

## Appendix 1

**Table 1 Major citrus postharvest diseases: typical symptoms on fruit, infection type, infection site and spread of infection with possible control strategies involved**

Disease	Causal agent	Typical symptoms on fruit	Infection type	Infection site	Spread to adjacent fruit	General control strategies	Reference
Anthracnose	<i>Colletotrichum gloeosporioides</i> (Penz) Sacc.	-Initially are silvery gray and leathery, and later the infected ring becomes brown to grayish black and softens as the rot progresses. The pathogen grows and sporulates in deadwood on the trees, with water transmitting spores to the immature fruit surface by forming appressoria. The structure remain latent, do not cause decay prior to harvest.	Quiescent, infective pathogen.	Injured rind.	Yes.	-Improved cultural practices such as removal of dead wood and twigs to reduce inocula. -Proper handling of fruits at harvest to minimize injury. -Cooling of fruits immediately after harvest at or below 10 °C. -Application of sanitary measures by removing infected fruits.	Brown, 1994.

Table ... continued

Black rot (stem-end rot)	<i>Alternaria citri</i> Ellis and Pierce	-Late infected fruit appear sound on the exterior and may escape. the attention of graders.	Quiescent, infective pathogen.	Natural openings at the stem-end.	No.	-Delay harvesting time until the infected fruit drop. -Application of postharvest treatments which delay fruit button (calyx) senescence may delay black rot development.	Brown, 1994.
183	-Cause premature coloring of a fruit on the tree (the most typical characteristic of the disease) and it causes fruit drop early in the season. -The fungus may cause stem-end rot infection of Valencia and grapefruits in long term cold storage	-Some times exhibits external symptoms at the blossom end (top), but is more often found in the core of the fruit bottom. -Infected fruit appear to have a dray, black, decayed area at or near the stylar or stem end.					Browning <i>et al.</i> , 1995.
Black spot	<i>Guignardia citricarpa</i> Kiely	-Variable in symptoms it may appear as hard freckle or virulent (spreading) spots.	Quiescent, infective pathogen.	Intact or injured rind.	Yes.		Kotze, 1993.

Table... continued

Blue mould	<i>Penicillium italicum</i> Wehmer	<p>-The decay first appears as watery discoloured spots that can easily punctured.</p> <p>-The white mycelium soon produces a mass of powdery blue coloured sporulating area surrounded by a white margin.</p>	Active, wound pathogen.	Injured rind.	Yes.	<p>-Mould sporulating may be inhibited by approved fungicide treatment.</p> <p>-Sanitation in the handling, packing and storage operations is very important.</p> <p>-Tests for pathogen resistance in the packinghouse-</p> <p>-Repacking <i>Penicillium</i> infected fruit is important and storage of packed fruits at or below 4.4 °C delay mould development.</p>	Whiteside <i>et al.</i> , 1993.  Browning <i>et al.</i> , 1995.
Greasy spot	<i>Mycosphaerella citri</i> Whiteside	<p>-Form necrotic specks on fruit rind between epidermis and oil glands</p> <p>-The lesions are pink at first and become brown or black with rind blotch in 3 to 6 months time.</p>	Quiescent, infective pathogen.	Intact or injured rind.			

Table... continued

Green mould	<i>Penicillium digitatum</i> Sacc.	<p>-First appears as watery discoloured spots that are easily punctured by finger pressure and later as white fungal mycelium producing a mass of powdery olive green or light to bright blue spores surrounded by a large white margin.</p> <p>-Finally, the decayed fruit becomes soft, shrunken, and shrivelled and entirely covered with spores.</p>	Active wound pathogen.	Injured rind.	No.	<p>-Minimize scratches, punctures and plugging ensuring careful harvesting and handling.</p> <p>-Sanitary practices must be applied to avoid resistant strains to fungicides.</p> <p>-Remove all debris and decayed fruit from the packing site.</p> <p>-Application of disinfectants.</p> <p>-Application of tests periodically to detect resistant strains.</p> <p>-Application of approved fungicides before or after harvest provide control of moulds.</p> <p>-At packhouse and transition store fruits at or below 4.4 °C.</p>	Brown, 1994.  Browning <i>et al.</i> , 1995.
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Table ... continued

Lime	<i>Gloeosporium limetticola</i>	Young fruits attacked by a	Quiescent,	Intact	or		Whiteside <i>et al.</i> ,
Anthracnose	Clausen	disease usually shed.	infective	injured			1993.
		-Fruits infected later develop	pathogen.	rind.			Browning <i>et al.</i> ,
		corky lesions that vary from					1995.
		slightly sunken spots to deep					
		cankers over much of the					
		surface but lack yellow haloes					
		unlike canker.					
186	<i>Geotrichum candidum</i> Lk ex	-Slightly raised, water-soaked,	Active,	Injured	Yes.	-Minimize scratches, punctures and	Brown, 1994;
Sour rot	Pers ( <i>Endomyces geotrichum</i> )	clear to yellow initial lesions,	wound	rind.		plugging ensuring careful harvesting and	Wills <i>et al.</i> ,
		which are confusing with	pathogen.			handling.	1998.
		those of <i>Penicillium</i> moulds				-Avoid harvesting fruits with high peel	
		are developed.				moisture early in the morning.	
		-At high relative humidity,				-Avoid fruit contact with soil during	
		yeasty layer may cover the				harvest.	

Table ... continued

		lesion and produces sour odour that attracts fruit flies which may enhance the spread of the fungus.				-Immediate cooling of picked fruits to below 10 °C will delay decay development.	
		-The infection quickly spreads into a soft decaying area favoured by moderate temperature 27 °C.				-Application of adequate sanitary practices (soak tanks with chlorine at proper pH). -Application of disinfectants. -Application of mixtures of fungicides.	
Stem-end rot 187	<i>Diplodia natalensis</i> P. Evans (syn. <i>Botryodiplodia theobromse</i> Pat.; <i>Physalospora rhodina</i> Berk and Curt	-Initially, decay occur at both ends of the fruit. -In infected fruit, lesions appear as dark discoloration	Quiescent, infective pathogen.	Natural openings at the stem- end.	No.	-Improve cultural practices such as removing dead trees, wood. -Harvesting by clipping rather than pulling.	Brown, 1994.

## Table continued

		within 1-2 weeks during storage time.				-Remove some buttons (sepal base) that harbour pathogen.	
		-Development of sour fermented odour as the fruit becomes black.				-Spot picking for natural colours and delaying harvest until more colour develops (reduce degreening time).	
						-In packhouse increase humidity to 90 – 95%.	
						-Maintaining of temperature at 82- 84 °F and ethylene formation at 1 – 5 ppm during degreening and storage.	
188	Stem-end rot	<i>Phomopsis citri</i> Faw	-Decay appears as a buff coloured to brown, leathery, pliable area encircling the button or stem-end of the fruit.	Quiescent, infective pathogen.	Injured rind.	Yes.	-Improved cultural practices such as Whiteside <i>et al.</i> , 1988. remove dead wood and twigs to reduce inocula.
		-The fungi colonize dead twigs and wood on the tree where spores are dispersed by rain and wind to fruit.	-Infection spreads through the core in a nearly even rind pattern from the stem-end to the surrounding.				-Proper handling of fruits at harvest to minimize injury.
		-Decay occurs after harvest when the fungus grows from the calyx (button) into the fruit.					-Cooling of fruits immediately after harvest at or below 10 °C.
							-Application of sanitary measures by removing infected fruits.

Table ... continued

Trichoderma rot	<i>Trichoderma viride</i> Pos ex Gray.  -The fungus is ubiquitous in soil growing on dead twigs. -Spores disseminated by contact with soil and / or infected wood. -The fungus mycelia are white and the conidia are globose with rough texture.	-Infection may be at any location of fruit rind. -Infected fruits develop cocoa brown colour with leathery and pliable appearances. -Decay on the fruit starts at the stem-end or stylar end. -Rotted fruits characteristically produce coconut odour.	Quiescent, infective pathogen.	Injured rind.	No.	-Improved cultural practices such as removal of dead wood and twigs is required to reduce inocula. -Proper handling of fruits at harvest to minimize injury. -Cooling of fruits immediately after harvest at or below 10 °C. -Application of sanitary measures by avoiding infected fruits.	Whiteside <i>et al.</i> , 1988.
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## Appendix 1

Table 2 Chemicals used as postharvest fungicides on citrus fruit and other crops

Name and formulation	Pathogens controlled	Host	Remarks	Extracted
<b>Alkaline inorganic salts</b>				
sodium tetraborate (borax)	<i>Penicillium</i> spp	Citrus	Only reasonably effective; Problem with residues	Willis <i>et al.</i> , 1998
sodium carbonate	<i>Penicillium</i> spp	Citrus	Only slightly effective	
sodium hydroxide	<i>Penicillium</i> spp	Citrus	Only slightly effective	
<b>Ammonia and aliphatic amines</b>				
ammonia gas	<i>Penicillium, Diplodia,</i>	Citrus	Good for fumigation of degreening and storage rooms	
sec-butylamine	<i>Rhizopus</i>			
	<i>Penicillium, stem-end rots</i>	Citrus	Good control as dip or fumigant	
<b>Aromatic amines</b>				
dichloran	<i>Rhizopus, Botrytis</i>	Stone fruits, carrot, sweet potato	Very effective	
<b>Benzimidazoles</b>				
benomyl, thiabendazole,	<i>Penicillium</i> spp	Citrus	Effective at low concentration;	
thiophanate methyl	<i>Colletotrichum</i> and other	Banana, apple,	resistance problem; residue	
carbendazim	fungi	pear, pineapple, stone fruit	tolerance 0-10µg/g	

Table ... continued

**Triazoles**

imazalil	<i>Penicillium</i> , stem-end rots	Citrus	Effective against benzimidazole-resistant strains and at low concentration
prochloraz guanidine	<i>Penicillium</i> spp	Citrus	Effective against benzimidazole-resistant strains
guazatine	<i>Penicillium</i> , <i>Geotrichum</i>	Citrus	Effective against benzimidazole-resistant strains

**Hydrocarbons and derivatives**

biphenyl	<i>Penicillium</i> , <i>Diplodia</i>	Citrus	Smell unpleasant
methyl chloroform	<i>Penicillium</i> , stem end rots	Citrus	Inhibits spore germination only

**Oxidising substances**

hypochlorous acid	Bacteria, fungi build up in wash water	Produce	Good sterilant, no penetration of injury sites, corrosive to metal
iodine	Bacteria, fungi	Citrus, grapes	Staining problem, expensive
nitrogen trichloride	<i>Penicillium</i> spp	Citrus, tomato	Hydrolyses to hypochlorous acid

**Organic acids and aldehydes**

dehydroacetic acid	<i>Botrytis</i> and other fungi	Strawberry	Dip not accepted by industry
sorbic acid	<i>Alternaria</i> , <i>Cladosporium</i>	Fig	Sterilant for picking boxes, storage rooms
formaldehyde	Fungi		

**Phenols**

Table ... continued

	o-phenylphenol	<i>Penicillium</i> spp	Citrus	Causes fruit injury
	sodium o-phenylphenate	<i>Penicillium</i> , bacteria and other fungi	Produce	pH control needed to prevent injury; residue tolerance 10-12µg/g
	<b>Salicylanilide</b>	<i>Penicillium</i> , <i>Phomopsis</i> , <i>Nigrospora</i>	Citrus, banana	Slight control
	<b>Sulphur (inorganic)</b>			
	sulphur dust	<i>Monilinia</i>	Peach	nd
	lime-sulphur	<i>Sclerotinia</i>		
192	sulphurdioxide gas, bisulphate	<i>Botrytis</i>	Grapes	Sulphur dioxide gas needs moisture to be effective;
	<b>Sulphur (organic)</b>			
	captan	Storage rots	Various produce	Nd
	thiram	<i>Cladosporium</i> , crown and stem-end rots	Strawberry, banana	Nd
	ziram	<i>Alternaria</i> , crown and stem-end rots	Banana	Nd
	thiourea	<i>Penicillium</i> spores	Citrus	Toxic to man
	thioacetamide	<i>Diplodia</i>		Nd

Legend: Nd = not determined

## Appendix 1

Table 3 Microbial antagonists registered as biopesticide for control of fungal diseases

Species name	Type	Target pathogen	Product name	Manufactured (Country)	Extracted from
<b>Bacteria</b>					Montesinos, 2003
<i>Bacillus popilliae</i>	I	<i>Popilla japonica</i>	–	–	
<i>B. thuringiensis</i> var. <i>aizawai</i>	I	<i>Galleria melonella</i>	–	–	
<i>B. thuringiensis</i> var. EG2348	I	<i>Lymantria dispar</i>	–	–	
<i>Burkholderia cepacia</i>	F	Soil borne fungi, nematodes	–	–	
<i>Pseudomonas fluorescens</i>	F	Soil borne fungi	–	–	
<i>P. syringae</i> ESC- 10, ESC-11	F	Postharvest Fungi	–	USA	
<i>P. chlororaphis</i>	F	Soil borne fungi	–	–	
<i>P. aureofaciens</i> Tx-1	F	Antracnose, soil borne	–	–	
<i>Pseudomonas aeruginosa</i> *	F	-Downy mildew of Grape, cucumber, pumpkin, pepper and melon; root rot by <i>Pythium spp.</i> ; late blight of potato by <i>Phytophthora infestans</i> . -To control <i>Geotrichum candidum</i> infection on pome and citrus.	Biosave 110, 111	USA	Shachnal <i>et al.</i> , 1996 Montesinos, 2003

Table ... continued

<i>Bacillus subtilis</i>	F	Pre-and postharvest disease of Avogreen avocado.		South Africa	Janisiewicz and Korsten, 2002; Montesinos, 2003
<i>B. subtilis</i> FZB24	F	Soil borne fungi	–	–	Motesinose, 2003
<i>B. subtilis</i> GB03	F	Soil borne and wilt	–	–	
<i>B. subtilis</i> GB07	F	Soil borne fungi	–	–	
<i>Streptomyces griseoviridis</i> K61	F	<i>Phythium</i> , <i>Fusarium</i> , <i>Botrytis</i> , <i>Alternaria</i> , <i>Rhizoctonia</i> and <i>Phytophthora</i> sp.	Mycostop	Kemira Argo of Finland	
<i>S. lydicus</i>	F	Soil borne fungi.	–	–	
<i>Agrobacterium radiobacter</i> K84, K1026	B	Crown gall <i>A. tumefaciens</i> .	–	–	
<i>Ralstonia solanacearum</i> non-pathogenic	B	Pathogenic <i>R. solanacearum</i>	–	–	
<i>Pseudomonas fluorescens</i> A506	B	Frost damage, fire blight ( <i>Erwinia amylovora</i> ).	–	–	
<i>Pseudomonas syringae</i> pv. <i>tagetis</i>	H	<i>Cirsium arvense</i>	–	–	
<i>Xanthomonas campestris</i> pv. <i>poae</i>	H	<i>Poa annua</i>	–	–	

Table ... continued

**Fungus biopesticides**

<i>Trichoderma polysporum</i> , <i>T. harzianum</i>	F	Soil borne fungi	–	–	Montesinos, 2003
<i>T. harzianum</i> KRL-AG2	F	Soil borne fungi	–	–	
<i>T. harzianum</i>	F	Foliar fungi	–	–	
<i>T. harzianum</i> , <i>T. viride</i>	F	Various	PlantShield	BioWorks, Inc.in Geneva	
<i>T. viride</i>	F	Phythium, Rhizocotonia, Fusarium and Botrytis	–	–	
<i>T. lignorum</i>	F	Vascular wilt	–	–	
<i>Trichoderma</i> spp	F	Soil borne	–	–	
<i>Ampelomyces quisqualis</i> M-10	F	Powdery mildew	–	–	
<i>Talaromyces flavus</i> V117b	F	Soil borne fungi	–	–	
<i>Gliocladium virens</i> GL-21	F	Pythium, Rhizocotonia and Sclerotinium sp. (Soil borne fungi).	SoilGuard	Cerit, USA	( <a href="http://www.cfgrower.com/tips/oct/biological.html">http://www.cfgrower.com/tips/oct/biological.html</a> ) Montesinos, 2003
<i>G. catenulatum</i>	F	Soil borne fungi	–	–	Montesinos, 2003
<i>Fusarium oxysporum</i> non- pathogenic	F	Pathogenic Fusarium	–	–	
<i>Pythium oligandrum</i>	F	<i>Pythium ultimum</i>	–	–	
<i>Phlebiopsis gigantean</i>	F	Heterobasidium	–	–	

Table ... continued

<i>Coniothyrium minitans</i>	F	<i>Sclerotinia sclerotiorum</i>			Montesinos, 2003
<i>Candida oleophila</i> 1- 182	F	Penicilium decay on citrus and pome fruits (postharvest decay).	Aspire™	USA	Shachnal <i>et al.</i> , 1996 Montesinos, 2003
<i>Cryptococcus albidus</i>	F	Postharvest disease of apples and pears (postharvest decay).	Yield plus	USA	Shachnal <i>et al.</i> , 1996 Montesinos, 2003
<i>Phytophthora palmivora</i> MWV	H	<i>Morrenia odorata</i>	–	–	Montesinos, 2003
<i>Colletotrichum gloeosporioides</i>	H	Cuscuta and various	–	–	
<i>C. gloeosporioides</i> f. sp. <i>malvae</i>	H	<i>Malva pulsilla</i>	–	–	
<i>C. g. f.</i> sp. <i>aeschynomene</i>	H	<i>Curty indigo</i>	–	–	
<i>C. coccodes</i>	H	<i>Abutilon theophrasti</i>	–	–	
<i>C. truncatum</i>	H	<i>Sesbania exalta</i>	–	–	
<i>Aiternaria cassia</i>	H	<i>Senna obtusifolia</i>	–	–	
<b>Viruses .</b>					
<i>Pine sawfly</i> NPV	I	<i>Diprion similes</i>	–	–	
<i>Heliothis</i> NPV	I	<i>Helicoverpa zea</i>	–	–	
<i>Gypsy moth</i> NPV	I	<i>Lymantria dispar</i>	–	–	
<i>Tussok moth</i> NPV	I	<i>Orgyia pseudotsugata</i>	–	–	
<i>Mamestria brassicae</i> NPV	I	<i>Heliothis</i>	–	–	
<i>Spodoptera exigua</i> virus	I	<i>S. exigua</i>	–	–	
Bacteriophage of <i>P. tolaasii</i>	F	Bacterial rot of mushroom	–	–	

**Legend:** B = bactericide; F = fungicide; H = herbicide; I = insecticide; N = nematicide.

\* A winner of the 2004 Presidential Green Chemistry Challenge Award.

– = status not known.

## Appendix 1

Table 4 Microbial antagonists and their mode of action on fruits

Microbial antagonists	Pathogen	Mode of action	Compound/ Metabolite produced	Commodity/ fruit	References
<b>Bacteria</b>					
<i>Bacillus subtilis</i> *	<i>Penicillium digitatum</i>	-Antibiosis -Competition for nutrients and space	Iturin -	Peaches Avocado	Pusey and Wilson, 1984; Demoz and Korsten, 2006
<i>Burkholderia (Pseudomonas) cepacia</i> *	<i>P. digitatum</i>	-Antibiosis -Competition for nutrients and space.	Pyrrolnitrin	Apple, pears and citrus	Smilanick and Denis-Arrue, 1992.
<i>P. syringae</i> (ESCO-10 and ESC-11)*	<i>P. digitatum</i>	-Antibiosis -Competition for nutrients and space	Syringomycin	Citrus	Bull <i>et al.</i> , 1997.
<i>Enterobacter cloacae</i>	<i>P. digitatum</i>	-Competition for nutrients and space	-	Citrus and pome, peach.	Wilson <i>et al.</i> , 1987.
<b>Yeasts</b>					
<i>Pichia guilliermondii</i> Wicker*	<i>P. italicum</i>	-Competition for nutrients and space -Directly parasitizing the pathogen	-	Citrus	Arras <i>et al.</i> , 1998.
<i>Candida saitoana</i> *	<i>P. italicum</i>	-Competition for nutrients and space. -Directly parasitizing the pathogen when co cultured with <i>Botrytis cinerea</i> .	-	Citrus/apple	El-Ghaouth <i>et al.</i> , 2000.
<i>Debaryomyces hansenii</i> *	<i>P. italicum</i>	-Competition for  -Directly parasitizing co cultured with <i>Botrytis cinerea</i> .	-	Citrus	Droby <i>et al.</i> , 1989.



Table ... continued

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<i>Cryptococcus laurentii</i>	<i>P. italicum</i>	-Competition for nutrients and space.	-	Citrus/ apple	Roberts, 1990.
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	<i>P. italicum</i>	-Competition for nutrients and space	-	Citrus	Janisiewicz <i>et al.</i> , 2000.
<i>Sporobolomyces roseus</i>	<i>P. italicum</i>	-Competition for nutrients and space	-	Citrus	Janisiewicz, 1994.

Legend: \* = antagonists with multiple mode of actions.

- = status not known.

**Table 5** Categories of information gathered in the informally structured questionnaire on citrus cultivation, pre- and postharvest disease control practices in Ethiopia

<b>Category information</b>	<b>Information requested</b>	<b>Dominant Response</b>
Geographical aspects	Summer and winter temperature	Table 2
	Water source relation: Rainfall	79.2%
	Irrigation	100%
	Altitude	Table 2
	Humidity	Table 2
History of citrus farm/ orchards	Ownership: Government	97%
	Private (Individual)	2.6%
	Private (Association)	0.4%
	Farm size (ha)	Table 3
	Cultivar variety:	
	Valencia	35.8%
	Washington navel	23.9%
	Hamlin	19.4%
	Pineapple	7.5%
	Shamuti	4.5%
	Jaffa	1.5%
	Robbins blood	1.5%
	Unknown cultivars	5.9%
	Scion/ root stock sources move in:	
	From certified growers (California (USA), Israel and Asmara (Erteria)	21.1%
	From local growers (UAAIE, Ghibe, Error)	36.8%
	Material source unknown	42.1
Orchard establishment, age in:		
Old orchards (>20 years)	70%	
Young orchards (<20 years)	30%	
Type of crop used in	Cereals, vegetables, fibre crops, oil crops	
Soil type and nutrient status	Table 2	
Fertilizer used	Table 2	
Preharvest epidemiology and disease/ pest management practices	Disease type:	
	Gumosiss (bark irruption)	In all citrus farms (>70%)
	Leaf and fruit spot	Ghibe citrus farm Tisablaima association citrus farm
	Insect pests	Reported 50% fruit damage
	Nematodes	12.1%
	Control measures:	Mostly by UAAIE, Hursso, Ghibe and Error Gota farms
Chemical spray (Insecticides)		
Plant decoction with animal urine and planting of a legume ( <i>Lablab purpureus</i> L.) between citrus trees	Tisabalima association farm	

Average input cost statistics in major Government citrus farms as compared to annual gross income	Labour cost	7.76%
	Agrochemicals (Pesticides and Fertilizers)	38.3%
Fruit harvesting	Peak time of harvest	Table 4
	Harvesting temperature	Ambient average temperature (18-25 °C)
	Harvesting techniques	Hand picking, tree shaking and pulling with long stick
	Persons involved in fruit picking	Temporary workers
Fruit transportation and storage facility	Fruit storage facility after picking	None
	Means of transportation	Open private and air-conditioned Efruit trucks.
	Storage facility, general impression	Untidy and with no temperature control in private and Efruit storage houses
	Postharvest disease incidence Pathogens Disease control methods	Fig. 10 and 11 <i>Penicillium</i> spp. Sorting out and remove decayed fruits
Fruit marketing	Local markets	Towns around farms and Addis Ababa, Harar and DireDawa
	Export markets	Djibouti and Somalia
Others	Pack house facilities	None
	Overall farm experience on pre- and postharvest disease control activities:	Involved cultural practices such as field sanitation and use of pesticides in Government farms and animal urine + plant decoction in private farms.

**QUESTIONNAIRE: I****Code “A” 2003/04****PREHARVEST CITRUS DISEASE MANAGEMENT PRACTICES  
ASSESSMENT**

This questionnaire was translated to the local language (Amharic) for fieldwork

**SITE: Citrus Production units in Ethiopia****Brief description:****Date:** \_\_\_\_\_

This questionnaire was designed as part of a PhD study that will focus on citrus fruit diseases and its control in Ethiopia. The first part of the questionnaire deal with pre-and postharvest factors, citrus diseases, crop management, and the second section dealing with fruit handling, storage, distribution and marketing. Therefore, we are kindly requesting your sincere response in replaying to the questions. Your input and time is much appreciated. All data will be held confidential and will only be used for research purposes.

Thank you!

Region: \_\_\_\_\_

Farm Name: \_\_\_\_\_

Farm Address: \_\_\_\_\_

**I. Geographical aspects**

1. What is the average summer and winter temperatures? (mark the applicable answer with an “X” )

1-a: Summer (day)

15-20°C	21-25°C	26-30°C	>30°C	<input type="checkbox"/>
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1-b: Summer (night)

<10°C	10-15°C	16-20°C	<input type="checkbox"/>
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1-c: Winter (day)

<10°C	10-15°C	16-20°C	21-25°C	<input type="checkbox"/>
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1-d: Winter (night)

<10°C	10-15°C	16-20°C	<input type="checkbox"/>
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2. Water source used in the farm

Rainfall	irrigation	Both	<input type="checkbox"/>
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3. How often do you irrigate your orchards?

Twice in a week	Once in a week	Twice in a month	Once in a month	Any other	<input type="checkbox"/>
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4. What is the average rainfall per a year in ml?

<250ml	250-500ml	501-750ml	751-1000ml	>1001	<input type="checkbox"/>
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5. What is the general altitude in ft above sea level?

0-300	301-600	601-900	901-1200	1201-1500	1501-1800	>1800	<input type="checkbox"/>
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6. What is the average humidity of the farm?

<30%	31- 50%	51-70%	71-90%	>90%	<input type="checkbox"/>
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**II. History of citrus farm and its orchards**

7. How big is a farm in hectares?

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8. How many types of sweet orange cultivars are produced on the farm? Can you name them and put in order of their importance in terms of area planted percentage composition?

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9. From where did you purchase the planting material?

Certified growers	Local growers	Any other source. Name the name	<input type="checkbox"/>
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10. How was the citrus seedlings/trees produced?

By seeding	By grafting	<input type="checkbox"/>
------------	-------------	--------------------------

11. How old are the orchards?

0-10 years	10-20 years	20-30 years	>30 years	<input type="checkbox"/>
------------	-------------	-------------	-----------	--------------------------

12. What is the ownership status of the citrus plantation site

Government	Association	Private (own)	<input type="checkbox"/>
------------	-------------	---------------	--------------------------

13. Are there any other crops growing in/ or around the citrus farm?

Yes	No	<input type="checkbox"/>
-----	----	--------------------------

14. For question number 12 above, if your answer is yes, what type of crop is it?

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15. What is the soil type of the farm?

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16. Have you ever determined the nutrient status of the soil?

Yes	No	<input type="checkbox"/>
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17. For the above question number 16, if your answer is yes, are their deficient chemical elements identified so far? List their names.

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18. For question number 16, if your answer is no, how did you managed diseases and or disorders associated with mineral deficiencies?

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19. Do you use a fertilization program?

Yes	No
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20. For question number 18, if your answer is yes, what type of fertilizer do you applied?

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21. Referring to question number 19, how often do you apply fertilizer to a farm with in a year?

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### III. Pre-harvest Epidemiology and Disease management practices

22. Do you have problems of diseases on your citrus trees?

Yes	No
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23. Which part of the tree is attacked with the most common diseases?

Root	Stem	Leaf	Fruit
------	------	------	-------

24. Which type of infection is most prevalent? Put in order (1-4) according to their importance

Fungal infection	Bacterial infection	Virus infection	Nematode attack	Insect problems
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25. If the disease has a microbial origin, which type? Can you name/ describe the type of disease and its pathogen in order of its importance?

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26. If insects are important, what type of insects?

Write their names in order of importance

a) \_\_\_\_\_

b) \_\_\_\_\_

c) \_\_\_\_\_

d) \_\_\_\_\_

e) \_\_\_\_\_

f) \_\_\_\_\_

27. If nematodes are present, which type? Mention by name according to their importance.

- a) \_\_\_\_\_  
 b) \_\_\_\_\_  
 c) \_\_\_\_\_

28. Referring to question number 25, when do you think does the fungal infection start to appear on the orchards?

- a 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- b 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- c 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- d 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- e 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------

29. With reference to question 26, when do you think does insect problem start to appear on the orchard?

- a 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- b 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- c 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- d 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- e 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- f. 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- g. 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------

30. Referring to question number 24, when do you think does the respective virus infection start to appear on the orchards?

- a 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- b 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- c 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- d 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------
- e 

At grafting	At flowering	Just at fruiting	At fruit ripening
-------------	--------------	------------------	-------------------



31. Referring to question number 24, when do you think does the respective nematode infection start to appear on the orchards?

a	At grafting	At flowering	Just at fruiting	At fruit ripening	<input type="checkbox"/>
b	At grafting	At flowering	Just at fruiting	At fruit ripening	<input type="checkbox"/>
c	At grafting	At flowering	Just at fruiting	At fruit ripening	<input type="checkbox"/>
d	At grafting	At flowering	Just at fruiting	At fruit ripening	<input type="checkbox"/>
e	At grafting	At flowering	Just at fruiting	At fruit ripening	<input type="checkbox"/>

32. How do you control disease? Explain your experience on the farm.

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33. Did you apply pesticides on your farm?

Yes	No
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34. Referring to the above question number 25, if you have applied pesticides, mention their names and application involved.

Name the commercial pesticides	What concentrations are being applied?	When do you start spraying pesticides?	How often do you spray during the growing season	What is the type of insect controlled?

**III. Input costs and production statistics**

35. How many workers are there in the farm?

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36. What is the average working hours of the farm worker per day?

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37. What is the average salary of a farm worker per month?

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38. What is the average input invested for pesticides purchase per year?

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39. What is the average input invested for fertilizer purchase per year?

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QUESTIONNAIRE: II

Code “B” 2003/04

**Postharvest citrus (sweet orange) fruit handling and disease management practices**

**SITE:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Name:** \_\_\_\_\_

**Region:** \_\_\_\_\_

**Packhouse/Market Name and address:** \_\_\_\_\_

**I. Fruit harvesting**

1. What is the daily temperature of a farm?

<10°C	10-15 °C	16-20 °C	21-25 °C	>25°C	<input type="checkbox"/>
-------	----------	----------	----------	-------	--------------------------

2. When is the peak time for harvesting fruit?

Jan.	Feb.	May	Apr.	Ma.	Jun.	Jul.	Au.	Sep.	Oct.	Nov.	Dec.	<input type="checkbox"/>
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3. What is the average temperature in the region?

**a) Day time**

<10°C	10-15 °C	16-20 °C	21-25 °C	>25°C	<input type="checkbox"/>
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**b) Night time**

<0°C	0-5 °C	6-10 °C	11-15 °C	>15°C	<input type="checkbox"/>
------	--------	---------	----------	-------	--------------------------

4. What is the average relative humidity (RH)?

<29	30-40	41-50	51-60	61-70	71-80	>81	<input type="checkbox"/>
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5. How do you pick fruit from the orchard?

Hand picking with gloves	Without gloves	By pulling with long sticks	By climbing in the tree
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Describe if you have another method of harvesting \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

6. Who are picking your fruits?

Farm workers	Retailing market dealers	Part time workers
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Mention if there are any \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

7. Referring to question number 5, how many fruits are harvested at a time in a day? \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

8. Where do you put fruits while collecting?

In a sack (1/2 a quintal size	In plastic crates	Openly on the ground/soil
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Mention if there is any fruit collection method

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

9. At what temperature do you store fruits?

<0°C	0-5°C	6-10°C	11-15°C	>15°C
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Mention if there is any

\_\_\_\_\_

\_\_\_\_\_

## II. Fruit Transportation

10. How do you transport fruits from farm to packhouse?

By vehicle	By cart	By human labor
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Explain if there are any other methods used in your farm to transport fruits?

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11. For how long do you store fruit in the packhouse?

<6hrs	A day (24hrs)	48hrs	A week	More than a week	We don't store fruits at all
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12. Storage conditions of fruits during transit?

<0 °C	0-5 °C	6-10 °C	11-15 °C	>15 °C
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## III. Postharvest Diseases

13. Do you have problems of postharvest diseases in the storage /packhouses?

Yes	No
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14. For the above question (9), if your answer is no, what do you think the case is? May you explain the detail?

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15. For question (13) above, if your answer is yes, what are the major diseases associated? May you write down their names in order of importance?

a) \_\_\_\_\_

b) \_\_\_\_\_

c) \_\_\_\_\_

d) \_\_\_\_\_

e) \_\_\_\_\_

f) \_\_\_\_\_

16. Referring to question number 15, how do you control postharvest diseases and their dissemination? Explain your experience?

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17. Do you apply commercial chemicals to control postharvest citrus diseases?

Yes	No
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18. For the above question (17) if your answer is yes, mention the type of chemical applied and how frequently used?

Chemical name	Formulation	Application (how frequently used)	Remarks

19. For the same question number 17, if your answer is no, why? May you explain the reason?

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20. Referring to question 18, the spray of chemicals, by what equipment and is the machine calibrated?

Yes	No
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21. For question number 20, if your answer is no, what is the hindrance?

- a) lack of knowledge
- b) lack of training
- c) lack of economy
- d) mention if there are any other factors

22. How far is the average distance to your local market? Name market places and their distance from the farm packhouse.

Name of market places	Approximate distance (km)	Safety measures taken to keep quality of fruits	Remarks

23. How do you transport the packed fruit to the local market?

By vehicle	On the back of animals	By human labor
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Mention if there are any means of fruit transportation:

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24. Do you have international market access for the fruit produced and or its product?

Yes	No
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25. For the above question (24), if your answer is yes, where? Mention the name of the country according to their market importance?

Country	Fruit market	fruit product	Remarks

24. How do you transport the packed fruit to the international market?

By air	By ship	By train
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25. What is the transit temperature used during export? If others, give

4°C	7°C	10°C	>11°C	
-----	-----	------	-------	--

26. Do you apply chemicals to control decay of fruits during transit?

Yes	No
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27. For the above question number 26, if your answer is yes, what chemicals do you use? List the name of commercial chemicals applied?

Chemical name	Formulation	Application (how frequently used)	Remarks

28. For question number 24, if your answer is no, what is hindrances to export?

- a) lack of knowledge, know how, contact
- b) quality guarantee because of diseases and associated problems
- c) mention if encountered other factors

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29. Write any experiences of your farm (cultural, physical, biological or a combination of them) in postharvest disease handling and management practices to control citrus fruit disease.

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**IV. Fruit Price and marketing**

30. What is the average price of fruit per kilogram in the local market?

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31. What is the average price of fruit per kilogram in the export market?

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**APPENDIX III**

**SENSORY EVALUATION FORM**

Name \_\_\_\_\_

Date \_\_\_\_\_

Time \_\_\_\_\_

Parameters	A	B	C	D	E	F	G	H	I
Skin colour									
Colour of the edible portion									
Colour of the flavedo									
Appearance									
Flavour									
Odour or smell									
Juiciness									
Sweetness									
Sour or Bitterness									
Overall acceptability									

**Attribute**

**Definition**

Smell	Total intensity of smell
Fresh	smell of fresh oranges
Flavour	Total intensity of flavour during the first chewing
Sweetness	Sweet taste
Bitterness or sourness	bitter or sourness
Appearance	whether it is fresh, shriveled, firm, soft
Colour	natural colour of orange or presence of browning.

Hedonic scale 1-5, where,

1=very poor, 2 poor, 3 fair, limited acceptability, 4- good, 5= excellent

Signature of the participant

\_\_\_\_\_

# Identification of citrus (*Citrus sinensis*) Postharvest Pathogens from Ethiopia and their Control

By

**Sissay Bekele**

**Promoter:** Prof. Lise Korsten  
**Co-promoter:** Dr. Thierry Regnier  
**Department:** Microbiology and Plant Pathology  
**Faculty:** Natural and Agricultural Sciences  
**Degree:** Ph.D. (Plant Pathology)

## SUMMARY

From a world prospective, the continuous application of chemical pesticides has serious long-term effects on human health and environmental pollution, and can result in resistant pathogen strains. However, postharvest diseases cause major losses on the markets and need to be controlled effectively. The search for biopesticides using microbial antagonists and natural plant products has subsequently become more important as viable alternatives to control postharvest diseases. Currently, little information exists in terms of citrus production practices, disease management measures and postharvest losses in Ethiopia. The aim of this study was therefore to determine what the current situation in the country is in terms of production, disease management and postharvest disease incidence, disease management practices in Ethiopia and to develop an effective and safe disease control strategy for the industry. Citrus production in Ethiopia is mainly done by Government enterprises with little technical expertise. Disease control strategies are ineffective with postharvest losses exceeding 46%. The most important postharvest pathogen identified was *Penicillium digitatum*. In development of biopesticides, three yeast antagonists [*Cryptococcus laurentii* (strain MeJtw 10-2 and strain TiL 4-3) and *Candida sake* (TiL 4-2)] and plant leaf extracts of *Acacia seyal* and *Withania somnifera* were found to have some potential to

control *Penicillium* in *in vitro* and *in vivo* trials and ensure fruit quality. The modes of action of the yeast antagonists were not based on antibiosis. Instead, it involved competitive colonization where the antagonists inhibited *P. digitatum* spore germination and reduced mycelial growth by 75-100%. Extracts from the two plant species showed broad-spectrum antimicrobial activity against a range of several fungal and bacterial pathogens. The semi-commercial application of the antagonists and plant extracts improve fruit quality and the integration of these biopesticides were found effective in semi commercial trials and may provide a commercial solution for the citrus industry.