INVESTIGATION OF SCAB DISEASE OF MANDARIN IN KAVRE DISTRICT AND CHEMICAL CONTROL

A
Dissertation
Submitted to the Central Department of Botany
Tribhuvan University

In Partial Fulfillment of the Requirements for the Award of the Degree of
Master of Science in Botany
(Plant Pathology)

Submitted by
SUNIL MAHARJAN
(2005/2007)
Roll No. : 1229
Regd No. 5-2-33-482-99

Tribhuvan University
Central Department of Botany
Kirtipur, Kathmandu, Nepal
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TRIBHUVAN UNIVERSITY

INSTITUTE OF SCIENCE AND TECHNOLOGY

CENTRAL DEPARTMENT OF BOTANY

ef. No.

Kirtipur, Kathmandu
NEPAL

RECOMMENDATION

This is to certify that Mr. Sunil Maharjan has completed this dissertation work entitled "INVESTIGATION OF SCAB DIAEASE OF MANDARIN IN KAVRE DISTRISCT AND CHEMICAL CONTROL" as a partial fulfillment of Master of Science Degree in Botany under our supervision. To our knowledge this work has not been submitted for any other degree.

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LETTER OF APPROVAL

This is to certify that the dissertation work entitled "INVESTIGATION OF SCAB DIAEASE OF MANDARIN IN KAVRE DISTRISCT AND CHEMICAL CONTROL" submitted by Sunil Maharjan has been accepted for the partial fulfilment of requirement for Master of Science in Botany.

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ACKNOWLEGEMENT

I would like to express my heartfelt gratitude to my respect supervisor Prof. Dr. Usha Budathoki, Central Department of Botany, Tribhuvan University for her guidance, supervision and co-operation throughout my work.

Again it's my pleasure to express my sincere gratitude to my Co- supervisor Dr. Chiranjivi Regmi, Senior Scientist and Chief of Technology, Nepal Academy of Science and Technology for his conscience effort, guidance, valuable suggestions, supervision and co-operation in completion of my Dissertation.

I am grateful to Prof. Dr. Krishna Kumar Shrestha, Head of Department, Central Department of Botany, T.U. for providing the laboratory facilities.

I would also like to express my sincere appreciation and gratitude to Dr. Chandra P Pokharel, Lecturer, Central Department of Botany, T.U. for valuable instruction, comments and encouragement throughout this thesis work. I am also grateful to Mr. Shiva Devkota, Lecturer, Central Department of Botany, T.U., Dr. Rosemary Shrestha, Crop Research Informatics Laboratory (CRIL), CIMMYT for their valuable suggestions and instructions and also Dr. Bhusan Shrestha Senior Researcher,Green Energy Mission Nepal for support and guidance during this research work.

I am thankful to Mrs. Saradha Joshi and Mrs. Gyanu Manandhar (Senior Scientific Officer, NARC) for identification of pathogen and providing journals and valuable suggestions. I would also thank Dr. Krishna P Paudyal for providing necessary literature material.

I am also thankful to NAST, Khumaltar for providing laboratory facilities throughout this work.

I would like to heartfelt thank my friend Mr. Basant Khatri Chhetri, without whom this thesis would not be complete. I also thank to friends Ms. Sajana Shrestha, Ms. Pushpa Panti, Mrs. Sarika Vaidya, Ms. Madhu K.C, Mr. Khila Devkota, Mr. Nabin and Mr. Rishi for their kind co-operations.

Last but not the least I am grateful towards my family for their constant inspiration, support and best wishes.

Sunil Maharjan

ABSTRACT

Present study was made on citrus scab disease of *Citrus reticulata* in Pipal Bot (Bakal) Kavre. Observation was made from December 2007 to December 2008. Infected fruits show somewhat circular, elevated spots. The lesions may be single or irregularly grouped. Severe infection of the disease produces premature fruit drop, severely external blemishes on fruit, reduce acceptability of the fruit in the fresh market, including deformation of leaf and defoliation of leaf.

The causative agent of the disease was isolated from the diseased plant parts and pure culture was prepared. The conidial measurement was 7-10.75 X 2.75-5.25 µm. To confirm it, pathogenicity test was conducted on host plant and after the appearance of the typical citrus scab lesion on host, the fungus was re-isolated, thus proving the Koch's postulates. The pathological and morphological characteristics confirm *Elsinoe fawcetti* as the causative agent of the citrus scab disease.

Disease development was found to be proportionate to the time of fruit maturity and size of the fruit. The number of lesions increases with fruit size and maturity

Two controlling chemicals, Carbendazim 50% w/p and Bordeaux mixture (1%) were sprayed on the plants in the citrus orchards in Pipal Bot Kavre. There was decrease in disease severity after a spraying of the chemical. The severity of the disease was calculated with reference to the plants that were not sprayed with chemical. It was observed that spraying of the chemical helped in deceasing the disease severity, Carbendazim 50% w/p was better in controlling the disease over the Bordeaux mixture. Integrated disease management is recommended in controlling disease rather than by spraying chemical fungicides only.

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ACRONYMS

µm =	micron
^{0}C =	degree Celsius
C =	centimeter
C =	*Citrus*
CFDD =	Citrus Fruit development Division
CMI =	Commonwealth Mycological Institute
DST- PDA =	Dodine Streptomycin Tetracycline PDA
E =	East
EPPO =	European and Meditarrian Plant Protection Organization
g =	gram
h =	hour
ha =	hectare
I_1, I_2, I_3 ..... =	Inoculated fruits that shows the symptoms
m/s =	meter per second
m =	meter
mm =	millimeter
mt =	metric ton
N =	North
PDA =	Potato Dextrose Agar.
Rs.=	Rupees
V.D.C =	Village development committee

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CHAPTER-I

1.1 INTRODUCTION

Horticulture is one of the main sources of income for farmers. It plays a great significant role in uplifting the socio-economic status of the people. The geographical and climatic conditions of mid-hill region of Nepal are very favourable for cultivation of different crops like citrus.

The citrus species grow best in subtropical environment though their origin is in the tropical region. Citrus endures the maximum and minimum temperature ranges within 14^0C – 40^0C and best growth occurs at 30^0C (Whiteside *et al*. 1993).

Citrus is perennial shrub or tree that belongs to the family Rutaceace. The centre of origin of citrus species is Asia, extending from the Himalayan foothills of Nepal, Bhutan, North-East India to North-Central China and South and New Caledonia (Lama and Kayastha, 1999). Among citrus species in Nepal, mandarin ranks first in terms of both production and acreage. (Hockey *et al*. 1998).

In the fiscal year 2007/08, the total area of 30790 ha has been used for the cultivation of citrus fruit, out of which 19915 hectare is described as productive area. The total production was 226404 mt, and the yield 11.37 mt/ hectare. According to the statistical data, the production and productive area of Citrus in Nepal is increasing every year. The productivity has increased to 11.37 mt/ ha in 2007/08 from 8.9mt/ha in 1975. Citrus species are mainly cultivated in 64 districts out of 75 of the country. The productivity area and production of citrus fruits in Kavre during 2006/07 was 495 hectare and 6116 mt. respectively (Stat. info. Nep. Agri. 2007/08).

The total annual production worldwide is about 115.65 million mt and the top ten citrus producing countries are Brazil (20.68 million mt), China (19.62 million mt), U.S.A (10.02 million mt), Mexico (6.85 million mt), India (2.86 million mt), Spain (5.70 million mt), Iran (3.73million mt), Italy (3.58 million mt), Nigeria (3.33 million mt) and Turkey (3.10 million mt) (FAO 2007).

Citrus is rich in vitamin C, organic acids, inorganic constituents, nitrogen compounds, enzymes, pigments, lipids, volatile flavouring constituent, minerals, and sugars and is highly recognized for its nutritional and medicinal value.

Per capita consumption of citrus fruit in developed countries is about 10 kilogram per year where as in Asian countries it is about 4 kilogram per year (Aubert et al, 1990). This figure is increasing with growing urban population.

According to FAO (2007), fresh orange consumption is declining in developed countries mainly due to two reasons: it is being replaced by orange juice consumption and improvements in transportation and storage favour wider and longer availability of substitute fruits. However, fresh orange consumption expanded in many developing countries, especially in emerging economies such as Mexico, India, Argentina, Brazil and China

In Nepal, citrus crop is infected by various diseases. Some of the diseases decrease the production where as other lower the quality and market price/value. Citrus fruit development division has the record of 20 different diseases caused by various pathogens. Citrus scab is caused by *Elsinoe*. This disease is most prevalent in citrus tree in many Asian countries, Australia and America.

Three different pathogens are responsible in causing the scab disease. They are as follows.

1. Citrus scab (sour orange scab) caused by *Elsinoe fawcetti* (Bitanc. and Jenkins) anamorph, *Sphaceloma fawcettii* (Jenkins).
2. Sweet orange scab caused by *E. australis* (Bitanc. and Jenkins) anamorph, *S. australis* (Bitanc. and Jenkins).
3. Tryon's scab (Australian citrus scab) caused by *S. fawcettii* var. *scabiosa* (McAlp and Tryon) Jenkins.

Inoculums for new infection consist of conidia and presumably ascospores, from scabs formed on leaves, twigs and fruits. Conidia are formed abundantly on wet scabs, in a nearly saturated atmosphere, between 20^0C and 28^0C.

Germination of conidia and infection do not require liquid water, both processes being possible with dew, fog or under high moisture conditions. A wet period of 2.5-3.5 hour is needed for conidial infection. The temperature range required for germination of conidia is

13-30^{0}C, but infection does not take place below 14^{0}C or above 25^{0}C. The incubation period is at least 5 days. The optimal temperature for disease development is 20-21^{0}C. Leaves shoot and fruits are infected when young (Whiteside, 1975).

The pathogen is able to survive in scab pustules on fruits remaining on the trees and on other plant organs providing inoculums for the next season. Even in resistant cultivars, the fungus can survive on diseased shoots (Whiteside, 1988).

Sour orange scab produces the symptoms on both leaves and fruits. Scab symptoms can appear on leaves and fruits as small, pale and somewhat circular, elevated spots. The lesions may be single or irregularly grouped. The crests of these wart-like growths usually become covered with a scabby, corky tissue, pale in colour but sometimes dark if colonized by other fungi. The disease on the fruit appear forming irregular scabby spots or caked masses which vary from cream-coloured to pale in young fruits to drab or olive with age (Timmer *et. al* 1992).

Scab generally do not affect yield but have serious effect on the external appearance of fruit and thus on the utilization of fruit for the fresh market. Scab and Melanose are two of the most important diseases that must be controlled on fresh-market fruit. A 10 percent reduction in fresh market fruit was estimated to reduce the profitability of grapefruit production in Florida by $866 per hectare (Timmer and Zitko ,1996)

In Nepal the detail study of the effect of the scab have not been seriously conducted. So actual loss due to the scab is not well known but according to farmers loss due to scab was Rs. 10 to 20 per kilogram of mandarin.

Citrus scab can be controlled by using chemical fungicides. According to Timmer and Dancan (1999) scab can be controlled by using regulatory measures, disease resistant verities, and biological control and chemical fungicides.

1.2 OBJECTIVES

The objectives of the present study are

- To find out the status of the disease in the study area.
- To study the dynamics of disease development.
- Identification and characterization of pathogen responsible for causing scab.
- Assess the effectiveness of fungicides to control the disease.
- To compare the fruit quality between infected and healthy fruit.

1.3 JUSTIFICATION

Citrus is the major cash crop in the world and in Nepal as well. Citrus scab is one of the major problems in citrus fruit reported from Kavre. Due to scab, the citrus of Kavre fetch Rs. 10 – 20 less per kilogram than those of the other parts of country. The disease has not been studied in detail so far in Nepal. Detailed study on pathogen and controlling measures will help to control the disease. By developing control procedures we can increase fruit production, ultimately uplifting the economical status of farmer.

1.4 LIMITATION

- Due to time and economic factors, present study suffers from following limitations.
- Study was carried out only for 13 months.
- Study was concentrated only in Kavre.
- Only two different control measures were used.

CHAPTER-II

2 LITERATURE REVIEW

2.1 Taxonomy of Citrus

Oliver (1860) attempted to treat the genus *Citrus.* This was the first of the modern high-grade taxonomic works on the orange subfamily.

In 1875, Hooker recognized thirteen genera in the orange subfamily with 43 species (in *Citrus* he gave only four species) in the account of the family Rutaceae in his Flora *of British India* (vol. 1).

In 1896, Adolph Engler published in the first edition of *Die natürlichen Pflanzenfamilien* an account of the plant family Rutaceae. His treatment of the orange subfamily included fourteen genera, and he estimated the total number of species at about seventy-one, of which six were in the genus *Citrus*.

In 1910, A. W. Lushington published "The Genus *Citrus*" (*Indian Forester* 36:323-53) and gave names to many of the Indian cultivated varieties studied by Bonavia. He also named some of the citrus fruit trees figured and described by Rumphius in the seventeenth century.

The taxonomy of citrus has not been resolved completely. There are various systems of classification; however, most citrus taxonomists accept two classification systems which were proposed about 50 years ago. The first system was proposed by Swingle in 1943 and second by Tanaka 1954 (Verdi and Splegel-Ray 1978). There are many differences between these two systems, especially with the number of species within the genus *Citrus*. The classification system proposed by Swingle recognize 16 species and 8 of them, including mandarin, are cultivated where as in Tanaka's system, the genus *Citrus* is composed of 159 species. Of these two classification systems, the former was adopted by most *Citrus* specialists (Verdi and splegel-Ray 1978).

According to Swingle's system of classification, mandarin (*Citrus reticulata*) belongs to the family Rutaceae, sub-family Aurontioidae, tribe Citreae, sub-tribe Citriane. The genus *Citrus* consists of two sub-genera, *Eutus* and *Papeda*. The former includes all the edible species, their hybrids and cultivars of *Citrus*. Papeda consist of 6 species which has non-edible fruits.

The two classification models of *Citrus* proposed by classical taxonomists in the past were solely based on few morphological characters and their geographical distribution (Gulan 1988). Although these models are basically valid at present and reflect taxonomic relationship among proposed species, the classification complexity of the genus *Citrus* is not yet resolved. Several factors such as evolution of new hybrid due to the natural intra- and intergeneric hybridization, asexual seed production and lack of specific criteria for recognizing species rank have been considered as complicating factors for citrus taxonomy (Scora 1988). As a result, there has been much disagreement among the taxonomists concerning the number of taxa which should have been given species rank within the genus *Citrus*. Therefore, in recent years a number of studies have been carried out using modern techniques such as numerical taxonomy, isoenzymes and DNA markers to determine the phylogenetic relationship among citrus fruits crops.

Barrett and Rhodes (1976) used 146 morphological characters to evaluate the phyogenitic relationship among *Citrus* species. Based on cluster analysis (numerical taxonomy), they recognized only three: Citron (*Citron medica*), pummel (*Citrus grandis*), and mandarin (*Citrus reticulata*) as basic and true species of genus *Citrus*. Zhong and Ye (1993) also reached the same conclusion based on the result of hierarchical cluster analysis of 86 morphological characters involving 59 taxa of *Citrus, Poncirus* and *Fortunella.*

Yamamoto *et al* (1993) reported a phylogenetic relationship of *Citrus* species using Restriction Fragment length Polymorphism (RFLP) analysis of mitochondrial DNA. He studied the RFLD of 28 species of *Citrus, Poncirus* and *Fortunella* using eight mitochondrial DNA probe/restriction enzyme combinations.

2.2 Morphology of *Citrus sp.*

Small trees; branches usually armed with straight spines. Leaves simple; petioles winged and articulated with lamina. Flower bisexual, rarely unisexual, solitary or in short axillary racemose. Calyx 4-5 lobed. Petals 4-5, free, gland-dotted, imbricate; stamens numerous free or united at base. Ovary globose or cylindrical; style distinct or tapering into ovary; stigma captitate. Fruit a berry, with segments filled with stalked pulp vesicles and bearing seeds at inner angles and the whole being surrounded by gland- dotted peel, green, often turning yellow or orange at maturity (and Long. 1991a).

2.3 Morphology of *C. reticulata* Blanco. (Nep: Suntala, Eng: Mandarin orange).

Tree 3m tall; twigs mostly spineless. Leaves ovate-lanceolate, 5-7 X 2-3 cm, acute but with blunt tip, base cuneate, margin obscurely crenate; petiole c 0.7 cm, narrowly winged. Flower solitary similar to those of *C. sinesis*. Fruit globose or depressed-globose, 5-10 cm diameter; peel thin, loosely attached, easily separating from 8-9 segments, bright orange or scarlet orange. Cultivated for its edible fruits (Grierson and Long 1991b).

Major Common Mandarin Varieties.—the major varieties of common mandarins are described below.

Algerian, Beauty (Beauty of Glen Retreat, Glen), Campeona, Clementine (Algerian), Clementino de Nules, Cravo (Laranja Cravo), Cravo Tardia, Dancy (Dancy Tangerine), Ellendale (Ellendale Beauty), Emperor (Emperor of Canton), Encore, Fagan, Fairchild, Fewtrell (Fewtrell's Early), Fortune, Fremont, Frost Dancy, Glen, Grant, Hearne, Imperial (Early Imperial), Kara, Kinnow, Kosho Tankan, Laranja, Cravo, Lee, Monreal, Murcott (Murcott Honey, Smith), Nagpur, Nova, Oneco, Ortanique, Osceola, Page, Ponkan (Nagpur, Warnurco), Robinson, Smith, Tankan, Trimble, Warnurco, Weshart, Wilking.There are many other minor commom

mandarin verities. These are of lesser commercial importance, or local interest, or still have not proven themselves (Reuther *et al* 1989).

2.4 Distribution of scab

Bio security Australia (2006) listed the countries where scab is prevalent.

EPPO region

Apart from its occurrence in Spain (Canary Islands only), all records of this pathogen in the region (Greece, Italy, Lebanon (potential EPPO country), Morocco, USSR) are dubious. The well documented record in the Mediterranean area is the scab on lemon fruits in Sicily caused by *E. australis*, not *E. fawcettii* (Ciccarone, 1957).

Asia

Bangladesh, Brunei Darussalam, China (Fujian, Guangdong, Guangxi, Guizhou, Hong Kong (restricted), Hubei, Hunan, Jiangxi, Sichuan, Taiwan (restricted), Yunnan, Zhejiang), India (Assam, Karanataka, Madhya Pradesh, Maharashtra, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal), Indonesia, Japan (Honshu, Ryukyu Archipelago), Kampuchea, Korea Democratic People's Republic, Korea Republic, Lebanon, Maldives, Malaysia, Myanmar (Burma), Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam, Yemen.

Africa

Ethiopia, Gabon, Ghana, Kenya, Madagascar, Malawi, Morocco, Mozambique, Nigeria, Sierra Leone, Somalia, South Africa, Tanzania, Uganda, Zaire, Zambia, Zimbabwe.

North America

Bermuda, Mexico, USA (including Hawaii).

Central America and Caribbean

Barbados, Costa Rica, Cuba, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Nicaragua, Panama, Puerto Rico.

South America

Argentina, Bolivia, Brazil, Colombia, Peru, Uruguay, Venezuela.

Oceania

Australia (*Sphaceloma fawcettii* var. *scabiosa* only), Fiji, New Caledonia, New Zealand, Papua New Guinea.

2.5 Taxonomic position of *Elsinoe fawcetti*

Elsinoe is a perfect stage. Taxonomic position based on Sparrow et al (1973a).

Division - Mycota

Sub-division - Ascomycotina

Class - Lacoloascomycetes

Order - Myriangiales

Family - Elsinoeacease

Genus - *Elsinoe*

Species - *E. fawcetti* Bitanc. And Jenkins

Its imperfect stage is *Sphaceloma fawcetti* Jenkins. Classification of this imperfect stage also given by Sparrow et al (1973b) and the taxonomic position is as follows.

Division - Mycota

Sub-division - Duteromycotina

Class - Coelomycetes

Order - Melanconiales

Family - Melanconiaceae

Genus - *Sphaceloma*

Species - *S. fawcetti* Jenkins

2.6 Synonyms.

E. fawcetti has the following synonyms.

1. *Cladosporium citri* sensu auct. NZ fide NZ fungi (2008)
2. *Sphaceloma citri* (1938) E. E. Butler Cif Annls.Mycol. 36: 240
3. *Sphaceloma citri* (E.E Butler) Cif. 1938 var. *citri* Annal. Mycol. Xxxvi p. 240
4. *Sorotrichum citri* E. E. Butler 1925 Trans. Brid. Mycol. Soc. 10: 121
5. *Sphaceloma fawcettii* Jenkins (1925) Phytopathology 15: 101

2.7 Symptoms

According to Whiteside (1988) infection of actively emerging shoot apices of highly susceptible cultivars causes much distortion. A protuberance develops on the invaded side of leaf, and a corresponding depression appears on the opposite side. As the leaves mature, they develop resistance against the pathogen. When infection occurs near the time of leaf resistances, the pustules are smaller and little or no leaf distortion occurs.

Scab pustules consist of a stroma, which contains mycelia and spores of the pathogen and dead cells, and hyperplastic host cells which contain few or no chloroplast. Infection of very young fruit promotes the formation of relatively large volume of hyperplastic tissue in conical or warty outgrowth (Whiteside *et al* 1993).

The scurfy type of scab symptoms may be confused with wind scar. However, in scab infection, some identifiable discrete, round pustules are usually present on the periphery of the confluent scrufy area. In wind scar injury, the periphery of the affected area may also contain some minute island of scar tissue, but these are usually elongated or run in lines. Wind scar and scab commonly occur together where the leaf comes in contact with the surface of the fruit (Whiteside *et al* 1993).

Scab generally does not affect yield but produces serious external blemishes on citrus fruit, thus effecting on the utilization of fruit and reducing acceptability in the fresh market (Agostini *et al* 2003 b).

2.8 Morphology of pathogen

Fawcett (1906) isolated the actual pathogen and reported as *Cladosporium citri* Massee.

Elsinoe causes the scab in different plants including citrus. According to Whiteside *et al* 1993 generally three types of scab occur in Citrus.

Citrus scab or sour orange scab cause by *E. fawcetti*,

Sweet orange scab cause by *E. australis*, and

Tryon scab *Sphaceloma fawcettii* var. *Scabiosa.*

According to the Sivanesan and Critchett (1972 a, b, c.), the conidia of *E. fawcetti* is spindle shaped, coloured conidia measuring 5-10X2-5µm. *E. fawcetti* has bigger conidia than *E. australis* which measure 4-6X2-4 µm. And smaller than Tryon scab, causes by *S. fawcettii* var. *scabiosa. E. fawcetti* produce the ascospores, having measurement 10-12X5-6 µm. The imperfect stage of *E. fawcetti* is *S. fawcettii* which is described by Jenkins.

Jenkins (1931) had obtained conidia from the *Sphaceloma* consisting of (i) ovoid elliptical hyaline conidia, (ii) ovoid elliptical or spindle- shaped coloured conidia, and (iii) small spherical conidia not previously reported. The coloured conidia ranged from pale yellowish to reddish brown or nearly black in coloured. Elongated hyaline conidia were usually seen in preparations made during periods of precipitation or when the leaves were wet with dew. Associated fungi were *Fusarium fructigenum* fr. *Collectotrichum gloeosporioides* Penz and *Cladosporium herbarum* Massee.

Bitancourt and Jenkins (1936) reported perfect stage of citrus scab and called as *Elsinoe fawcetti*.

Due to advancement in molecular technology, identification of different species of scab inducing pathogen has become easy and more reliable compared to the traditional methods.

Tan *et al*. (1996) conducted studies on three citrus scab pathogens, *E. fawcetti, E. australis, Sphaceloma fawcettii* var. *and scabiosa*. Morphologically the pathogens were difficult to differentiated, but by the use of molecular analysis techniques, the pathogens were readily differentiated. Restriction analysis of the amplified internal transcribed spacer (ITS) ribosomal DNA with several endonuclease and sequence analysis of the ITS readily differentiate *E. australis* from *E. fawcetti* and *S. fawcettii* var. *scabiosa*.

Hyun *et al.* (2007) conducted PCR assay on two species of *Elsinoe*, namely *E. fawcetti* and *E. australis.* The two species could not be readily distinguished by morphological or cultural characteristics and can be distinguished only by host range and the sequence of the internal transcribed spacer (ITS) region. Random amplified polymorphic DNA (RAPD) assays clearly distinguished *E. fawcetti* and *E. australis*, and the sweet orange and natsudaidai pathotypes within *E. australis* could be differentiated.

2.9 Disease cycle

Peltier (1923) showed that susceptibility to scab varies not only from season to season depending on weather conditions but varies even within the same season. Thus a bad scab year is determined largely by weather condition just at the time of formation of the leaves and fruit.

Whiteside (1986) had developed a semi selective media for the culture of pathogen. The slow-growing pathogen is confined to the pustule's stromatic portions which are colonized by many fast growing organisms including *Cladosporium* spp. and yeasts. *E. fawcetti* was isolated successfully by scraping small fragments from the pustules onto the semi selective media in isolation plates. Dodine was the only material found that suppressed yeasts and *Cladosporium* spp.

Agostini *et al*. (2003a) had studied Citrus scab, caused by *E. fawcetti*, on rough lemon seedlings. Conidia of E. fawcetti were inoculated on rough lemon and exposed to a range of temperatures and durations of leaf wetness. Scab was most severe at temperatures from 23.5 to 27°C and much less severe at 17, 20, 30, or 32°C. Leaf wetness duration of 4 h was sufficient for some infection, but 12 h of leaf wetness were needed for maximum infection with scab.

2.10 Transmission

Whiteside (1975) demonstrated disperse of conidia; 2m/s velocity is enough for the dispersal of coloured conidia by both wind and water. In addition to high humidity, the conidiophores require some unknown factors to produce coloured conidia. These conidia are dispersed by dry wind; most coloured conidia remain viable at least until the following night and then germinate with dew. Because of the ability of coloured conidia to survive post dispersal desiccation until dew was avaibility for germination, some infection still ensued when the periods of canopy wetting by rain, overhead irrigation, or non fungicide sprays were too short to permit infection prior to drying. The splash distribution dispersal is more significant epidemiologically than dry, air borne dispersal. The later, however, might cause more distance dispersal than wind-blown splash-liberated inoculums.

2.11 Varietal susceptibility

Winston *et al* (1925) had made extensive study on susceptibility of scab on rutaceous plants. He gives a more extensive list indicting the relative susceptibility to the disease as it occurs on its host in central and south Florida. The citrus scab attacks a comparatively narrow range of plants in the family rutaceous, as indicated by observation and inoculation made on 2 sub-families, 4 sub-tribes, 22 genera, 35 species, 71 varieties, and 47 hybrids combinations. Many citrus forms appear to be immune while the susceptible ones exhibit all grades of resistance.

Varietal differences in susceptibility, within a species, have been reported to exist. Chowdhary (1955) found that rough lemon, adajmir, lime, karna, citron and one mandarin cultivar were highly susceptible to scab; pumelo and Kata-jamir were less susceptible and Kashi mandarin was found to be immune. Observations recorded for five years indicated that the incidence and severity of disease depend largely on moisture and to a lesser degree on the critical growth phase. Also young leaves and tissues were found highly susceptible.

Ieki (1982) studied resistance of 43 cultivars and some seedlings to *Elsinoe fawcetti,* using artificial infection. Thirteen cultivars were resistant (hypersensitive reaction) and 12 cultivars were moderately resistant. Segregation ratios for resistant and susceptible seedlings among hybrid progenies are presented. Resistance of hybrid seedlings was strongly affected by the resistance of the parents, suggesting that resistant genes might be dominantly inherited.

Reddy *et al.* (1986) found that rough lemon (*Citrus jambhiri*) strs. Including Shomyndong, Milam, Khattazamir, Brazilian rough lemon and Chase rough lemon were immune to *Elsinoë fawcetti* and may be useful in breeding for scab resistance verities.

2.12 Disease management

The scab disease must be managed properly rather than controlling by using the chemical fungicides only. There are following steps for the purpose (Timmer and Duncan 1999).

2.12.1 Regulatory measure

Disease avoidance has been widely practiced to limited problems with citrus pathogens. These pathogens are readily spread on vegetative materials such as nursery trees and bud wood. Most important citrus growing areas have strict regulations on movement of vegetative materials, and long periods of quarantine are required to ensure the health of introduced materials. Diseases of fruit and foliage can also be

transported in shipments of commercial fruit. Movement of fresh fruit from areas with canker, sweet orange scab, and black spot is often restricted or prohibited.

The key to eradication seems to be the availability of accurate survey information on disease distribution prior to the initiation of a vigorous eradication campaign. Eradication of localized outbreak of diseases which have water-borne inoculums, such as canker, scab and post bloom fruit drop, is probably feasible.

2.12.2 Disease resistant verity

The specific resistance exists in certain pathotypes of the fungi causing scab. With the progress in genetic engineering in recent years, it is conceivable that gene for resistance for disease could be introduced into susceptible cultivar or that resistant line could be selective or breeds. The development of Murcott resistant to scab seems within the realm of possibility.

2.12.3 Biological control

There are currently no known biological controls of foliar disease of citrus, and little research has been conducted. There is some possibility of identification of organism which is antagonistic to citrus pathogens or competes with them on the leaf or fruits surface.

Highland and Timmer (2004) had studied on biological control of scab. Serenade® Bacillus subtilis biofungicide (QRD 137, QRD 131, QRD 132, QRD 141) is a new biologically based fungicide/bactericide registered for use against a variety of fruit pathogens, scab Serenade has been shown to inhibit plant pathogens by stopping spore germination, disrupting germ tube and mycelial growth and producing a zone of inhibition through secondary metabolites to restrict pathogen growth. Serenade bio fungicide can be viewed as a viable, effective, safe, and IPM acceptable alternative for foliar disease control in Florida citrus

2.12.4 Cultural practices

Management practices have been manipulated to reduce the impact of disease for many years. While these measures are seldom effective enough to eliminate the need for chemical control. They can make disease more manageable. In Florida, the shift from overhead irrigation to under-tree micro sprinklers had reduced the severity of scab and post bloom fruit drop.

2.12.5 Fungicides

Whether desirable or not, chemical control is still the main stay of management of foliar and fruit disease.

Copper products are less effective for control of scab, black spot etc. but in many areas the development of resistance by the pathogen causing there diseases has limited its usefulness.

Dithiocarbamates are still used to some extent. Ferbam is effective against scab but the short residual activity of these products often makes frequent application necessary.

Difenoconazole is very active against scab.

According to the Moherek (1970) on over head irrigated citrus, a single application of Difolatan at elevated rates applied during the delayed dormant or pin-point stage affords suppress or control of scab as compared to standard schedules of Ferban and/ or copper. 'This single application technique' concept with Difolatan could conceivably replace the two or more fungicide sprays normally applied to fresh fruit varieties for disease control.

Whiteside (1974) had evaluated fungicides for citrus scab control. The relative effectiveness of fungicide sprays for controlling sour orange scab '*E. fawcetti*' was determined for foliage on a highly susceptible clone of rough lemon and for fruit on the 'temple' orange variety. Difolatan or Benlate when applied at the appropriate times gave better scab control than copper fungicides or Ferbam. High rates of Difolatan provided sufficiently long action to reduce infection of fruit that set 6 -8

weeks after spraying. In contrast, Benlate gave consistently good scab control only when applied shortly before fruit set.

Gonzalez (1980) conducted experiment on the effectiveness of various fungicides against Scab in Persian lime. Persian lime trees were sprayed four times a year (twice towards the end of each flowering period) with commercial rates of six fungicides. TMTD [thiram] and ferbam reduced scab (*Elsinoe fawcetti*) infection from 11.4-9.9% in unsprayed controls to 2.8-0.65%and 2.9-1.93%, respectively. Fundazol [benomyl], Blue Cupravit [copper oxychloride] and Cufran-z [cufraneb] also gave satisfactory control (5.9-2.1% infection), but zineb was ineffective. Fungicide application did not affect fruit quality and no residues were found in fruit at harvest.

Reddy *et al* (1983) studied on ccontrol of scab. In fungicide tests Difolatan [captafol], Bavistin [carbendazim], copper oxychloride and Daconil [chlorothalonil] were equally effective against *Elsinoë fawcetti* on fruit.

Whiteside (1990) states that because of fungal tolerance problem with benomyl and unavailability of Captofol, Florida citrus grower have no consistently effective, registered materials for scab control. Diathianon perform better than currently recommended copper fungicides treatments. However the sterol inhibiting fungicides, difenoconazole usually gave better control of scab than dithianon. Where spray treatments were delayed till after some fruits had already become infected, difenocozole reduce scab severity even more than Captafol, because of its unique ability to inhibit the further development of existing pustules.

Bushong and Timmer (2000) states the effectiveness of Benomyl, fenbuconazole and azoxystrobin is evaluated on rough lemon seedling of scab. Benomyl was effective if applied up to 72 h after inoculation and fenbuconazole and azoxystrobin were effective if applied within 16 to 48 h after inoculation.

According to Agostini *et al* (2003b) products that induce disease resistance in plants were evaluate on potted seedling of rough lemon for citrus scab. Products like Oxycom, Nutriphite, Messenger, Goemar H11, Serenade, Rezist, Prophyte, Aliette, Actigard and Key plex were evaluated and compared with Benomyl or Strobilirin fungicide as standard. Most product reduced disease severity compared with the

untreated control, but were less effective than standard fungicide Oxycom and Messenger controlled scab well in some tests.

Mondal *et al* (2005) states that the baseline sensitivity for mycelia growth of foliar fungal pathogen of Citrus could be determined in vitro for Azoxystrobin, pyraclostrobin, and fenbuconazole. The effective dose to reduce growth by 50% (ED 50 values) was determined for each pathogen fungicide concentration. A discriminatory dose for each combination was selected near the ED 50. The effect of salicylhydroxamic acid (SHAM) on the sensitivity of the five fungal species to azoxystobin and pyraclostrobin was determined.

2.12 Study in Nepal

Khadka and Shah (1967) and Lama (1976) observed *E. fawcetti* on citrus species of Rapti and *Citrus sinensis* of Malepatan respectively.

Pawsey (1989) listed various pathogens from different citrus species of Nepal. He had reported *Elsinoe fawcetti* on *Citrus sinensis* (L.) Osbeck.

According to the citrus development project 1989 scab disease is present in many districts like Ramechap, Sindhuli, Kavre, Kaski, Gorkha, Syanja, Nawalparasi etc, among which citrus of Kavre and Kaski are seriously infected.

CHAPTER-III

3 METHOD AND MATERIALS.

A list of materials, chemicals, equipments and media required for the study is presented in Annex-1.

3.1 METHODS

3.1.1 Study area

Geographically, the Kavre district is located between 27^0 20' N to 27^0 45' N latitude and 85^0 21' E to 85^0 59' E longitude. This is representative of mid hill district of Nepal. The elevation ranges from 2000 to 3018m above the sea level. The average temperature ranges in high hill from 9-10^0 C and 17-19^0C maximum and in low lands (below 900m) it is 14-15^0C minimum and 27-28^0C maximum. The average rainfall of the district is about1757mm. The district has the privilege of having sub tropical climate which is favourable for growing citrus in large area (Regmi 2000).

According to statistical information on Nepalese agriculture (2007/08) citrus fruits are cultivate in Kavre where total productive area is 495 ha and total production of 6116 mt.

The temperature of the area, its relative humidity and annual rainfall play an important role for the survival and dissemination of the pathogen. The map of the study area and table showing average temperature precipitation and relative humidity of Dhulikhel is presented in Annex. The study was carried out in 3 orchards of citrus in Kavre V.D.C. ward number one, Pipal Bot (Bakal) during Dec 2007 to Dec 2008.

3.1.2 Survey of disease

Survey of the disease was carried out in different mandarin orchards in Pipal Bot. In the survey, the citrus plants were observed for the symptoms which include irregular scabby spots. Colours of the spots vary from cream to pale brown in young fruits, where as the colour changes to olive gray as the fruits mature.

3.1.3 Status of disease

Status of the disease was found out by calculating frequency and severity. Disease frequency was calculated by using following formula (Johnson and Booth, 1983).

$$\text{Disease frequency} = \frac{\text{Number of infected plants X } 100}{\text{Total number of plants}}$$

Severity of the disease of each plant was calculated by counting the number of fruits showing more than 10 scab lesions out of total fruit (Johnston and Booth modified).

$$\text{Disease severity} = \frac{\text{Number of fruits with lesions X } 100}{\text{Total number of fruits}}$$

3.1.4 Dynamic of disease development

Dynamics of the disease development was studied on the basis of increase in disease severity of control plot which was sprayed with water.

3.1.5 Sample collection

Random sampling method was applied for the collection of the infected citrus fruits.

3.1.6 Preparation of media

Fresh and healthy potatoes were peeled into small pieces and 200 g of the finely sliced potato were boiled in a beaker containing 500ml of distilled water till the slices became soft. The infusion was cooled and filtered through fine muslin cloth. Then the final volume was made to one litre by adding distilled water. The extract was taken in a conical flask. 20g of agar and 20 g of dextrose were added. The culture was then sterilized by autoclaving at 121^0C under 15 lbs pressure for 30 minutes, and 100 mg of tetracycline was added, after the media was cooled.

3.1.7 Culture of the pathogen

The isolation of the pathogen was done in lab of NAST and Central Department of Botany; T.U. Whiteside (1978) suggested the method for isolation of fungi. For this process the small young lesion was first sterilized with absolute alcohol. This is done to remove any other external pathogens like bacteria and other fungi and also help to remove debris. Thin tangential sections were then cut with scalpel from the scabby tissue and deposited on sterile petri-plate. Sections were mashed with bend spatula and the resulting fragments were deposited in petri-plates containing water agar and incubated at $25^{o}C \pm 2^{o}C$. After 3-4 days all the fungus were sub-cultured in PDA media and incubated. All the fast growing fungi were discarded and slow growing fungi were again sub-cultured. For this process only young lesions were used. It was comparatively quite easy to isolate the pathogen in general PDA medium from young lesions. But it was almost impossible to isolate the pathogen from old lesions without using the semi selective DST- PDA medium, which was developed by Whiteside (1986). This process was adopted in this research because in semi-selective DST-PDA medium dodine, one of main constituent is not available in our local market. This dodine helps to suppress other fast growing fungi like yeast and *Cladosporium* without affecting *Elsinoe fawcetti*.

3.1.8 Observation of culture

After 3-4 days of incubation, each colony was re-cultured in sterilized petri plates containing the PDA medium to prevent the pathogen being over grown by saprophyte fungi and other fast growing fungi. And after one week, colonies were observed under the microscope.

3.1.9 Identification and characterisation of fungi

The fungus was identified as *Elsinoe fawcetti* in laboratory of NARC on the basis of colour of colony and size of the conidia. Commonwealth Mycological Institute (CMI) book, other international journals and standard mycological books were also consulted for identification.

3.1.10 Pathogenicity test

Pathogenicity test is most important and essential step for the conformation of the pathogen. For the pathogenicity test, the fruit must not be older than two months and the diameter must be below 15 mm (Whiteside 1988).

For this process, three disease free mandarin plants were selected. Two plants were selected for the pathogenicty test and one plant was taken as control. Fruits were surface sterilized with 95% ethyl alcohol. With the help of sterile sand paper, portion of the fruit was rubbed. Small piece of red/orange colour colony was transferred to the rubbed portion and covered by the cotton. The inoculated fruits were covered with sterilized paper and polythene bag to maintain the moisture content of the fruits. After one week the polythene bags were removed and observed. Observation was taken every weeks and time taken for the appearance of the first symptoms in the fruits were noted. The fruits that showed the lesions were selected for further culture. The re-cultured fungus was confirmed to be *Elsinoe fawcetti* by comparing its colony and spore measurements with the original culture of pathogen.

3.1.11 Chemical spray for disease control

Three different orchards were selected for this study from Kavre V.D.C ward no. 1 Pipal Bot (Bakal). Five plants from each orchard were selected on Randomized Complete Block Design (RCBD) basis where each plant was considered as a replication for each treatment. The third orchard was taken as a control and only water was sprayed.

The selected plants were treated using the following fungicides.

(i) Carbendazim 50% w/p.
(ii) Bordeaux mixture 1%.

Spraying was carried out every month. The first spraying was done during the emergence of new flushes, on 2nd April 2008 and the last treatment was done on 13th December 2008.

3.2 MATERIALS

3.2.1 Sprayers.

Manual sprayer was used to spray all the fungicides.

3.3 Citrus fruit quality test

Fruit quality test is one parameter that helps to find whether the scab disease decreases the quality of fruits or not. The citrus fruit quality test was performed in Horticulture Development Research and Training Centre, Kirtipur. For the fruit quality test different parameters like skin colour, fruit size (in diameter and in height), weight in grams, peel thickness in millimetre, no. of segments, pulp weight in grams, juice weight in grams, no of seeds (perfect and imperfect), brix value and citric acid value were taken.

CHAPTER-IV

4 RESULT

4.1 Status of the disease in the study area

The study was conducted from Dec 2007 to Dec 2008. Survey was conducted in three orchards of Pipal Bot.

In first orchard 26 citrus plants were cultivated, 21 plants were found infected with different severity level. In second orchard out of 13 citrus plants, 12 cultivated plants were infected. In third orchard 9 plants out of 10 were infected.

The result of severity is given below in table.

Table 1: Species of Citrus fruits infected with Citrus Scab in Survey Area.

Species of citrus fruits	Total no. of plant observed	No. of infected plant with scab disease.	No. of non infected plants with scab.	Percentage of infection.
Citrus reticulata	47	41	6	87%
C. maxima	1	X	1	0%
C. jhambhiri	1	1	X	100%

Table 2: Citrus fruits infected with Citrus Scab in different orchards.

Orchards No.	Total no. of plant observed	No. of infected plant with scab disease.	No. of non infected plants with scab.	Percentage of infection.
1	26	21	5	80%
2	13	12	1	92%
3	10	9	1	90%

It shows that the disease scab severity is widely spread in this area as the percentage infected trees is 80% - 100%.

4.2 Dynamics of disease development.

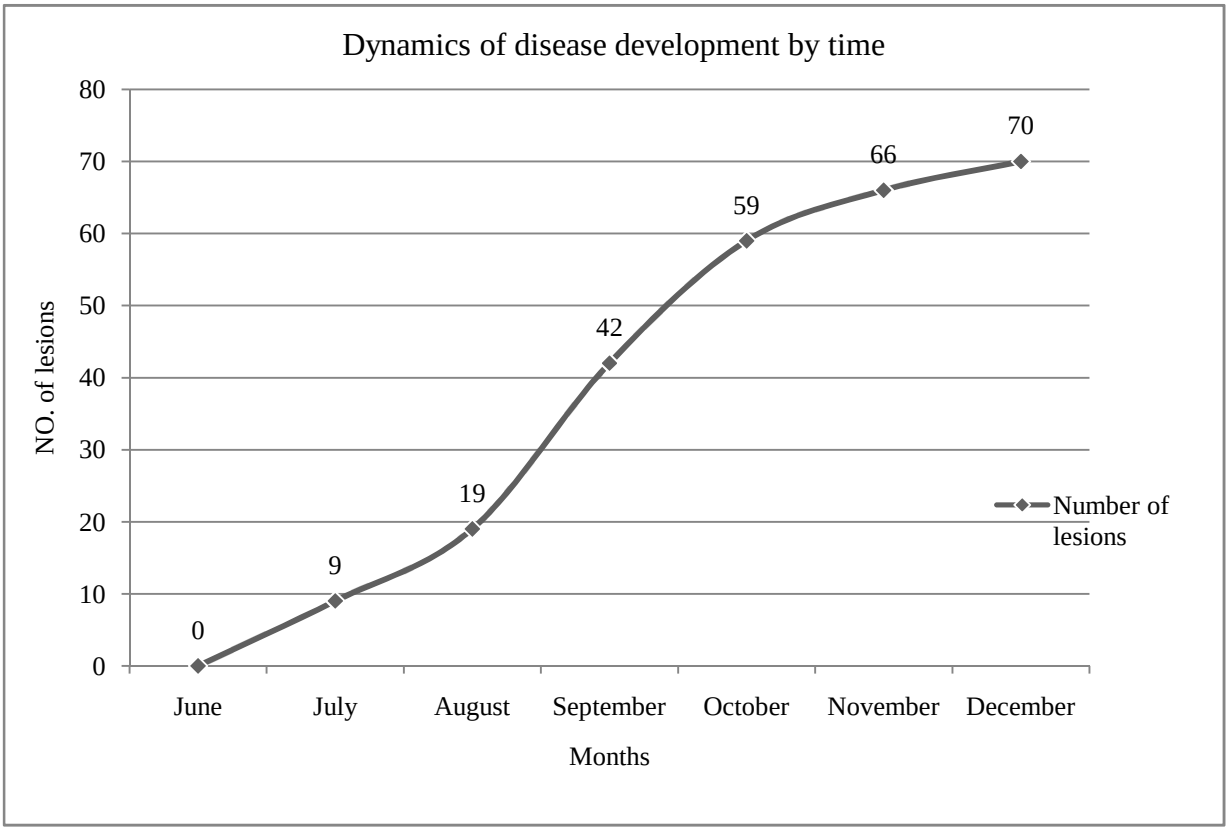

Fig 1: Disease development by time.

The average severity, in terms of number of lesions on fruits, of five plants was plotted against the month. It was found to gradually increase to the higher level in the beginning. In June there were no lesions where in July, 9 were seen. From August the lesions increased to 19 and sharply increased from 42 to 69 from September to November and in December the lesions increased up to 70 (Fig. 1).

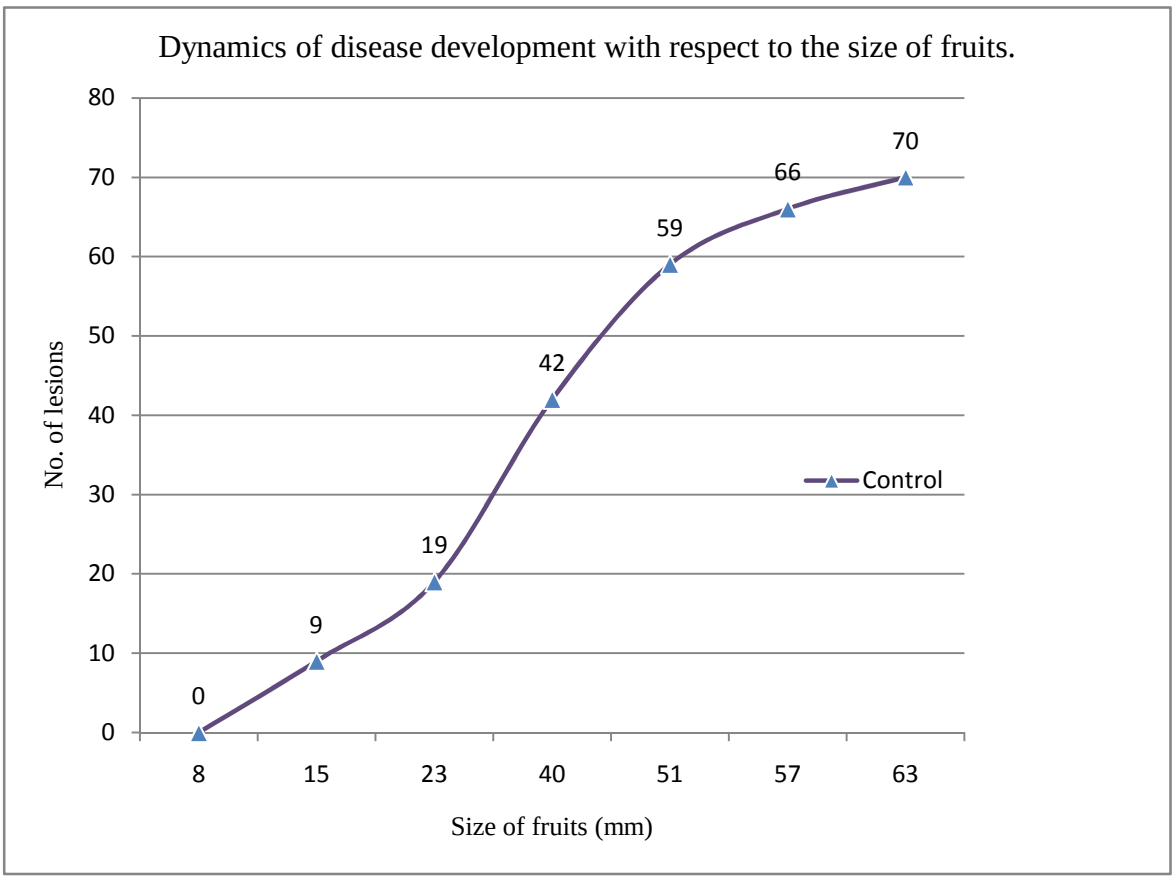

Fig 2: Disease development with respect to the size of fruits.

The average severity, in terms of number of lesions on fruits, of five plants when plotted against size of the fruit , it was found to gradually increase to the higher level in the beginning. There were no lesions seen when diameter of the fruit was 8 mm. When the diameter of the fruit reaches15 mm, the lesions were 19 and then sharply increased from 42 to 69 when fruit size became 23 to 57 mm. The lesions increased up to 70 when diameter reached 63mm (Fig. 2).

4.3 Characterization of pathogen.

Conidia single celled, thin layered, oval, small in size and ranges from 7-10.75 X 2.75-5.25 µm. The sizes of conidia are slightly larger than described by Sivanesan and Critchett (1972b). Colony appeared red/orange coloured on the plates (Liao and Chung 2008).

Thus, the character of *Elsinoe fawcetti* was similar to that described by Sivansen and Critchect (1972b) and Whiteside 1988. Therefore, the isolated fungus was identified as *Elsionoe fawcetti* Bitancourt and Jenkins.

Table 3: Comparison of different *Elsinoe* spp.

S. No.	Pathogens	Length (µm)	Breadth (µm)
1	*Elsione fawcetti*	5-10	2-5
2	*E. australis*	4-6	2-4
3	*Sphaceloma fawcettii* var. *scabiosa*	8-20	2-6
4	Isolated pathogen (*Elsione fawcetti*)	7-10.75	2.75-5.25

4.4 Pathogenicity test

The symptoms seen after conducting pathogenicity test were similar to the symptoms first seen on the fruits. Thus the fungus was found to be pathogenic and was able to produce disease on inoculated fruits which were disease free prior to the inoculation. The symptoms appeared from 14 – 28 days of inoculation of the test fruits while the control showed no symptoms.

Table 4: Time taken (days) to appear typical scab lesions.

S. No.	Inoculated fruits that shows typical scab lesions	Time taken to produce typical scab lesions.
1	I_1	14
2	I_2	21
3	I_3	28
4	I_4	28
5	I_5	14
6	I_6	28

The pathogenicity test of the isolated fungi, inoculated on fruit showed the diseased symptoms with the typical scab lesions. Time durations to produce symptoms for I_1, I_2, I_3, I_4, I_5, and I_6 were 14,21,21,28,14,28 days, respectively and control fruits did not produce any symptoms.

Cultures obtained from these infected fruits showed that the size of conidia were similar to the conidia of the pathogen from the original culture.

4.5 Effectiveness of fungicides.

For the control of scab, Bordeaux mixture and Carbendazim were used. Bordeaux mixture and Carbendazim showed the significant decrease in scab disease (Table 5).

Table 5: Effectiveness of fungicides before and after spraying.

No. of Orchard	P1		P2		P3		P4		P5		Average	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
I	85%	12.5%	94%	15%	64%	9%	75%	10%	70%	11.8%	77.6%	11.64%
II	40%	12%	71%	9%	84%	10%	75%	11%	96%	46.6%	73.2%	11.7%
III	86%	84%	76%	72%	40%	66.67%	66%	60%	76%	77.27%	68%	72%

The table 5 showed that there was significant decrease in the disease severity treated with Carbendazim and Bordeaux mixture except in the control plot III in which average severity increased from 68% to 72%.

In first plot, sprayed with Carbendazim the disease severity significantly decreased from 77.6% to 11.64%.

In second plot, sprayed with Bordeaux mixture the disease severity significantly decreased from 73.2% to 11.7%.

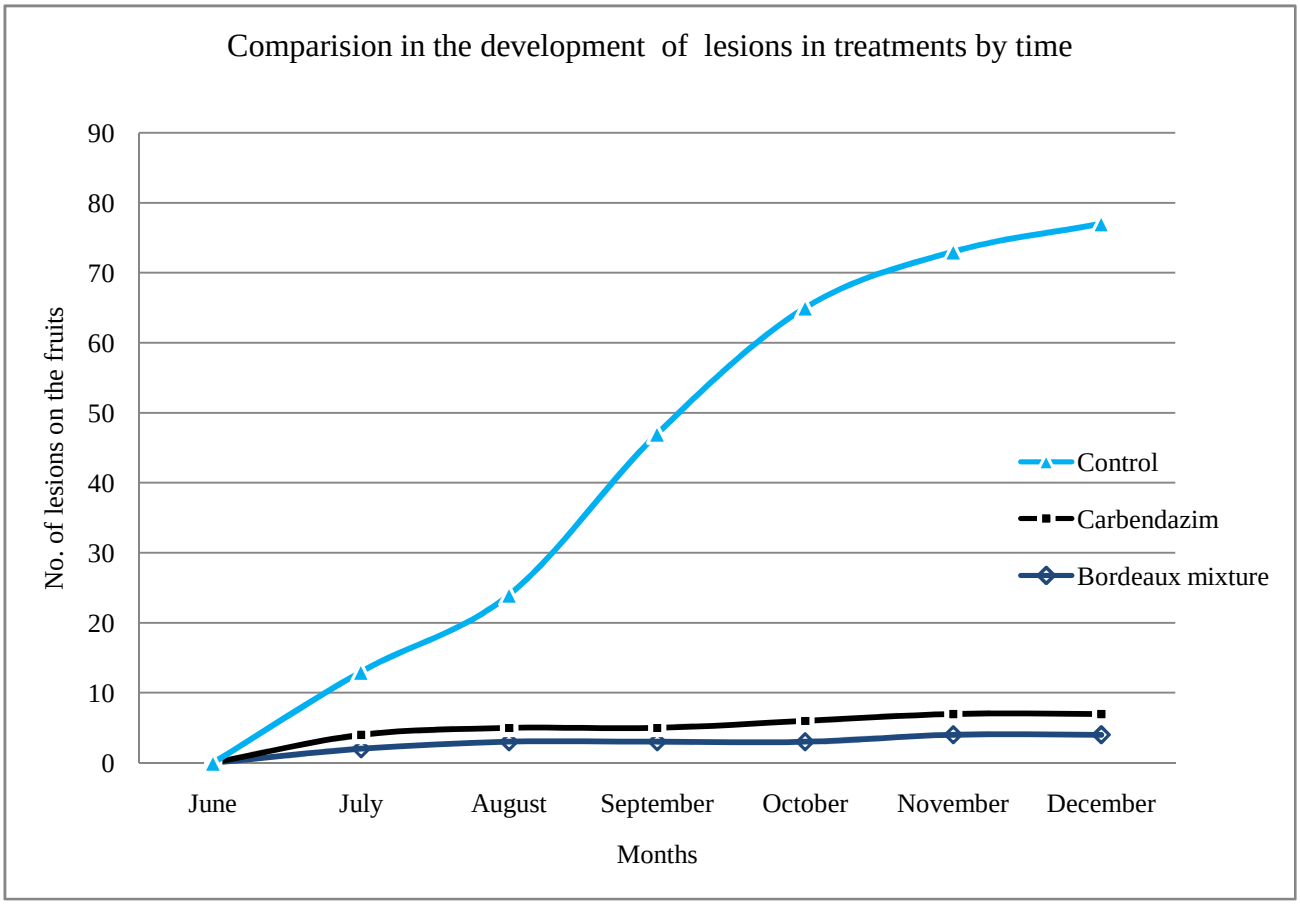

Fig 3: Comparison of disease severity of the treated plants and the plant of the control.

Number of lesions on the fruits was plotted against months for control and treated plots (Fig 3)

In June lesions were not seen in any of the orchards. Lesions were seen from July onwards. In July number of lesions in control plot was found to be 9, and the lesions gradually increased till August. In August number of lesions was 19, in September 59, in November 66. This showed that number of lesions in fruit increased rapidly from August to November where as in December the number of lesions increased from 66 to 70.

In Bordeaux mixture treated plot, the first lesions appeared in July and number of lesions was found to be 2. From July to December the number of lesions increased from 2 to 4.

In Carbendazim treated plot, the first lesion appeared during July and the total number of lesions was 2. From July to December the number of lesions increased from 2 to 3. Compared

to above there was only an increment of one lesion. This indicates that the Carbendazim is very useful to control the scab disease

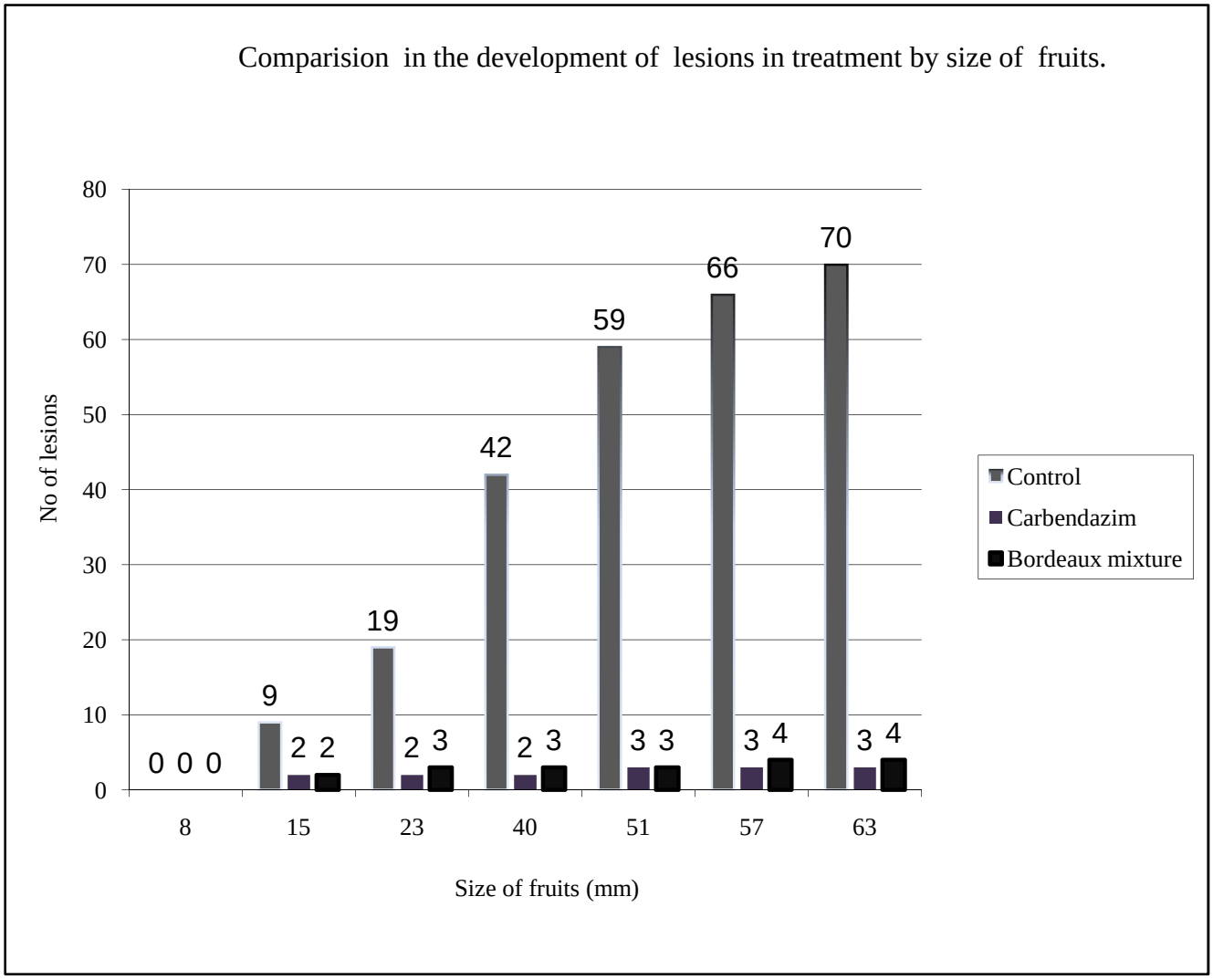

Fig: 4 Comparison of Disease Severity of the treated plots and control plot.

The fig 4 shows the comparison between the treated plots (i.e. Bordeaux mixture and Carbendazim) with that of control plot.

In fig 5 the observation is shown from June to the December.

Fruits in the control plot which were sprayed with water only showed the greatest number of lesions. In June the average diameter of the orange was 8 mm and no lesion was seen. From July the fruits started to show the lesions. In July the diameter of fruits was 15 mm , in August 23 mm, in September 40 mm, in October 51 mm, in November 57 mm and in December 63 mm and the respective number of lesions were 9, 19, 42, 59, 66, and 70. The

diameter rapidly increased from August to October and number of lesions also increased simultaneously from August to October.

In the plot, treated with the Bordeaux mixture the diameter of the fruits from June to the December were 9, 18, 25, 34, 42, 51 and 57 mm respectively. Lesions were not seen in June. From July to December number of lesions were 2, 3, 3, 3, 4, and 4 respectively. In this plot there was no drastic increase in the number of lesions. In average, from June to December only two new lesions could be seen.

In the plot, treated with the Carbendazim the diameter of the fruits from June to the December were 8, 15, 25, 44, 53 and 58 mm respectively. Lesions were not seen in June. From July to December number of lesions were 2, 2, 2, 3, 3, and 3 respectively. In this plot also no drastic increase in the number of lesions seen. In average from June to December only one lesion increased. Comparison to above here was only an increment of one lesion, this indicates that the Carbendazim is very useful to control the disease.

4.6 Fruit quality test

The table 6 shows the fruit quality test between fruits collected from control plot and treated plots (i.e. Bordeaux mixture and Carbendazim). From the present study it was found that, the quality of fruits from none of the plots had significant difference. So it is clear that the scab disease does not decrease the fruit quality, but it only causes external blemishes which reduces the value of the crop in the fresh market.

Table: 6 Fruit quality test

S. No		Skin surface	Skin Colour	Fruit size		Weight (g)	Pill Thickness (mm)	No. of segment	Pulp weight (g)	Juice weight (g)	No. of Seeds		Brix	Citric acid
				D/mm	H/mm						Perfect	Imperfect		
CARBENDIZAM	1	Rough	7.5	59.2	52.1	100	3.7	9.0	60	30	9	2	12.9	1.36
	2	”	9.0	64.9	50.0	100	3.6	10.0	60	20	13	4	12.0	1.50
	3	”	8.0	66.2	49.6	100	5.6	11.0	60	20	15	3	9.4	4.34
BORDEAUX MIXTURE	4	”	7.5	58.1	52.1	70	2.6	9.0	60	20	10	2	10.2	0.9
	5	”	9.0	55.1	47.1	50	3.3	9.0	40	20	13	1	10.8	1.12
	6	”	9.0	54.1	45.7	50	3.2	10.0	20	10	12	2	10.2	1.3
CONTROL	7	”	9.0	59.0	49.8	75	3.6	10.0	60	30	13	5	11.3	1.42
	8	”	7.5	55.8	48.4	60	3.7	10	50	30	9	1	11.5	1.24
	9	”	7.5	55.1	44.5	50	3.4	10.0	40	20	12	1	10.4	1.12

CHAPTER - V

5.1 Discussion.

The study was limited to survey of the scab disease, isolation and characterization of pathogen, trial on some fungicides and effect of scab on the quality of fruits. The finding on the disease development and characterization of pathogen are important to enrich the scientific knowledge about the disease. The result of the experiments to control the disease has revealed the effectiveness of some fungicides that might be recommended for the citrus scab to the farmers.

A general survey was conducted to find out the status of the disease in Pipal Bot of Kavre district. Owning to time limitation only Pipal Bot was selected for this research work. Mandarin (*Citrus reticulata*) was found to be predominantly cultivated and highly infected by the scab.

In all three orchards the severity percentage was very high i.e. 80% to 92%. This signifies that the agro climatic condition of Pipal Bot is also very favourable for the disease, survival of the pathogen and its dissemination. It is also clear that the mandarin planted in this place is a highly susceptible one.

According to different views of the local people and orchard owners the disease was not seen before the commercial cultivation of mandarin. All the plants were seed propagated, so the disease might have been transmitted from the nursery from where it was brought.

Average number of lesions in a control plot in July was 9. In August the number of lesions was 19. From August to November the numbers of lesions drastically increased from 19 to 66. In December only four lesions increased and reached up to 70. This signifies that even though the fruit is no longer vulnerable to the pathogen, number of lesions increases till the fruit attain maturity. From the result it is also clear that number of lesions mostly increase in rainy season.

Various researchers likeTimmer *et al* (1992) Whiteside *et al.*(1993) states that the susceptible period of the fruit is up to 2 months old from the petal fall. After 2 months, fruits become resistant to the scab pathogen. This may be due to the fact that, the cells of the fruits peel starting to secrete essential oils. These essential oils act as protectant against the pathogen. So pathogen does not invade and infect the fruit externally. Even though the fruit is no longer

susceptible, the lesions or fungus continue to show more lesions because the mycelium start to spread within the fruits that entered before the fruits acquired resistance.

From June, the rainy season starts, during this season the pathogen gets the favourable environmental conditions and start to produce lesions rapidly. As the lesion gets the favourable condition, it produces large amounts of conidia which disperse by splashes of rain water or rain drops to the twigs, branches and leaves. In these plant parts conidia remain dormant or produce acervuli that act as carrier for the next season.

The disease symptoms were very confusing with the citrus canker and wind scar. Wind scars are formed due to collision of oranges with each other and with twigs and scar form brown patches which is almost similar to that of scab. While in citrus canker the spots were brown in colour with crater like lesions. These symptoms were almost similar to that of scab but in citrus scab the diagnostic symptoms was brown to creamy brown in colour and conical shape with outgrowths.

The colony of the *Elsinoe fawcetti* is slightly red/orange with slow growing property as described by Liao and Chung (2008). However, Timmer (personal conversation) suggested that colony colour is highly variable. Colour can vary from buffy orange to reddish black and tends to get darker if sub-cultured many times.

In case of size of the conidia, present study revealed the diameter as 7.00 – 10.25µ X 2.75-5.25 µ which was slightly bigger than organism producing similar symptoms as stated by Sivansen and Critchect (1972b).

No authentic record regarding the size of conidia of the causal organism was recorded from Nepal before its study.

Thus conidia were identified as *E. fawcetti* in laboratory of NARC. Due to the lack of the molecular tools, we have to rely on the symptoms and conidial measurement and pathogenicity test to identify the pathogen.

Pathogenicity test is also one of the most important steps for the confirmation of the pathogen that causes the scab in citrus. Pathogenicity test was performed as describe by Whiteside (1978).

In first week, there were no visible symptoms seen. From second week fruits start to show small brown spots at the inoculated part. After 2 weeks the plants showed the characteristics

symptoms of the disease. However Timmer (personal conversation) suggested that symptoms should begin to appear in a week and should fully develop after 10 days or 2 weeks. For the maximum spread of the disease and maximum number of lesions the fruits were left in tree for further observation.

During pathogenicity test 4 out of 10 inoculated fruits withered in the tree and dropped. The possible reason for such high rate of fruits fall may be high amount of inoculums during inoculation or may be due to production of high amount of ethylene gas when covered by the polythene bag. To avoid the high rate of fruits fall, the amount of inoculums should be reduced during inoculation. *Elsinoe* requires only a short time of moisture to cause infection. It is better to inoculate in late afternoon or evening and then remove the bags in the morning. That usually avoids the fruit drop problem (Timmer personal conversation).

The difference in time period to show the lesions in different tree and fruits may be due to different level of resistance to the scab.

After observation, fruits showing scab symptoms were used to re-isolate the pathogen because only re-isolation of the pathogen from the inoculated plants can establish that a particular organism is the causative agent of the particular disease. For re-isolation process, previously mention method was adopted and the fungus identified.

According to the various researchers Fawcetti (1906), Peltier (1925), Timmer *et al* (1992), Whiteside *et al*.(1993), the plant is more vulnerable from the time of appearance of new flushes till fruits reaches a maturity of 2 months old or 15 mm in diameter till the fruits developed resistance. For the control two chemicals were taken one is Bordeaux mixture and next is Carbendazim. Three orchards were selected for research and from each orchard five plants were selected. It was done to compare the efficacy of each of the chemical sprayed. The third plot was considered as control so that the efficiency of the chemical fungicides can be compared. After each spray the symptoms on fruits virtually did not increases as compared to control one. The fungicides were sprayed from the time of appearance of first flushes and was continued till the fruits were harvested. From this research it is clear that after fruit attaining resistance spraying of fungicides is useless. This is because after attaining the 15 mm diameter or 2 months old fruit itself becomes resistant toward the disease and continuous spraying of fungicides may increase the cost for farmers. Whiteside (1988), Kucharek and Whiteside (2000) suggested that fungicides should be applied only for about 2 months, till the fruits develop resistivity against the pathogen.

The 1st plot was sprayed with the Carbendzium, a systemic fungicide. The Carbendazim acts systemically inside the plant body thus protecting the plant against the pathogen. From the first spray to the last spray there was no significant increase in disease lesions on the fruits except two fruits out of 10. From July to December the average number of lesions increased from 2 to 3 only. The frequency of spot appearance was much lower in the treated plants than those used as control. But Gonzalez (1980), Rao (1983), Reddy et al (1983) states that the systemic fungicides should be applied 1 or 2 times where as protectant fungicides should be applied 2-3 times before flushing and after petal fall.

In that area none of the farmer had used any control measure so the effectiveness of Carbendizum could be due to the application of the fungicide for the first time.

The continuous use of only one kind of fungicides should be avoided because the fungi may develop resistance against the fungicides as mentioned by Whiteside (1980) in which Benomyl -tolerant strains of the pathogen were found. Sharma (2006) suggested that systemic and protectant fungicides when used in combination with over alternate application help in preventing resistance development in apple scab. So this method may be useful in preventing the development of resistance in *Elsinoe* strains in citrus scab.

Bordeaux mixture is a cupper based contact fungicide. This fungicide helps to suppress the growth and development of inoculums on plants thus giving resistance against pathogen. Bordeaux mixture also gave almost same result as Carbendizum. The increment in number of lesions from July to December was 2 to 4. In this study 1% Bordeaux mixture was used however, Citrus Development project (1989) suggested that 2% Bordeaux mixture must be used for controlling the scab disease. Due to the phytotoxic nature of Bordeaux mixture it must be prepared in appropriate concentration and used in proper ways. According to Moherek (1970) and Whiteside (1974) ferbam and difolotan give better result than Cupper base fungicides.

Regular use of chemical fungicide as well as sanitary measures like collecting and destroying all the scab affected plant debris, dropped leaves and fruits, pruning all the diseased branches that carry the causal organism are important in order to reduce the incidence of scab in the orchard. These methods should be adopted because in latter season scab lesions produce the acervulli which survive in the plant debris. Similar suggestion was also given by Citrus Development Project (1989).

The fruit quality test of mandarin was done in Horticulture Development Research and Training Centre, Kirtipur. Citric acid value, brix value, colour of skin, number of skin, fruit size, fruit weight, number of segments, pulp weight, and number of seeds did not show much difference among the studied plots.

The brix value and citric acid content which was presented in result among all three plots do not show much differences. From the result it is clear that this disease does not affect the quality of citrus fruit however this disease only causes the external blemishes. Scab apparently does not affect the general health of the sweet orange. Severely diseased fruits often reach normal size, but the rind is greatly disfigured which was also reported by Jenkins (1937). Due to the scab disease, the citrus of Karve fetch Rs. 15-20 per kilogram less than those from the other parts of country. This is because scab causes external blemishes and deform the external appearance of the fruits thus, reducing the market value as reported by Agostine *et al.* (2003).

CHAPTER - VI

6 Conclusion and Recommendation

6.1 Conclusion

It was found that the disease was prevalent and quite sever in mandarin. The pathogen was isolated in the lab and confirmed by conidial measurement and pathogenicity test. Furthermore, field study was conducted to find the effective control measure of the disease and it was found that both i.e. Bordeaux mixture and Carbendazim were effective in controlling the disease. But in term of economy and user friendly Carbendazim should be prescribed.

6.2 Recommendation.

- The disease is transmitted by planting materials; so there should be strict internal quarantine to prevent the spread of scab from Kavre to other districts.
- For the control of the disease, Carbendizum is best recommended in term of economy
- Further research should be carried out by CDB and other concerned institutions with mutual collaboration.
- No authentic records regarding to the size of conidia of *Elsinoe fawcetti* from Nepal. So CDB and academic institutions must take initiation for authentic data record.

CHAPTER - VII

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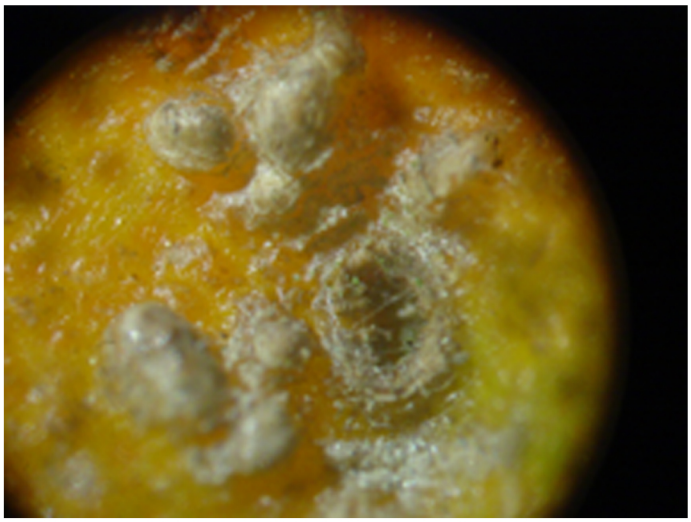
Photo Plate



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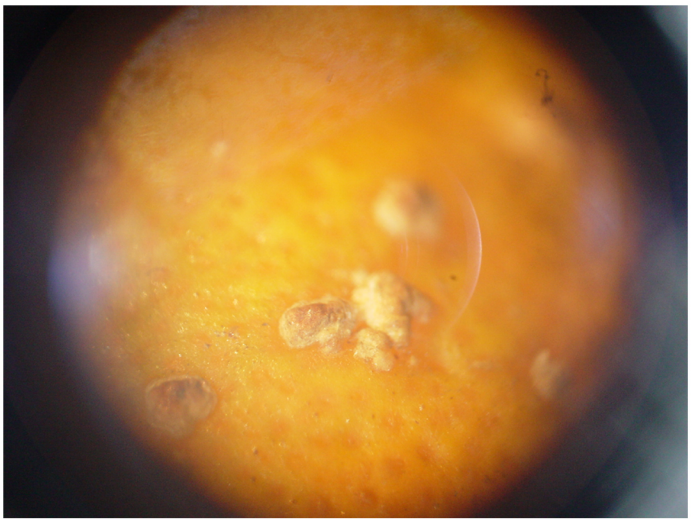

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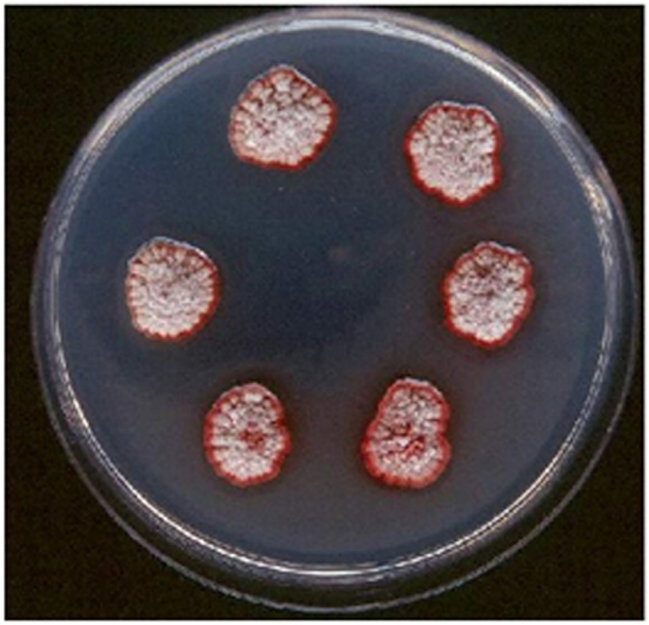
Infected fruit use as fodder

Infected fruit

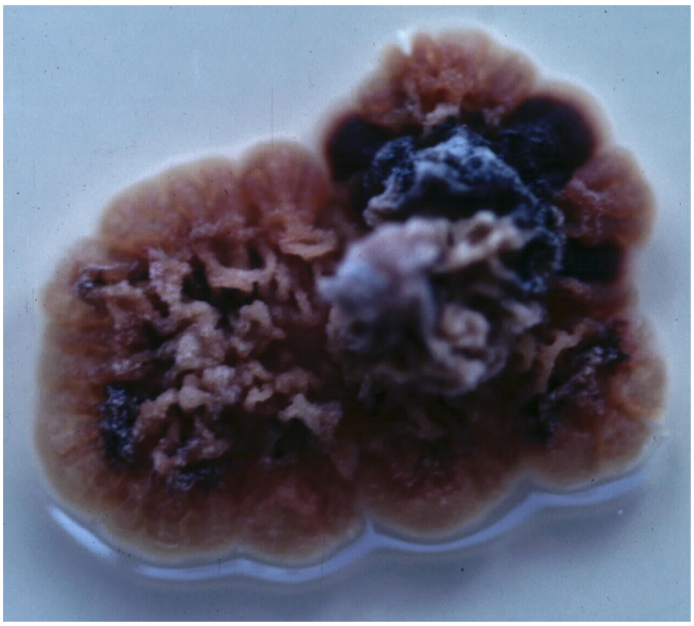
Spraying Bordeaux Mixture

Spraying Carbendazim fungicide

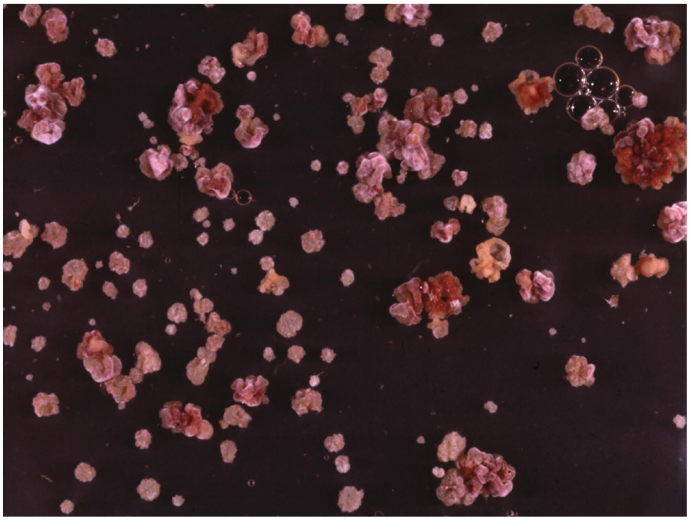
Photo Plate



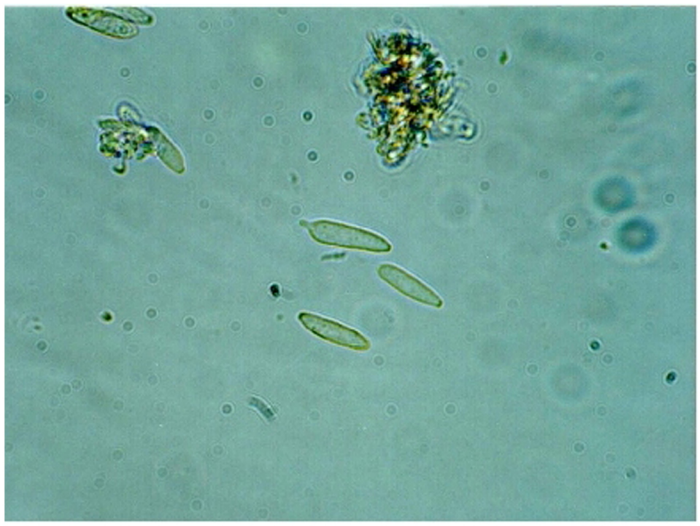
Colonies in Petri plate



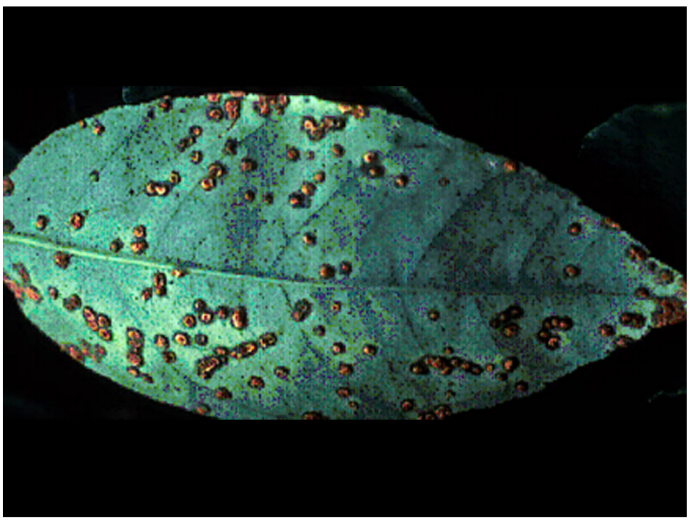
A single colony



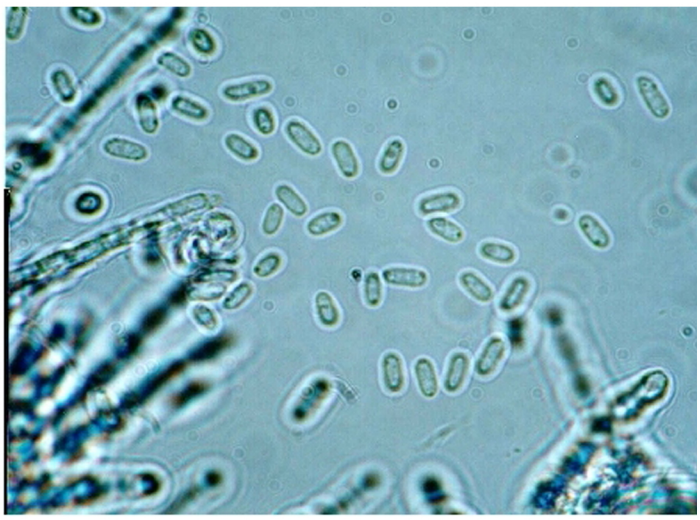
Colonies of *Elsinoe fawcetti*



Macro conidia of *Elsinoe*



A highly infected leaf



Micro conidia of *Elsinoe*

ANNEX-1

The chemicals and media that were used through out this experiment are as follows.

a. Bordeaux mixture.
b. Cotton blue
c. Lacto Phenol
d. Potato Dextrose Agar (PDA).
e. Spirit.

Equipments / Instrument

a. Beakers
b. Conical flask
c. Coverslips
d. Electric balance
e. Glass rod
f. Graduate cylinder
g. Petridishes
h. Slides

Equipments

a. Autoclave
b. Bunsen Burner
c. Digital camera
d. Heater
e. Incubator
f. Laminar air flow
g. Microscope
h. Oven
i. Spirit lamp
j. Sprayer
k. Vernier caliber

Other equipments

a. Blade
b. Foreceps
c. Needles
d. Plastic bags
e. Rope

Preparation of fungicide.

Bordeaux mixture

Composition :

Copper sulphate ($CuSO_4$) :100g

Quick lime :100g

Water :1litre

Preparation

First Copper Sulphate and Quick lime dissolved in water separately then mixes in a bucket.

Micrometer and standerization

The sporangia spores, conidia, hyphae are measured by the help of ocular and stage micro metre. Ocular micrometer is unitless micrometer which was kept inside in the eye piece, stage micro metre is placed on the stage of the micrometer which has definite units in micron (µ). Then the scale of two micrometer are coincide each other. Then stage micrometer was removed and placed the slide. 1 division of stage micrometer is equivalent to 0.01 mm.

$$\text{One ocular division (in } \mu\text{m)} = \frac{\text{No. of division on stage micrometer}}{\text{No. of division on ocular micrometre}} \times 100$$

Annex-2

Tangerines, (mandarin oranges) (raw) Nutritional value per 100 g (3.5 oz)	
Energy 50 kcal 220 kJ	
Carbohydrates	13.34 g
- Sugars 10.58 g	
- Dietary fiber 1.8 g	
Fat	0.31 g
Protein	0.81 g
Thiamin (Vit. B1) 0.058 mg	4%
Riboflavin (Vit. B2) 0.036 mg	2%
Niacin (Vit. B3) 0.376 mg	3%
Pantothenic acid (B5) 0.216 mg	4%
Vitamin B6 0.078 mg	6%
Folate (Vit. B9) 16 µg	4%
Vitamin C 26.7 mg	45%
Calcium 37 mg	4%
Iron 0.15 mg	1%
Magnesium 12 mg	3%
Phosphorus 20 mg	3%
Potassium 166 mg	4%
Sodium 2 mg	0%
Zinc 0.07 mg	1%

(http://en.wikipedia.org/wiki/Mandarin_orange**)**

ANNEX-3

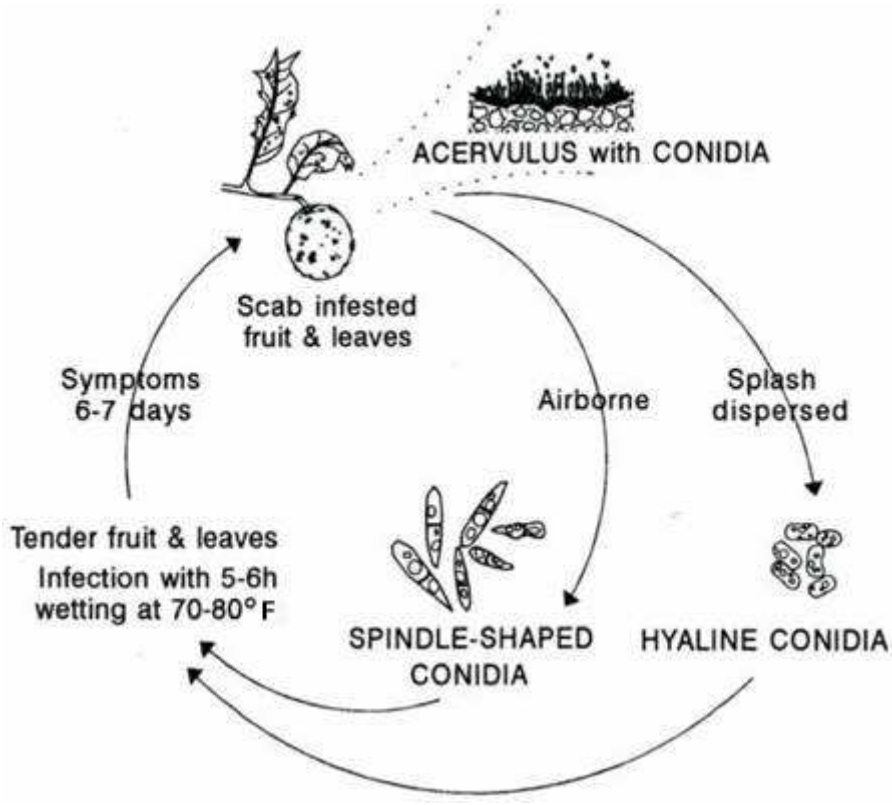


Fig: Life cycle of the *Elsinoe fawcetti* (From Citrus Health Management Drawing by Diana Drouillard.)

ANNEX-4

Area, Production and Yield of fruits in Nepal since 1993 - 2008.

Year	Total area (ha)	Productive area (ha)	Production (mt)	Yield (mt/ha)
93/94	13544	7899	76471	9.68
94/95	14629	8488	83375	9.82
95/96	15244	8977	88635	9.87
96/97	45924	9330	92994	9.97
97/98	17026	10034	100352	10.00
98/99	18007	10592	107250	10.13
99/'00	19018	11277	115067	10.20
00/01	20673	11892	121665	10.23
01/02	22423	12615	130928	10.38
02/03	23663	13312	139110	10.45
03/04	24799	13931	148010	10.62
04/05	25910	14606	156959	10.75
05/06	26681	15206	164075	10.79
06/07	27980	15832	171875	10.86
07/08	30790	19915	2264404	11.37

Source: Statistical Information on Nepalese Agriculture 2007/2008

ANNEX-5

Area under Citrus fruits (2007/08).

District	Mandarine	Sweet Orange	Lime	Lemon	Other	Total
C. Mountain	462	102	153	52	48	817
Ramechap	15	1024	23	-	-	1062
Sindhuli	172	1329	40	0	-	1540
Kavre	850	70	90	25	52	1087
Bhaktapur	114	11	46	1	1	174
Lalitpur	160	25	33	20	28	266
Kathmandu	199	20	33	20	10	329
Nuwakot	149	16	52	1	-	219
Dhadhing	757	30	92	25	17	920
Makwanpur	53	5	16	5	5	84

Source: Statistical Information on Nepalese Agriculture 2007/2008

ANNEX-6

Productivity Area and Production of Citrus Fruits.(2007/08)

District	Mandarine (A/P)	Sweet Orange (A/P)	Lime (A/P)	Lemon (A/P)	Others (A/P)	Total (A/P)
C. Mountain	215/2177	37/385	39/304	42/297	28/196	361/3359
Ramechap	8/98	925/12950	17/136	-	-	950/13184
Sindhuli	115/1418	1264/17692	24/226	-	-	1404/7106
Kavre	495/6116	43/538	52/451	0/1	5/59	590/7106
Bhaktapur	10/119	3/36	1/9	1/-	1/-	15/164
Lalitpur	150/1802	22/257	29/248	21/209	20/194	241/2710
Kathmandu	140/1695	16/191	72/628	5/49	10/97	243/2660
Nuwakot	90/1080	-/-	5/43	1/7	-/-	96/1131
Dhading	706/8634	27/297	88/831	22/198	18/158	860/10118
Makwanpur	42/498	3/29	9/98	6/30	3/14	62/669

A: area of cultivation,P: production of Fruits.

Source: Statistical Information on Nepalese Agriculture 2007/2008

ANNEX-7

List of Different Citrus Disease Responsible For Damaging Citrus Of Nepal

S.No.	Disease Name	Causal Organisms
1	Powdery mildew	*Oidium tingitaninum* Carter *
2	Sooty mould	*Capnodium citri*
3	Scab	*Elsione Fawcetti* Bitancourt and Jenkins
4	Foot rot	*Phytophthora nicotianawe*
5	Root rot	*P. citropthora* (sm.and sen.) Leaon
6	Brown rot gummosis	*P.citopthora* (sm.and sen.) Leon.
7	Dilodia Gummosis	*P. palmivora* Butl./P parasite
8	Melanose	*Diplodia natalensis* Pole Evans
9	Twing blight	*Sclerotina solerotiroum* (lig) Cabbage group. Stalk rot
10	Anthracnose / wither tip	*Colletotrichum gloeosporides* (penz)
11	Greasy spot	*Mycoxphaerella citri* Whiteside / *Cercospora citrigrisea* Fisher
12	Damping off	*Rizocotonia solani* Kuhn *Phytium ahanidermatun* (Eds) Fit.
13	Felt Disease	*Septobasidrum psudopedicellatum* Burt
14	Blue mould	*Penicillium lialium* Whemer
15	Citrus canker	*Xanthomaonas citri* (Hasse) Dawson
16	Pink disease	*Peticulazia (corticium) salmonicolor* Berj and Br.
17	Green mould	*Penicillium digitatium* Sall
18	Huanglongbing/ Greening	*HLB/ Candidatus Liberibacter asiaticus*
19	Tristeza/ quick decline/ stem pitting	CTV Mild virulent strain.
20	Exocortis	*Exocortis viroid*

Source: Citrus Fruit Development Division (CFDD), Kirtipur, Nepal.

ANNEX-8

CITRUS SPECIES FOUND IN NEPAL

S.No.	Local Name	Common Name	Scientific Name
1	Kagati	Acid Lime	*Citrus aurantifolia* Swingle
2	Junar/ Mausami	Sweet orange	*C. sinensis*
3	Nibuwa/ Chasme Kageti Eureka	Hill lemon/ Nepali oblong lemon/ Eureka lemon	*C. pseudolimon* Tanaka , *C. limon* (L.) Burn. F.
4	Bhogate	Pummelo	*C. grandis* Osbeck, *C. maxima*
5	Kali jyamir	Sour orange	*C. aurantium* L.
6	Keep	Bitter orange	*C. aurantium* L.
7	Seti jyamir	Rough lemon	*C. janbhiri* Lush.
8	Suntala/ Kamala	Mandarin/ tangerine	*C. reticulata* Blanco *C. tangerine*
9	Bimiro	Citron	*C. medica* L.
10	Chaski	Sweet lime	*C. limettioides* Tanaka
11	Sankhatro	Possible hybrid of shaddock or pummelo	
12	Chaku paw	Possible hybrid of grapefruit	
13	Tinpate suntala	Trifoliate orange	*Poncirus trifoliate* L.
14	Muntala	Kumquat	*Fortunella japonica* Swingle / *F. margarita*
15	Kinnow suntala	Kinnow mandarin	*C. nobilis X C. deliciosa* Hybrid
16	Satusma suntala	Satusma orange	*C. unshiu* M.

Source: Citrus Fruit Development Division (CFDD), Kirtipur, Nepal

ANNEX-9

Fruit Diameter and Number of Symptoms of Carbendazim Treated Plot

June

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	8	-	12	-	12	-	13	-	10	-	9	-	12	-	13	-	13	-	10	-
P_2	6	-	9	-	7	-	6	-	8	-	12	-	9	-	11	-	6	-	7	-
P_3	7	-	6	-	7	-	12	-	9	-	8	-	7	-	12	-	9	-	9	-
P_4	8	-	12	-	8	-	9	-	3	-	15	-	8	-	10	-	11	-	9	-
P_5	10	-	6	-	8	-	4	-	9	-	9	-	6	-	10	-	44	-	9	-

July

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	17	1	23	-	26	-	26	-	21	-	19	-	23	3	25	3	26	2	20	1
P_2	13	-	20	-	12	-	12	1	15	-	24	1	20	3	21	-	13	1	13	-
P_3	15	4	12	1	11	1	22	5	16	-	17	2	18	3	20	2	18	2	18	-
P_4	15	2	22	-	17	-	15	-	7	-	25	3	18	-	20	-	19	2	19	1
P_5	20	5	7	-	15	-	8	-	15	1	17	-	12	-	20	-	15	-	15	-

August

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	30	1	31	5	30	-	35	-	33	-	28	-	29	3	26	3	32	2	28	1
P_2	33	-	32	-	18	-	24	1	23	1	31	1	23	3	Fall		22	1	26	-
P_3	22	5	24	2	31	1	28	5	25	-	29	2	30	6	28	2	28	-	Fall	
P_4	29	2	18	-	Fall		22	-	24	-	23	5	Fall		18	-	25	2	19	1
P_5	19	5	27	3	22	-	31	-	31	1	59	-	21	-	12	-	28	-	25	-

September

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	40	1	41	3	40	-	45	-	41	-	37	-	38	4	38	3	42	2	39	1
P_2	32	-	40	-	28	-	32	1	32	-	39	1	35	3			30	1	34	-
P_3	35	5	35	-	36	1	39	7	35	3	38	3	40	3	35	2	35	-	Fall	
P_4	38	2	28	2	Fall		30	-	32	6	33	6	Fall		27	-	35	2	28	1
P_5	Fall		26	3	31	-	40	-	39	-	37	-	32	-	30	-	38	-	39	-

October

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	53	1	52	3	52	-	53	-	52	-	44	-	35	5	49	3	49	2	49	1
P_2	44	-	44	-	35	-	40	-	39	-	39	1	42	3	Fall		38	1	43	-
P_3	34	5	34	3	45	2	48	8	48	2	48	7	44	4	43	2	45	Fall		-
P_4	39	2	35	3	Fall		44	-	45	-	43	4	Fall		38	-	44	2	35	1
P_5	Fall		52	3	53		52	-	45	-	43	-	41	-	39	-	45	-	47	-

November

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	62	1	60	4	60	-	62	-	60	-	52	-	60	5	65	3	57	2	56	2
P_2	52	-	52	-	43	-	49		48		47	1	50	3	Fall		45	2	52	1
P_3	53	6	54	-	53	2	57	9	50	2	55	8	57	4	52	2	53	-	Fall	
P_4	45	2	43	3	Fall		56	-	51	-	41	4	Fall		45	-	55	2	42	1
P_5	Fall		60	3	61	-	60	-	52	-	50	-	50	-	48	-	53	-	56	-

December

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	67	1	64	4	65	-	66	-	66	-	57	-	65	5	69	3	62	2	60	2
P_2	60	-	59	-	49		56	-	55	-	53	1	55	3	Fall		50	2	58	1
P_3	60	6	60	-	61	63	9	55	2	60	8	-	60	4	57	2	57	-	Fall	
P_4	52	2	50	3	Fall		62	-	56	-	46	4	Fall		50	-	63	2	47	1
P_5	Fall		65	3	66	-	65	-	58	-	56	-	58	-	53	-	58	-	61	-

ANNEX-10

Fruit Diameter and Number of Symptoms of Bordeaux Mixture Treated Plot

June

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	7	-	15	-	15	-	8	-	8	-	9	-	15	-	14	-	16	-	7	-
P_2	8	-	8	-	10	-	8	-	10	-	6	-	15	-	9	-	9	-	12	-
P_3	8	-	9	-	6	-	10	-	15	-	10	-	7	-	10	-	4	-	4	-
P_4	7	-	6	-	8	-	6	-	9	-	15	-	15	-	6	-	7	-	6	-
P_5	10	-	8	-	8	-	45	-	8	-	9	-	4	-	7	-	8	-	7	-

July

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	15	-	24	1	27	-	17	1	25	-	18	1	27	-	25	3	27	3	28	4
P_2	28	3	15	-	19	5	16	3	15	5	14	-	28	4	18	3	18	1	22	2
P_3	17	-	18	1	12	1	18	-	18	2	18	1	12	-	20	-	7	-	7	-
P_4	15	-	10	-	17	-	10	1	25	-	25	-	28	2	10	1	12	1	12	3
P_5	20	1	17	3	18	-	25	-	18	-	12	-	10	-	15	-	15	4	12	-

August

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	25	-	30	1	22	-	28	1	30	-	26	1	32	-	30	4	32	5	33	5
P_2	32	3	29	-	39	5	26	3	21	5	Fall		28	5	27	5	26	1	29	3
P_3	25	-	Fall		20	1	26	-	24	2	27	1	21	-	27	-	Fall		22	-
P_4	21	-	23	-	27	-	Fall		30	-	32	-	30	3	19	2	20	1	22	4
P_5	29	1	25	3	23	-	32	-	25	-	19	-	12	-	20	-	21	4	18	-

September

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	33	-	40	1	31	-	37	1	36	-	35	2	40	-	36	5	36	6	38	6
P_2	40	3	39	1	35	7	35	3	33	6	Fall		37	5	35	5	36	1	37	3
P_3	35	-	Fall		30	1	36	-	33	2	32	1	31	-	35	-	Fall		31	-
P_4	31	-	33	-	36	-	Fall		39	-	41	-	37	3	27	2	30	1	31	5
P_5	37	1	31	5	32	-	39	-	32	-	35	-	20	-	29	-	29	6	27	-

October

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	40	-	48	-	43	-	46	1	42	-	45	2	49	-	44	6	43	7	44	6
P_2	49	4	47	-	45	10	43	3	45	8	Fall		46	5	42	4	43	1	43	4
P_3	45	-	Fall		49	1	50	-	41	2	44	1	43	-	41	-	Fall		47	-
P_4	42	-	39	-	44	-	Fall		46	-	49	-	44	4	34	2	45	1	37	5
P_5	48	2	35	5	38	-	37	-	38	-	32	-	28	-	38	-	38	6	Fall	

November

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	48	-	58	1	52	-	54	1	51	-	54	1	57	-	52	6	51	7	52	7
P_2	58	4	55	-	54	10	51	3	53	8	Fall		53	5	50	5	51	1	52	4
P_3	53	-	Fall		58	2	59	-	50	2	53	1	50	-	50	-	Fall		53	-
P_4	51	-	49	-	52	-	Fall		54	-	57	-	52	5	42	-	53	1	45	5
P_5	56	2	43	6	46	-	46	-	45	-	41	-	35	-	45	-	45	6	Fall	

December

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	54	-	62	1	59	-	60	1	57	-	61	1	62	-	58	6	57	7	56	7
P_2	64	4	60	-	60	10	57	3	60	8	Fall		60	5	55	5	57	1	56	4
P_3	60	-	Fall		63	3	65	-	55	2	59	1	55	-	55	-	Fall		58	
P_4	56	-	53	-	56	-	Fall		60	-	62	-	57	5	47	-	58	1	51	5
P_5	62	2	50	6	51	-	51	-	51	-	46	-	41	-	51	-	51	6	Fall	

ANNEX-11

Fruit Diameter and Number of Symptoms of Control Plot

June

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	6	-	7	-	8	-	10	-	8	-	8	-	10	-	44	-	5	-	8	-
P_2	10	-	8	-	9	-	10	-	9	-	6	-	10	-	7	-	6	-	6	-
P_3	7	-	9	-	7	-	7	-	10	-	4	-	10	-	7	-	6	-	5	-
P_4	10	-	8	-	10	-	6	-	12	-	6	-	9	-	9	-	10	-	13	-
P_5	6	-	8	-	11	-	7	-	6	-	7	-	7	-	8	-	10	-	7	-

July

	F1		F2		F3		F4		F5		F6		F7		F8		F9		F10	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P1	12	9	13	10	15	11	20	9	15	11	15	10	20	8	19	12	10	14	16	10
P2	18	9	18	10	16	12	18	15	17	12	12	7	20	10	48	14	13	10	12	10
P3	13	7	16	8	12	12	15	10	20	8	7	8	18	7	15	6	13	7	10	9
P4	18	8	14	10	47	6	14	3	25	14	10	8	16	13	10	6	20	9	23	7
P5	11	10	14	11	20	12	15	12	13	10	13	9	12	3	14	3	19	4	12	15s

August

	F1		F2		F3		F4		F5		F6		F7		F8		F9		F10	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P1	20	20	Fall		25	10	27	19	24	20	24	22	28	16	25	20	Fall		Fall	
P2	25	18	Fall		25	20	Fall		26	19	20	15	27	19	Fall		20	19	Fall	
P3	22	18	25	15	27	22	20	19	28	12	14	13	26	16	19	12	18	10	20	18
P4	23	15	22	18	23	10	20	8	30	22	18	15	24	20	17	10	25	17	29	14
P5	19	19	24	20	25	20	21	20	28	18	19	19	19	8	19	18	23	8	Fall	

September

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	38	36	Fall		40	39	42	47	39	48	41	48	45	48	40	50	Fall		Fall	
P_2	45	30	Fall		41	49	Fall		43	40	44	40	43	50	Fall		39	50	Fall	
P_3	Fall		43	39	43	50	32	48	35	39	Fall		43	40	37	43	37	39	39	48
P_4	41	30	40	45	38	38	42	30	Fall		35	40	37	50	34	40	47	48	38	49
P_5	Fall		42	45	38	49	38	50	42	35	38	35	35	30	37	39	39	35	Fall	

October

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	49	57	Fall		52	53	54	62	52	65	54	63	55	62	54	63	Fall		Fall	
P_2	58	53	Fall		52	61	Fall		53	60	55	59	55	70	Fall		51	71	Fall	
P_3	Fall		53	51	55	60	43	65	45	57	Fall		54	59	50	58	50	52	52	63
P_4	50	51	51	53	Fall		53	45	Fall		46	60	45	68	50	56	53	58	50	61
P_5	Fall		58	60	56	60	50	69	52	50	50	50	45	48	49	50	41	55	Fall	

November

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	55	63	Fall		57	60	62	70	57	72	58	72	63	70	60	70	Fall		Fall	
P_2	65	60	Fall		55	66	Fall		58	68	63	65	61	75	Fall		55	77	Fall	
P_3	Fall		60	61	60	68	49	71	51	63	Fall		60	65	56	65	53	60	59	70
P_4	57	63	56	63	Fall		61	53	Fall		53	65	52	73	55	65	55	67	56	66
P_5	Fall		65	62	53	68	56	75	62	58	54	58	51	56	53	52	50	63	Fall	

December

	F_1		F_2		F_3		F_4		F_5		F_6		F_7		F_8		F_9		F_{10}	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
P_1	59	58	Fall		62	65	67	73	61	75	63	76	67	73	65	76	Fall		Fall	
P_2	69	67	Fall		59	70	Fall		62	72	68	69	65	80	Fall		60	80	Fall	
P_3	Fall		65	63	64	72	54	75	56	69	Fall		64	70	60	69	57	63	67	75
P_4	61	63	60	63	Fall		64	60	Fall		59	67	59	78	60	70	60	70	64	70
P_5	Fall		70	66	58	73	60	80	66	63	60	65	50	60	60	64	55	66	Fall	

ANNEX-12

Temperature, Relative humidity and Annual rainfall of Dhulikhel of Kavre during study period.

Year	Month	Temperature in ^{o} C		Relative humidity (%)	Rain fall (mm)
		T max	T min		
2007	Dec	16.66	4.40	81.55	00.00
2008	Jan	15.00	3.70	48.75	6.00
”	Feb	17.40	3.60	70.20	00.00
”	Mar	22.20	9.00	64.65	17.00
”	Apr	27.00	11.60	64.65	37.40
”	May	27.10	13.80	75.25	105.30
”	June	26.70	18.00	88.75	216.00
”	July	26.90	18.90	90.05	174.10
”	Aug	25.90	18.60	91.55	317.40
”	Sept	24.70	17.10	90.15	239.20
”	Oct	22.80	12.70	82.35	18.00
”	Nov	19.50	8.60	80.35	00.00
”	Dec	15.90	6.4	84.40	2.00

Source: Department of Hydrology and Meterology.

KABHREPALANCHOK DISTRICT

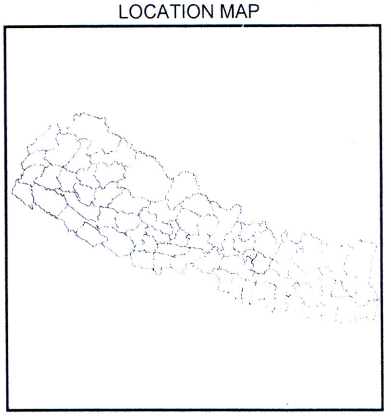
ZONE : BAGMATI

District Code : 24

SCALE 1 : 325000

6500 0 6500 13000 Meters

LOCATION MAP



LEGEND

———	District Boundary
———	VDC Boundary
MORANG	District Name
BUKHEL	VDC Name

DISTRICT : KABHREPALANCHOK

Area :1396 Sq.Km.(Approx.)

HORIZONTAL DATUM

Spheroid Everest 1830
Projection MUTM
Origin Longitude 84° E., Latitude 0° N.
False coordinates of origin 500 000 m. Easting, 0 m. Northing
Scale Factor at Central Meridian 0.9999

Map compiled from National Topographic Database at scales 1:25 000 and 1:50 000, 2002. Internal administrative boundaries are not demarcated on the ground. Map produced by the Survey Department, National Geographic Information Infrastructure Programme, (NGIIP). Kathmandu.