abu-Tarboush and Ahmed, (1996)
Florida	Cranberry	Halimatul *et al.*, (2007)
Gambia	Wonjo	Omemu *et al.*, (2006)
Indian Subcontinent	Mesta, Meshta	Halimatul *et al.*, (2007) and Udayasekhara Rao, (1996)
Indonesia	Rosela	Halimatul *et al.*, (2007)
Latin America	Jamaica	Halimatul *et al.*, (2007)
Malaysia	Assam paya, Asam susur	Halimatul *et al.*, (2007) and Tee *et al.*, (2002)
Mali	Dah, Dah bleni	Omemu *et al.*, (2006)
Myanmar	Chin baung	Omemu *et al.*, (2006)
Namibia	Omutete	Halimatul *et al.*, (2007)
Nigeria	Zoborodo	Omemu *et al.*, (2006)
Panama	Saril	Halimatul *et al.*, (2007)
Senegal, Congo and France	Bissap	Omemu *et al.*, (2006)
Thailand	Krajeab	Omemu *et al.*, (2006)

2.2 Origin, Distribution and Ecology

Roselle, which originated from Africa, was planted in Sudan about 6000 years ago (McClintock & Tahir, 2004). Sudan is currently the major producer of Roselle as a source of Sudanese tea (Mohammad *et al.*, 2002). It was introduced to India and South America as a vegetable in the 17th century and was domesticated in Asia for use in fibre production (McClintock & Tahir, 2004). There were reports of its cultivation, in India, Sri Lanka, Thailand, Malaysia and Indonesia at the beginning of the 20th century. According to Appell (2003), the extensive cultivation of this plant under a government subsidized programme was established to obtain fibre for sugar-sack manufacturing in the Dutch East Indies (now Indonesia) in the 1920s. Nowadays, it is found throughout the tropics especially in the Savannah region of West and Central Africa (McClintock & Tahir, 2004).

The warm and damp tropical climate is suitable for Roselle plants as it is exceptionally susceptible to frost and mist (Morton, 1987; Qi *et al.*, 2005). The temperature range within which Roselle thrives is between 18 and 35°C, with an optimum of 25°C. Growth of the plant ceases at 14°C (McClintock & Tahir, 2004). In tropical and subtropical regions, an altitude 3000 ft. (900 m) above sea level is suitable for growing this plant (McClintock & Tahir, 2004). Annual rainfall between 400 and 500 mm is necessary throughout the Roselle growing season (Augstburger *et al.*, 2000). Roselle is a short-day plant that is very sensitive to the photoperiod. In the first 4-5 months of its growth, Roselle requires a daily light phase of 13 hours (McClintock & Tahir, 2004). According to Duke (1983), flowers would not appear if there were more than 13 hours of sunlight in a day, while McClintock and Tahir (2004), reported that flowering of Roselle plants was excellent when daylight was shorter than 12 hours.

According to Duke (1983), Roselle plants prefer well-drained humus and rich-fertile soils with a pH of 4.5 to 8.0. It tolerates floods and heavy winds.

2.3 Nutritional Values, Health Benefits and Usages

The young shoots, leaves and calyces of Roselle are used as a cooked vegetable or cut and used as vegetable sauce (Amusa, 2004; Komarov, 1968). According to Fasoyiro *et al*. (2005), the dried red calyces have been used to prepare tea, syrup, jams and jellies as beverages. Leaves and young shoots of Roselle are eaten raw in salads, and the red fleshy calyx lobes are chopped and used in fruit salads in the United States (Facciola, 1990). McClintock and Tahir (2004), observe that the calyces are harvested as fodder for livestock in West Africa and Roselle seed oil is used in soap and cosmetics industries. In addition, the seed oil is extracted and used for cooking and as an ingredient in paints (Halimatul *et al.*, 2007). Roselle seeds are pounded into meal, added to cereals, or roasted and boiled as a coffee replacement in some parts of Africa (Amusa, 2004; Augstburger *et al.,* 2000). The seeds are also eaten roasted as snacks or ground into meal to make cakes. Nutrient compositions of different parts of Roselle are presented in Table 2.2.

Table 2.2: Nutritional information from different parts of Roselle

Nutritional value (unit/100g)	**Flowers**	**Red Calyces**	**Green Calyces**	**Seeds**
Ash Content (g)	9.75±0.59	12.24	6.83	6.89
Fat Content (g)	0.59±0.06	2.01	2.17	21.60
Crude Fibre (g)	33.9±3.59	4.69	6.75	4.12
Protein Content (g)	9.87±0.28	4.71	6.45	31.02
Moisture content (g)	4.38±0.05	7.60	6.24	9.25
Carbohydrate (g)	4.38 ±0.05	68.75	71.56	36.37
Sodium (mg)	ND	96.66	48.1	ND
Potassium (mg)	ND	49.35	49.59	ND
Calcium (mg)	ND	12.65	21.58	6.6
Magnesium (mg)	ND	38.65	47.54	ND
Iron (mg)	ND	3.22	3.37	ND
Zinc (mg)	ND	12.22	16.28	ND
Manganese (mg)	ND	2.39	5.61	ND
Nickel (mg)	ND	1.78	3.57	ND
Phosphorus (mg)	ND	36.30	15.05	6.8
Ascorbic acid (mg)	ND	16.67	12.50	ND

Sources: (Sáyago- ayerdi *et al*., 2007; Ismail *et al*., 2008; Adanlawo and Ajibade, 2006).

ND: Non Determinate

Roselle is an attractive garden plant. The cut flowers and also the decorative red stalks with ripe red fruits have been exported to Europe (Grubben *et al.*, 2004). More importantly, Roselle has been used in medicinal treatment (Lawton, 2004). Roselle tea is used to control high blood pressure and its leaves are used as a source of mucilage in pharmacy and cosmetics (McClintock & Tahir, 2004). According to McClintock and Tahir (2004), extractions of Roselle have been used medicinally to treat colds, toothaches, urinary tract infections and hangovers. In Senegal, the juice from Roselle leaves has been used to treat conjunctivitis. Roselle leaves have also been applied as a poultice to treat sores and ulcers, besides being used as an antiscorbutic for the treatment of scurvy, a refrigerant to relieve fevers, an emollient, a diuretic, and a sedative (Duke, 1983). The leaves are not the only Roselle part that is useful for the treatment of scurvy. Gallaher *et al*. (2006) and Komarov (1968), report that a root decoction of Roselle has also been used for a similar application. Besides that, the bast fibres, and sometimes the whole stem, have been used in the paper industry in the United States and Asia (McClintock & Tahir, 2004).

2. 4 Propagation

Roselle is commonly propagated by seeds, but it is also readily grown from cuttings (Rolfs, 1929). Sowing is at the beginning of the rainy season in India. There are two approaches for sowing: sowing directly in the field and sowing in seed beds (Augstburger *et al.*, 2000).

2. 5 Harvesting and post-Harvest Handling

The fruits should be gathered sufficiently early before any woody matter forms in the pods, or in the calyxes (Rolfs, 1929). Harvested stems are steeped in water for two weeks, followed by stripping of the bark and subsequently the stems are beaten to discrete their fibres. The beaten stems are washed, dried and sorted according to length, colour and stiffness to produce fibre (Augstburger *et al.*, 2000). The seed capsules are removed from the calyces by round and sharpened metal tubes (Rolfs, 1929).

2. 6 Roselle in Malaysia

Roselle is a relatively new plantation crop in Malaysia, having been introduced in the early 1990s (Halimatul *et al.*, 2007). Today, Roselle is recognised as a health drink in Malaysia owing to its high contents of vitamin C and anthocyanin (Bajaj, 1993). Roselle has been commercially grown by small farmers in Terengganu, especially in bris soils, and also in many parts of Johor (Mohammad *et al.*, 2002). Early research on Roselle planting was carried out by the University of Malaya, and later by the Malaysian Agricultural Research and Development Institute (MARDI). Its first commercial planting was promoted by the Department of Agriculture (DOA) in Terengganu (Mohammad *et al.*, 2002). In 1993, the planted hectarage was only 12.8 ha. Planting increased steadily, reaching a peak of 506 ha involving more than 1,000 farmers, before declining (Mohammad *et al.*, 2002). Currently, the crop is planted only on a small scale in Setiu district, Terengganu, with planting now spreading to parts of Kelantan (Bachok district), Pahang (Romping district), Johor (Mersin district) and also Sarawak (Mohammad *et al.*, 2002).

2. 7 Pests and Diseases of Roselle

The expansion of Roselle planting has increased the threat of disease outbreak. As with other plants in the Malvaceae family, Roselle is at risk of diseases that affect the cotton crop. The major symptoms of Roselle diseases caused by fungi, nematodes, bacteria and virus are illustrated in Figure 2.2. According to McClintock and Tahir (2004), the most important diseases of Roselle are root rots and stale rots caused by *Phythophtora parasitica*, leaf fleck caused by *Phoma sabdariffa*, blackleg, stalk base rots and root rots caused by *Macrophomina phaseolina*, root rots and seed rots caused by *Rhizoctonia solani*, root rots caused by *Botrytis cinerea*, seed and stem rots caused by *Sclerotium rolfsii*, leaf spot caused by *Cercospora hibisci*; and powdery mildew caused by *Oidium abelmoschii*. In addition, vascular wilt of Roselle caused by *Fusarium oxysporum* was reported in Malaysia by Ooi and Salleh (1999) and Ooi *et al.* (1999). According to McClintock and Tahir (2004), Roselle plants are also prone to attack by several virus diseases such as leaf curl, cotton leaf curl and yellow vein mosaic. A hard-cracking leaves disease caused by virus has been reported in Nigeria on Roselle plants. A bacterial disease has been reported on Roselle plants caused by *Bacillus solanacearum* (McClintock & Tahir, 2004). Roselle plants have been seriously attacked by root-knot nematodes such as *Meloidogyne arenaria*, *M. incognita* and *M. javanica.* Another nematode, *Heterodera rudicicola,* has been recognised as a major pest affecting the roots of Roselle plants (McCaleb, 1996; McClintock & Tahir, 2004).

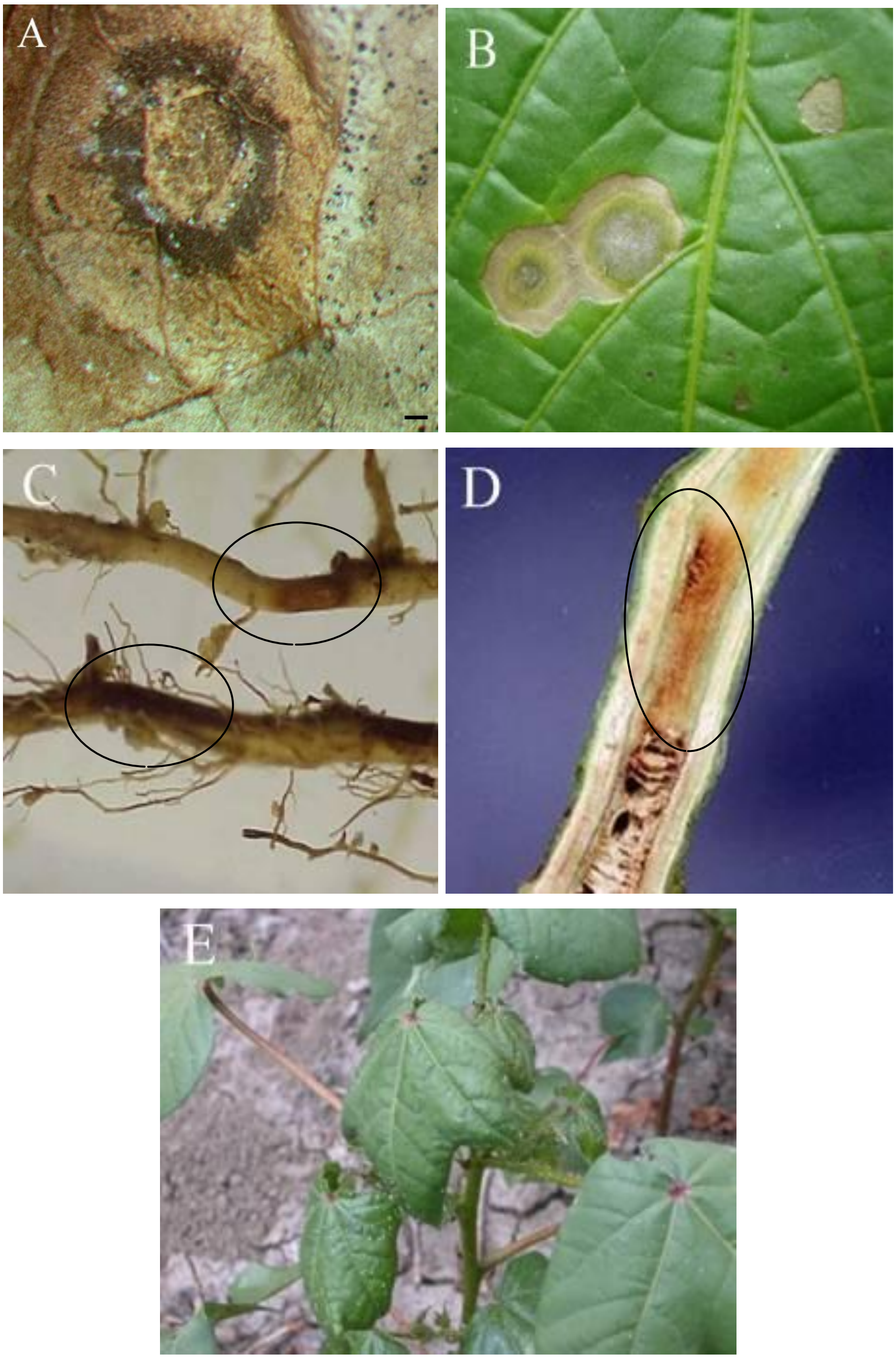

Figure 2.2: Symptoms of Roselle diseases caused by fungi, nematodes, bacteria and virus. (A and B) Fungi diseases, *Phoma* sp. and *Fusarium* sp. respectively; (C) Nematode attack of roots; (D) Bacterial disease of stem; (E) Virus disease. Scale bar; A=1000 µm. [Source; (C) ("Nematode Diseases of Pearl Millet," 2011), (D) ("*Ralstonia solanacearum*," 2011)].

2. 8 Fungal Diseases of Roselle

Vascular wilt of Roselle is caused by *Fusarium oxysporum* that comes from plant debris and infected soil. Infected plants in the field show wilting of the whole shoot with necrotic lesions seen at the stem base and extending upward to the branches (Amusa, 2004). In addition, young and mature plants are flaccid with the stem tissues showing discolouration on the wood. Amusa *et al*. (2005) investigated this disease on Roselle plants in south-western Nigeria. Ooi and Salleh (1999) also observed this disease in Malaysia while Ploetz *et al*. (2007) reported a similar disease for the first time on Roselle plants in the United States.

Foliar blight of Roselle plants caused by *Phyllosticta hibisci* was reported by Amusa *et al*. (2005). This fungus produces round black pycnidia in culture. Field symptoms of the disease include water-soaked necrotic spots on the young foliage. Pycnidia are often formed on the upper segment of the leaf spot in a ring around the centre of the lesion. According to Amusa *et al.* (2001) this disease is common in several tropical and sub-tropical areas.

Leaf spot and stem canker diseases of Roselle caused by *Coniella musaiaensis* were first reported by Persad and Fortune (1989) in Trinidad and Tobago. Westcott and Horst (2001), also reported *Cercospora hibisci* as the causal agent of leaf spots. Small irregular and light blight lesions were observed on the lower leaves, young growing tips and mature stems. Pycnidia were produced on both surfaces of the leaves and severely infected leaves defoliated (Persad & Fortune, 1989). According to Jeavons (1975), root rots and collar rots of Roselle in Trinidad are caused by *Phythophtora nicotianae*. Horst *et al*. (2008) have suggested that *Rhizoctonia bataticola* alone, or in combination with

F. oxysporum, causes this disease in Roselle plants. Powdery mildew of Roselle plants arising from infection by *Microsphaera euphorbiae* was reported by Westcott and Horst (2001). Morton (1987) found *Oidium* sp. and *Phythophtora sabdariffa* to be associated with mildew in Florida and the Philippines respectively.

In South Africa, botrytis blight of Roselle caused by *B. cinerea* was reported by Swart and Langenhoven (2000). The pathogen was isolated from affected stems and flower stalk tissues. It produced abundant conidia and mycelial on the surface of dead and infected tissues. This disease has significant impact on the yield of Roselle plants under cool and wet growing conditions. Swart and Langenhoven (2000), observed foot and stem rots caused by *Phythophtora parasitica* var. *Sabdariffa*, a soil or water-borne fungus. Infection often occurs when there is stagnant water in the fields. Symptoms of the disease include black stems which result ultimately in the death of the infected plant. A list of fungi that cause diseases on Roselle is shown in Table 2.3.

Table 2.3: Fungi-causing diseases in Roselle

Host	Fungi species
Roselle	*Aecidium garckeanum*
	A. hibiscisurattense
	Alternaria macrospora
	Cercospora abelmoschii
	C. malaysensis
	Corynespora cassiicola
	Cylindrocladium scoparium
	Diplodia hibiscina
	**Fusarium decemcellulare*
	F. sarcochroum
	F. solani
	F. vasinfectum
	Guignardia hibisci sabdariffa
	Irenopsis molleriana
	Leveillula taurica
	Microsphaera euphorbiae
	**Phoma sabdariffae*
	Phymatotrichum omnivorum
	Phythophtora parasitica
	Phythophtora terretris
	Pythium perniciosum
	**Rhizoctonia solani*
	Sclerotinia fuckeliana
	S. sclerotiorum
	Sclerotium rolfsii

*Referred to genera in this study
Source: (Orwa *et al.*, 2009).

2.9 *Phoma* spp.

2. 9. 1 History and Host Range

This genus was discovered more than 170 years ago and it has had a long and complicated history (Montel *et al.*, 1991). Sutton (1980) claimed "in excess of 2000 species have been described for this genus". *Phoma* is a harmless saprophytic species as well as an important plant pathogenic fungus with more than 110 species that are known as primary plant pathogens (Aveskamp *et al.*, 2008). It is also known as a useful bio-control agent of weeds and plant pathogens (Aveskamp *et al.*, 2008). For example, *P. herbarium*, *P. exigua* and *P. macrostoma* play a role as bio- herbicides against various broadleaf weeds (Aveskamp *et al.*, 2008). *Phoma exigua* is a fungus which is frequently encountered on leaves, stems and roots of herbaceous plants. Its diagnostic characteristics have been described by Boerema and Howeler (1967) and Boerema (1972). *P. exigua* has been isolated from more than 200 host genera (Marcinkowska *et al.*, 2005). It is both a plant pathogen as well as being saprotrophic on dead plant material (Bithell & Stewart, 2001). The fungus has also been described as a species complex with several varieties showing different cultural characteristics and specific host-relationships (Abeln *et al.*, 2002). Boerema (1972) listed soybeans, cowpea, okra, cotton, hollyhock, tobacco, tomato and eggplant as conventional hosts of *P. exigua.*

2. 9. 2 Biology

This genus is a member of the family Pleosporaceae in the order Pleosporales and class Euascomycetes. Primary infection of a host plant may occur through wounds that are caused by cultivation practices, weather conditions and interaction with other organisms. This might take place naturally through stomata or directly through the epidermis (Agrios, 2005; Graaf *et al.,* 2002; Roustaee *et al.,* 2000; West *et al.,* 2001; Williams, 1992). The fungal hyphae grow intercellularly through the plant tissues and the fungus becomes neurotropic (Hammond & Lewis, 1987). This development can be observed macroscopically as the formation of lesions. After a short period, dark coloured, mostly globosely or flask-shaped pycnidial conidiomata can often be observed within the lesions embedded in the plant's epidermis. These pycnidia contain numerous conidia in a pale white to pinkish coloured matrix. Conidia, and in some species, mycelial fragments, disperse easily by water-splash, misting or wind, and can thus infect new host plants. In the absence of a suitable host and during periods of stress, such as drought or extreme cold, most species persist as saprobes on the residue of plants that were previously infected in the soil as conidiomata, and the uni- or multicellular chlamydospores (West *et al.*, 2001; Williams, 1992). *Phoma* species overwinter as perithecia, pycnidia, and mycelium in infested plant residue (Nyvall, 1999). Its primary inocula are ascospores and conidia produced during moist weather. As with conidia, ascospores are disseminated by splashing rain and wind to host plants. *Phoma* infection in Roselle occurs under wet conditions during or after flowering (Nyvall, 1999).

2. 9. 3 Morphological and Cultural Characteristics

Phoma species are described as filamentous fungi that produce pycnidial conidiomata with monophialides, doliform to flask-shaped conidiogenous cells. A collarette is present at the apex of those cells after the production of the first conidia. *In vitro,* the hyaline conidia are mainly single-celled, although in several species a small percentage of transversely septate conidia may also be observed (Boerema *et al.*, 2004). Morphological and cultural characteristics used to identify *Phoma* spp. include the size and shape of the pycnidia, conidia and chlamydospores, growth characteristics on media, such as growth rate, pigment formation and colony outline and pattern. Pycnidia are highly variable in shape and size, but in most species they are globose or subglobose, or sometimes pyriform due to an elongated neck. In older cultures the pycnidia may aggregate. The colour varies with species from yellowish to brown-olivaceous or olivaceous-black, depending on the culture conditions and age. The use of biochemical reactions and physiological tests to indicate the presence of certain metabolites is a common practice in *Phoma* systematics. The application of alkaline reagent (NaOH) on fresh cultures is still used in identification to change the colour of pH-dependent metabolites and pigments (Boerema & Howeler, 1967; Dennis, 1946; Dorenbosch, 1970; Montel *et al.*, 1990; Noordeloos *et al.*, 1993).

2. 9. 4 Diseases Caused by *P. exigua*

As a plant pathogen, *Phoma* causes severe damping-off in seedling and also lesions on leaves, stems, stem-base and roots of older plants (Marcinkowska *et al.*, 2005). Bithell and Stewart (2001) reported that *P. exigua* was pathogenic to Californian thistle. It has also been reported as a disease causal agent in bean, soybeans, sunflower, corn and hop (*Humulus lupulus*) in Slovenia (Marcinkowska *et al.*, 2005; Radisek *et al.*, 2008). Necrotic bark lesions on poplars caused by *P. exigua* have been reported by Gruyter and Scheer (1998) in the Netherlands. In Spain, this pathogen was found by Alvarez *et al*. (2005) to be the causal agent of a new disease in Oleander (*Nerium oleander*). In North Carolina and Virginia, USA, stem canker on cotton caused by *P. exigua* was reported by Koenning *et al*. (2000).

Jellis and Richardson (1987) describe gangrene as a serious fungal disease of stored potato tubers caused by *P. exigua*. Small, dark round or oval depressions in the tuber surfaces are the first symptoms of the disease. Lesions steadily enlarge to give the characteristic "thumb-mark" depressions covered by smooth, darkened skins. Small dark pycnidia appear in lesions, and also large cavities lined with white fungal mycelium may be seen when affected tubers are cut open (Parry, 1990).

According to Koenning *et al*. (2000), leaf spots caused by *P. exigua* are brown or grey, usually 1-4 mm in diameter. The leaf spots could be larger in severe cases and pycnidia appear as brown to black specks which are visible on the lesions. As the leaf spots worsen, young leaves may turn brown and die while the lower leaves usually drop off. Koike *et al*., (2006) reported Phoma basal rots of Romaine and Crisp head lettuce in California caused by *P. exigua*. Symptoms of this disease are brownish black, sunken cavities on the crown and upper taproots. The sunken cavities are firm and there are no fungal mycelia on the crown of the plants. The affected plants are stunted. Schwartz, (2009) reported leaf blight caused by *P. exigua* on beans in Colombia. Symptoms of this disease appear as tan coloured spots, with minute dark circular specks on the surface of the spots. This disease can be distinguished by the existence of pycnidia on the spots. The damaged tissues inside the spots may become papery -thin and tear easily; the spots are ragged in appearance.

2. 10 *Fusarium* spp.

2. 10. 1 History and Host Range

The genus *Fusarium* was introduced by Link in 1809 (Leslie *et al.*, 2006). It is a fungus that is widely distributed throughout the world (Levic *et al.*, 2009). The genus *Fusarium* contains 100 species that include pathogenic and non-pathogenic forms (Alastruey-Izquierdo *et al.*, 2008). The pathogenic forms cause various diseases and are responsible for major economic losses in agriculture (Sun, 2008). The population of *Fusarium* species in agricultural fields is often as high as 100,000 propagates per gram of soil or more (Nelson *et al.*, 1981). *Fusarium* has caused diseases in various economically important crops such as bananas, cotton, legumes, maize, rice and wheat (Summerell *et al.*, 2003). According to Alastruey-Izquierdo *et al*. (2008), the most common pathogenic species are *F. solani* and *F. oxysporum.* The latter is a soil saprobe with the ability to cause vascular wilt and root rots diseases on plants (Sun, 2008). *F. solani* and *F. oxysporum* have been reported as the causal agents of die-back of *Dalbergia sissoo*, a timber-yielding tree, in the Nepal (Joshi & Baral, 2000; Parajuli *et al.*, 2000). Sudden death syndrome (SDS) is a fungal disease of soybeans which is caused by *F. solani* (Sanogo *et al.*, 2000). *Fusarium* has also been isolated from the human eye, food, soil, water and air (Alastruey-Izquierdo *et al.*, 2008; Levic *et al.*, 2009). Identification to specie level is based on morphological characteristics.

According to Burgess and Trimboli (1986) and Nelson *et al*. (1992), *Fusarium nygamai* was initially isolated from roots of grain in Australia. Leslie *et al*. (2006) reported the first observation of *F. nygamai* in New South Wales in the 1977 and 1978. It has also been found in soil samples in South Africa, Thailand and Puerto Rico (Burgess & Trimboli, 1986; Nelson *et al.*, 1992).

Fusarium camptoceras has been isolated from shattered grains of rice in Minnesota (Nyvall *et al.*, 1999). *F. camptoceras* has also been isolated from the external surfaces of the banana fruit (Jimenez *et al.*, 1993). Gu *et al*. (1990) conducted studies on *F. camptoceras* occurring on mulberry plants in Zhejiang Province in China.

2. 10. 2 Biology

This genus belongs to the order Hypocreales, family Hypocreaceae or Nectriaceae in class Sordariomycetes (Fry, 2004; Lacey & Kaya, 2007). *Fusarium* produces microconidia, macroconidia and chlamydospores. This soil-borne fungus survives as chlamydospores in soil and as mycelium in plant residues (Jameson-Jones, 2006). Chlamydospores are stimulated to germinate by host roots, extracts from the host roots, or contact with pieces of freshly colonized plant remains (Toussoun *et al.*, 1970). If the weather conditions are wet, spores from the soil level are splashed, causing disease on the upper parts of the plants. In the advanced stages of the disease, the fungus produces vast quantities of conidia and chlamydospores. The latter are returned to the soil when the dead plant decays. There, they remain dormant and are viable for several years (Toussoun *et al.*, 1970). *Fusarium* species overwinter as perithecia, chlamydospores, or saprophytic mycelial in infested plant residue (Nyvall, 1999). Disease is mainly spread by conidia, the primary inoculum, that are disseminated by splashing rain (as mentioned) and by wind (Toussoun *et al.*, 1970).

2. 10. 3 Morphological and Cultural Characteristics

Fusarium species grow on many artificial media. On potato dextrose agar (PDA), they produce white, lavender, pink, salmon or grey- coloured colonies with velvety to cottony surfaces (Anaissie *et al.*, 2009). In identifying *Fusarium* spp. by the morphological approach, carnation leaf agar (CLA) and PDA media are commonly used (Booth, 1971; Fisher *et al.*, 1982; Nancy *et al.*, 1982). The main characteristic of this genus is the production of microconidia in the early phase of growth followed by the production of multi septate sickle-shaped macroconidia, with a more or less pronounced foot cell in the later phase (Kavanagh, 2007). The presence of polyphialides and monophialides are important to separate sections and species within *Fusarium*. Length and shape of micro conidiophores are used to separate *Fusarium* spp. Presence or absence of microconidia and chlamydospores are important, as are the shapes of the microconidia and macroconidia (Nelson *et al.*, 1983).

2. 10. 4 Diseases Caused by *Fusarium* spp.

The phytopathogenic genus infect a wide range of plants and show symptoms such as root rots, vascular wilting, yellowing and foliar necrosis (Rodrigues & Menezes, 2005). Symptoms of *Fusarium* head blight on wild rice seed from both cultivated fields and natural stands are shrunken, light weight seeds which are discoloured light tan to light brown (Nyvall *et al.*, 1999). Fusarium wilt starts with a slender leaf vein and drooping of the petioles in tomato and potato (Sally *et al.*, 2009). The lower leaves wilt, turn yellow and later die. Fusarium wilt may kill the plants before they mature (Sally *et al.*, 2009). In this disease, a single shoot of the plant can be affected first, before the whole plant shows disease symptoms (Sally *et al.*, 2009). In the diseased plants, leaves start to yellow and dry slowly and the symptoms of the disease usually appear on one side of the plant. On the affected side of the plant, the wood beneath the outer bark has a uniform chocolate brown discoloration. This discoloration can be seen in the midribs of affected leaves and in the roots (Sally *et al.*, 2009).