ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

BIOTECHNOLOGY PROGRAM

Survey and Serological Identification of Viruses Infecting Garlic (*Allium sativum* L.) in Ethiopia

By

Kero Jemal

Advisor:- Tileye Feyissa (PhD)

Co-Advisor:- Adane Abraham (PhD)

A Thesis Submitted to School of Graduate Studies Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Biotechnology

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Acronyms

DADS	Diallyldisulphide
DAS-ELISA	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
DNA	Deoxyribonucleic Acid
EIAR	Ethiopian Institute of Agricultural Research
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization of the United Nations
GCLV	Garlic Common Latent Virus
GV-A	Garlic Virus A
GV-B	Garlic Virus B
GV-C	Garlic Virus C
GV-D	Garlic Virus D
GV-E	Garlic Virus E
GV-X	Garlic Virus X
HARC	Holetta Agricultural Research Center
HDL	High Density Lipoprotein
HMG CoA	3-hydroxy-3-methyl-glutaryl-CoA reductase
IgG	Immunoglobulin G
LDL	Low Density Lipoprotein
LYSV	Leak Yellow Strip Virus
MAb	Monoclonal Antibody
ORF	Open Reading Frame
OYDV	Onion Yellow Dwarf Virus
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline -Tween20
PCR	Polymerase Chain Reaction
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic Acid

RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SLV	Shallot Latent Virus
SNNP	South Nations and Nationalities People
ssRNA	Single stranded RNA
TAS-ELISA	Triple Antibody Sandwich Enzyme Linked Immunosorbent Assay
Vari	Variety
w/w	Weight by weight ratio
w/v	Weight by volume ratio

Abstract

Garlic (*Allium sativum* L.) is one of the most important bulb vegetables, which is used as spice and flavoring agent for foods and as medicinal plant. It is produced throughout the world including Ethiopia. Garlic is infected by numerous systemic viruses including the genus Potyvirus, Carlavirus and Allexivirus. There is no any research report available about garlic viruses in Ethiopia. Therefore, this study is aimed at survey of garlic virus diseases and detection of garlic viruses by DAS-ELISA. A survey was conducted in the year 2009 to identify viruses infecting garlic (*Allium sativum* L.) in different growing areas of Ethiopia. Surveys conducted to assess the status of virus diseases affecting garlic in the major growing areas of Ethiopia indicated that leaf yellowing; yellow mosaic, stripes and stunting were the most common disease symptoms observed. The highest visually observed disease incidence in a field was 93%, recorded in the Arsi zone of Oromia region. When 520 symptomatic and asymptomatic samples collected from 56 fields from major garlic growing areas of Ethiopia were tested by the double antibody sandwich enzyme linked immunosorbent assay(DAS-ELISA) for four viruses (OYDV, LYSV,GV-B and GV-C), 119 samples (23%) were found to be infected with at least one virus. GV-B, member of *Allexivirus* was the most frequent (17.7%), followed by OYDV, member of *Potyvirus* (5.6%) and GV-C (4.8%) which is *Allexivirus*. LYSV was detected only in 7 samples. Mixed infections were also very common. Among the mixed infections, the most common one was GV-B +GV-C(2.5%) followed by GVB+OYDV(1.7%) and GV- B +LYSV(0.8%). The incidence of GVB+GVC+OYDV was 0.4 %.

Key words: *Allium sativum, DAS-ELISA, Garlic, Garlic virus B, Garlic virus C, Leak yellow Strip Virus, Onion Yellow Dwarf Virus.*

1. Introduction

Garlic (*Allium sativum* L.) is one of the most important *Allium* plants widely cultivated throughout the world including Ethiopia. It is one of the most important bulb vegetables, which is used as spice and flavoring agent for foods and as medicinal plant (Velisek *et al.,* 1997). Viral diseases are one of the major causes of low yield and quality in garlic. A significant reduction in yield and quality due to virus infection is now a serious economic problem in many countries (Fujisawa, 1989; Hwang *et al* 1986; Ogawa *et al*, 1976; Walkey and Antil, 1989). Since a significant reduction in both yield and quality due to virus infection is currently a serious economic problem, garlic viruses have been intensively studied. More than eight viruses have been detected in garlic including the members of the genus *Potyvirus, Carlavirus* and *Allexivirus* forming a viral complex and causing a disease called garlic mosaic (Van Dijk, 1993; Dovas and Vovlas 2003). Garlic plant (*Allium sativum* L.) is a species that can only be vegetatively propagated by bulbs and this condition favors a process of virus accumulation overtime in plant materials.

Virus diseases of garlic are usually induced by mixed infections of several viruses belonging to different taxonomic groups, which are known as the garlic viral complex including: Potyvirus (*Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV)); Carlavirus (*Garlic common latent virus* (GarCLV), and *Shallot latent virus* (SLV)) and various *Allexiviruses* (Van Dijk, 1993). *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Shallot latent virus* (SLV), and *Garlic common latent virus* (GCLV) have been cited as aphid-borne viruses infecting garlic (Barg *et al.,* 1994; Conci *et al*, 1992). Other garlic viruses are transmitted by mites (Barg *et al.,* 1994; Helguera *et al.,* 1997; Van Dijk and van Der Vlugt, 1994) and have been grouped in the genus *Allexivirus* (Van Regenmortel *et al.,* 2000). Based on the nucleotide sequence, different virus species have been detected within the genus *Allexivirus*, such as *Garlic viruses A, B, C, D, E,* and *X* (GarV-A, B, C, D, E, and X) (Chen *et al.*, 2001; Chen *et al.*, 2004; Kanyuka *et al.,* 1992; Song *et al.*,1998; Tsuneyoshi and Sumi 1996).

Both potyviruses and carlaviruses have been previously detected in garlic plants from many countries. Details of the occurrence of OYDV and LYSV have been described in Greece (Dovas *et al.,* 2001), Italy (Dovas and Vovlas 2003), Brazil (Daniels 1999; Fajardo *et al.*, 2001), and

Japan (Takaichi *et al.*, 2001). Conci *et al*, (2002) studied LYSV in Argentina. GarCLV has been detected in Brazil (Daniels, 1999), Italy (Dovas and Vovlas 2003) and, along with SLV, in Greece (Dovas *et al*, 2001). In addition to potyviruses and carlaviruses, garlic plants have often been infected by *Allexiviruses*. Dovas *et al*, (2001) detected Allexiviruses in Greece and Takaichi *et al*. (1998) in Japan. More than six types of *Allexiviruses* have been discovered including *Garlic virus* A, B, C, and D, GarV-A, GarV-B, GarV-C, GarV-D, GarV-E and GarV-X, all of which have been abundantly detected in garlic plants (Koo *et al.*, 2002; Chen, and Adams, 2001; Park *et al.*, 2005; van Dijk, 1993).

These virus diseases of garlic (*Allium sativum* L.) are widespread throughout the world, causing serious losses in crop yields and deterioration of quality. They accumulate in bulbs because of the vegetative propagation of the crop. If the viruses are to be controlled effectively, it is first necessary to establish their identity and to determine their incidence and prevalence in a given area.

There is no any report on the presence or absence of these viruses infecting garlic. Therefore, the aims of this study are: - (i) to survey the status of garlic virus diseases in the fields of garlic major growing areas of Ethiopia. (ii) to study the occurrence of selected viruses that infect garlic such as members of *Potyvirus*; OYDV and LYSV which are aphid born and *Allexivirus*; GV-B, GV-C which are mite-borne mosaic viruses in Ethiopia serologically by Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) (iii) to determine the incidence and prevalence of virus disease symptoms in the fields and virus infections in different garlic growing regions of Ethiopia.

2. Literature Review

2.1. Origin and Distribution of Garlic (*Allium sativum* L.)

Garlic (*Allium sativum* L.) has been so extensively domesticated over so many thousands of years that there no longer are wild forms found anywhere in nature related to the type of garlic humans now use. That is, a truly wild species of garlic (*Allium sativum* L.) is not yet known but *Allium longicuspis* is considered to be the most closely related to the cultivated garlic and is considered to be the garlic's wild ancestor. Since *A. longicuspis* is endemic to central Asia, it is held that garlic originated there (Engeland, 1991).Therefore, although, because of its wide cultivation the place of origin of garlic is uncertain, the center of origin of garlic known today has been considered to be Central Asia (India, Afghanistan, West China, Russia e.t.c) (Preglove, 1972; Edwards *et al.*, 1997; Tindall, 1986). Etoh and Simon (2002) and Tindall (1986) also indicated that, the primary center of origin of garlic is Central Asia (Kazakhstan) and the secondary center is the Mediterranean and Caucasus zones. It was probably used in Central Asia since Neolithic times as a food flavoring and seasoning. Garlic was cultivated in the Middle East and Egypt since pre-historic times and now grown in all parts of the world (Edwards *et al*, 1997). It was also known by the advanced ancient civilizations of the Indus Valley, in what today is Pakistan and western India. From here it spread to China, and then Spanish, Portuguese and French introduced it to the New World (Jourdaion and Lavigne, 1987).

From the center of origin, garlic has been spread in ancient times to the Mediterranean region, to west, south and east through trade and colonization. Over the last five thousand years it has been distributed all over the world (Engeland, 1991; Maas and Klaas, 1995). Today, garlic is grown in temperate and tropical regions all over the world. It is grown in the Mediterranean area; tropical Asia (India, Philippines); China; West Africa; East Africa (Ethiopia, Kenya);Central and South America (Brazil, Mexico) (Tidal, 1983).

2.2 Description of Garlic Plant

2.2.1 Taxonomy of garlic

Allium sativum L, commonly known as garlic is a species in the onion family *Alliaceae*. Botanically, it belongs to the genus *Allium*, family *Alliaceae* of plants that produce organosulfur compounds, such as allicin and diallyldisulfide (DADS), which account for their pungency, lachrymatory effects and spicy aroma. Its close relatives include vegetable crop such as onion (*Allium cepa*), leek (*Allium ampeloprasum*), shallots (*Allium ascalonicum*) and chive (*A. schoenioprasum*) (Eric, 2010). Garlic is a diploid species with 2n = 2x = 16 of obligated apomixis. Therefore, it is a sterile species, produces sterile flowers and does not produce seeds and reproduces only by vegetative propagation through its bulbs or cloves (Mc- Collum, 1987; Figliuolo *et al*., 2001; Ipek *et al*., 2003; 2005).

2.2.2 Morphological description of garlic (*Allium sativum* L.)

Garlic is monocotyledonous biennial plant but production as annual for its bulbs or cloves is common practice. It is an erect or upright plant that can reach a height of 70 cm to 90 cm (Brewster, 1994). The crop consists of an underground bulb and above the ground vegetative part which consist of the leaves and flowers. The rooting system is adventitious while the bulbs comprise of small bulbils called cloves, which are the vegetative propagating materials of the crop (Pulseglove, 1972).The true stem is much reduced. The long, sword shaped leaves grow from the bulb beneath the surface of the soil are linear, flat and lance shaped. They are green, sometimes with a blue tinge. The bulbs are broadly ovoid two to four centimeters in diameter and consist of several, densely crowded, angular, truncated smaller bulbs called cloves. The garlic bulb consists of numerous cloves, which is the main economic organ both for consumption and propagation. The average number of cloves within each bulb varies from less than 8 to greater than 15 depending upon the strain of garlic. Cloves and bulbs are covered by a white papery coat and are used in both cookery and medicine. Flowers are in umbels, borne on smooth, round, solid scape; variable in number, on slender pedicels (Tindall, 1983). The umbels are globose, nearly always with bulbs and with many flowers. The flowers placed at the end of a stalk rising direct from the bulb on slim stalks, are small, white or pink but often never seen as they wither in the bud. The sepals are oblong, greenish white, or more or less tinged with purple.

The flowers are grouped together in a globular head, or umbel and the entire umbel is enclosed in a tear drop shaped membranous spathe. Flowers are usually sterile (Warrier *et al*, 1993).

2.2.3 Soil and climate requirements

Garlic can be grown in a wide variety of soil types, a wide range of soil conditions and pH levels, provided they are well drained, but prefer to grow in a soil with a high organic material content (http://www.sfc.ucdavis.edu/pubs/sfnews/archive/95071.htm). It requires well drained loamy soils rich in humus, with fairly good content of potash. Sandy loams are best because of their water holding capacity and generally good drainage. Clay soils are suitable if they can be loosened enough so as not to inhibit planting and bulb growth. Garlic has a relatively shallow and limited root system; therefore, plants are easily stressed by insufficient moisture and also by water logging (Rubatzky and Yamaguci, 1997). However, sandy, silt and clay loam are recommended for commercial production. The soil should be fertile, rich in organic matter, well drained, capable of holding adequate moisture during the growing period, and having soil pH ranging from 6.8 to 7.2. Lower pH levels inhibit plant growth, and soil pH below 5.0 can actually lead to plant death (Janet, 2008).

Garlic can be grown under a wide range of climatic conditions but prefers cool weather and grows at higher elevation (900 to 1200 meters) and grows best within the geographic areas having a mean monthly growing temperature ranging from 12^{o}C to 24^{o}C (Libner, 1989). In most areas elevation from 500-2000 m provide suitable growing conditions, particularly during dry periods (Tindall, 1983). Relatively high temperatures up to 30^{o}C are required for optimum bulb development but cooler conditions in the early stage favour vegetative growth (Tindall, 1983). Excessive humidity and rainfall are detrimental to both vegetative and bulb formation. The crop is therefore normally grown in low rainfall areas with irrigation during the early vegetable growth (Tindall, 1983).

Short days are very favorable for the formation of bulbs. In areas where there is seasonal variation in length of day, it is preferable to plant during short photoperiod so that maximum use will be made of this for vegetative growth. The total yield depends on the amount of vegetative growth made before bulbing begin (Tindall, 1983). In Ethiopia, garlic is cultivated in home

gardens, in small irrigated fields and by vegetable growers cooperative. Garlic grows probably throughout the cooler part of the country from altitude 1800-2800m (Edwards *et al.*, 1997).

2.2.4 Varietal Differentiation

Since the cultivated forms of garlic are all sterile, and have been propagated only vegetatively, it might be expected that they would show little intraspecific variation. However there are many strikingly distinct clones of garlic. Many selections and clones of garlic are in cultivation, mainly distinguished by the number of cloves produced per bulb which may vary from 16-50 and by the size of individual cloves (Tindall, 1983). Taxonomists have recognized at least four botanical varieties with in *Allium sativum* L., namely *Allium sativum* L. *vari. Sativum*; *Allium sativum* L., *vari. ophioscorodon; Allium sativum* L., *vari. pekinense; Allium sativum* L., vari. *nipponikum kitamura*. But generally there are two subspecies of garlic commonly known, most notably hard neck garlic (*sativum*) and soft neck garlic (*ophioscorodon*). The latitude where the garlic is grown affects the choice of type as garlic can be day-length sensitive. Hard neck garlic is generally grown in cooler climates; soft neck garlic is generally grown closer to the equator (http://www.natural-holistic-health.com/alternative-therapies/herbs-for-health/medicinal garlic).

2.2.5 Production Trend of Garlic

Garlic is the second most widely used Allium after onion (Rubatzky and Yamaguchi, 1997). According to FAO (2001), production of garlic stood at about 10 million tons per annum which is only about 10% that of bulb onion. Garlic is grown globally, but China is by far the largest producer and the largest consumer of garlic in the world today (Kamenetsky and Rabinowitch, 2001). China produces approximately 10.5 million tons of garlic annually, accounting for over 77% of world output and India (4.1%) and South Korea (2%) follow, with Russia (1.6%) in fourth place and the United States (where garlic is grown primarily as a cash crop in every state except for Alaska) in fifth place (1.4%). This leaves 16% of global garlic production in countries that each produces less than 2% of global output. The world average yield of garlic is about ten tons per hectare, but can go up to 19 tons per hectare (FAO, 2001).

World garlic cultivation was increased from 771,000 hectare of land in 1989/90 to 1,204,711 hectare of land in 2007 with total production from 6.5 million to 15.68 million tons, and

productivity from 8.43 ton per hectare and 13.02 ton per hectare, respectively (www.faostat.fao.org., 2007). In Ethiopia, the total area under garlic production in 2006/07 reached 9,266 hectares and the production is estimated to be over 683,000 quintals annually (MoARD, 2007).

2.3 Importance of Garlic Plant

2.3.1 Uses of garlic as food and spice

The value of garlic as a crop has been recognized from very ancient times. It is estimated that it has been cultivated for over 5000 years. During all this time it has been used as food, condiment and medicine by many civilizations in Asia and the Mediterranean region (Ipek *et al*., 2005). Garlic is one of the most important bulb vegetables, which is used as spice and flavoring agent for foods (Velisek *et al.*, 1997). It is widely used around the world for its pungent flavor as a seasoning or condiment. It is a fundamental component in many or most dishes of various countries in the world including Ethiopia. The pungency, lachrymatory effects and spicy aroma of garlic are due to the presence of organosulfur compounds such as allicin and diallyldisulfide (DADS). Although people have come to enjoy the taste of garlic, these compounds are believed to have evolved as a defensive mechanism, deterring animals like birds, insects, and worms from eating the plant. Garlic adds to taste of foods as well as it helps to make them digestible. The flavor varies in intensity and aroma with the different cooking methods. It is often paired with onion, tomato, or ginger. It is an important ingredient in the leading cuisines around the world. Garlic as a spice is utilized in both fresh and dehydrated state in the food industry. It is dehydrated into different products such as flakes, slices, and powders (Ahmad, 1996). In Ethiopia, garlic is used in preparing foods, particularly some kinds of stew and in making dried foods for storage (Edwards *et al.*, 1997).

Garlic has a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking. A bulb of garlic, the most commonly used part of the plant, is divided into numerous fleshy sections called cloves. The cloves are used as seed, for consumption (raw or cooked), and for medicinal purposes. The leaves, stems (scape), and flowers (bulbils) on the head (spathe) are also edible and are most often consumed while immature and still tender. The papery, protective layers of "skin" over various parts of the plant and the roots attached to the bulb are the only parts not considered palatable.

In addition to adding taste for foods, garlic contains different useful minerals, vitamins and many other substances used for health of human beings (Table 1). It is rich in sugar, protein, fat, calcium, potassium, phosphorous, sulfur, iodine fiber and silicon in addition to vitamins. It possesses high nutritive value. Its pungent flavor makes it used mainly as spice, seasoning and flavoring of foodstuffs involving both green tops and bulbs. Garlic is used for flavoring in cooking and is unique because of its high sulfur content. In addition to sulfur, garlic also contains arginine, oligosaccharides, flavonoids, and selenium, all of which may be beneficial to health (Milner, 1996). Even if it is most often used as a seasoning or a condiment, it is also believed to have some medicinal value (*http://www.garlic-central.com/garlic-health.html*).

Table 1. Nutritive Value of Garlic

Substance	**Amount found/100g**	**Substance**	**Amount found/100g**
Water(Moisture)	58.58%	Vitamin B6	1.235 mg
Energy	623 kJ (149 kcal)	Folate (Vitamin. B9)	3 μg
Carbohydrates	33.06 g	Vitamin C	31.2 mg
Sugars	1.00g	Calcium	181 mg
Dietary fiber	2.1 g	Iron	1.7 mg
Fat	0.5g	Magnesium	25 mg
Protein	6.39g	Phosphorus	153 mg
Beta-carotene	5 μg	Potassium	401 mg
Thiamine(Vitamin B1)	0.2 mg	Sodium	17 mg
Riboflavin (Vitamin. B2)	0.11 mg	Zinc	1.16 mg
Niacin (Vitamin. B3)	0.7 mg	Manganese	1.672 mg
Pantothenic acid (Vitamin B5)	0.596 mg	Selenium	14.2 μg

Source: USDA nutrition database (2009)

2.3.2 Medicinal importance of garlic

Medicinal use of garlic (*Allium sativum* L.) has existed for thousands of years (Dausch, 1990; Han, 1993), but there was little scientific support of its therapeutic and pharmacologic properties until recently. Some of the earliest references to this medicinal and culinary plant are found on Sumerian clay tablets dating from 2600-2100 B.C. Sanskrit records also show its medicinal use about 5,000 years ago and it has been used for at least 3,000 years in Chinese medicine. The

Egyptians, Babylonians, Greeks, and Romans used garlic for healing purposes during ancient times (Koch and Lawson, 1996). Garlic was an important medicine to the ancient Egyptians as listed in the authoritative medical text of the era *Codex Ebers* (a medical text dating to 1500 B.C.) especially for the working class involved in heavy labor (Lawson, 1998; Moyers, 1996). This earliest known reference indicated that garlic formed daily diet of many Egyptians. It was fed particularly to the working class involved in heavy labor, as in the building of pyramids, because it was an effective remedy for many aliments such as heart problems, headache, bites, worms and tumors (Moyers, 1996). There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina (Lawson *et al*., 1998).

Garlic has a folk history as an antibiotic and antifungal agent. This folk history was formalized when tincture of garlic was recommended for cholera in 1758 in Codex medicamentarius (Stoll and Seeback, 1951). In 1858, Pasteur noted garlic's antibacterial activity, and it was used as an antiseptic to prevent gangrene during World War I and World War II (Murray, 1995). The antibiotic effect was confirmed first with early reports by Dombray and Vlaikovich in 1924. Fungicidal properties were reported by biologists in the 1940s (Leshinkov, 1947). Starting with isolation of allicin from garlic by Cavallito *et al* (1944), the pharmacology of garlic has been actively investigated. Historically, garlic has been used around the world to treat many conditions, including hypertension, infections, and snakebites, and some cultures have used it to ward off evil spirits. Currently, garlic is used for reducing cholesterol levels and cardiovascular risk, as well as for its antineoplastic and antimicrobial properties (Koch, 1996). In Ethiopia, garlic is used as medically for a range of skin and stomach problems and also to treat many conditions such as common cold.

Much beneficial health related biological effects of garlic are attributed to its characteristic organosulphur compounds (Agarwal, 1996; Koch and Lawson, 1996). The best known and most extensively studied is allicin (diallyl thiosulphate), the principal active substance of fresh garlic extract, which is responsible for garlic’s typical pungent smell. When garlic cloves are chopped or crushed, the enzyme allinase converts alliin (a systeine-sulphoxide) in garlic to allicin (a thiosulphate) (Banerjee *et al*., 2003). The later compound is thought to confer many of garlic’s medicinal effects, but garlic has been also shown to be metabolized to a number of additional organosulphur compounds (Khanum *et al*., 2004).

Garlic contains more than 200 chemical compounds. Some of its more important ones include: volatile oil with sulphur containing compounds: allicin (diallyl thiosulphinate), alliin(S-allyl-L-cysteine sulfoxide), and ajoene and enzymes: allinase, peroxidase and myrosinase. Allicin is what gives garlic its antibiotic properties and is responsible for its strong odor. Ajoene contributes to the anticoagulant action of garlic. Garlic also contains citral, geraniol, linalool, A phellandrene and B phellandrene. Other major compounds present are diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide and allyl methyl disulphide (Husain *et al*., 1992).

Garlic is a widely studied species with many purported benefits and a long medicinal history dating back to Aristotle, Hippocrates and Aristophane (Ali *et al*., 2000). Actually, garlic contains a variety of effective compounds that exhibit anticoagulant or antithrombotic (Ali and Thomson, 1995; Bordia *et al*., 1996), antioxidant, (Augusti and Sheela, 1996; Anwar and Meki, 2003) antibiotic, (Bakri and Douglas, 2005; Rees *et al*., 1993; Yoshida *et al*., 1998) hypocholesterolaemic, (Ali *et al*., 2000) hypoglycaemic, as well as hypotensive activities (Banerjee and Maulik, 2002). Garlic has been used from the time when ancient times in India and china for a valuable effect on the heart and circulation, cardiovascular diseases (Yu-Yan and Liu, 2001; Gardener *et al*., 2003). It has also been proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effects against food borne pathogens (Teferi Gedif and Hahn, 2002).

Garlic can rightfully be called one of nature’s wonders. It can inhibit and kill bacteria, fungi, parasites, lower blood pressure, blood cholesterol and blood sugar, prevent blood clotting, protect the liver and contains antitumor properties (Sovova and Sova, 2004). Garlic can also boost the immune system to fight off potential disease and maintain health. It has the ability to stimulate the lymphatic system which expedites the removal of waste from the body. It is considered an effective antioxidant and can help protect cells against free radical damage. In addition, it nourishes and supports the heart, stomach, circulation and the lungs. Garlic has come to be seen as an all rounded treatment for preventing wound infection, common cold, malaria, cough and lung tuberculosis, hypertension, sexually transmitted diseases, mental illness, kidney diseases, liver diseases, asthma, diabetes. Current research suggests that garlic may help prevent some forms of cancer, heart disease, strokes and viral infections.

Garlic has antiseptic, antifungal and antimicrobial properties both internally as well as externally. Some of garlic's constituents possess broad spectrum antibiotic effects (Davis *et al*., 2003; McCann, 2003; Castleman, 2001; Schulz *et al*., 2004). A wide range of microorganisms including, bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations. The significant antibiotic compound of garlic is allicin. It has been shown to be effective against a broad range of dangerous bacterial species such as *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Salmonella typhimurium*. One underlying mechanism of action for garlic's antibiotic action is the stimulation of the immune system, both systemically and locally (Gardener *et al*., 2003).

Anti bacterial effects of garlic also results from interaction of sulphur compounds, like allicin, with sulphur (thio) groups of microbial enzymes such as, trypsin and other proteases, leading to an inhibition of microbial growth (Jonkers *et al*., 1999; Bakri and Douglas, 2005). Fresh garlic extracts have been found to inhibit many fungal species and have been used to protect plants and stored foods as well as in medicine. Garlic has been shown to be effective in suppressing infection caused by *Candida albicans*. Garlic allicins have also acted as a larvacide and bacteriostat, active against gram positive or gram negative microorganisms. Several other microbes are affected by garlic, including some viruses. Most researchers agree that the sulfur containing compounds of garlic, especially allicin, alliin, cycroalliin, and diallyldisulphide are the most biochemically active.

One of the best researched areas of garlic is its effects on the heart and circulatory systems. Garlic has been used since ancient times in India and China for a beneficial effect on heart and circulation. Recent studies suggest garlic has some properties, which help in reducing blood pressure. It slows down fast pulse and improves heart rhythm. Garlic can open blood vessels and reduce hypertension, eliminate intestinal parasites, lower cholesterol and reduce susceptibility to allergies (*www.ayurvedicmedicinalplants.com*).

According to Fulder (1989), garlic can lower blood lipid levels by inhibiting lipogenesis (formation of fats in the liver and adipose tissues). Garlic accelerates the breakdown and excretion of lipids and enhances the transfer of lipids from storage adipose tissues to the blood

stream. It also has medical benefits in lowering total plasma cholesterol and triglyceride levels while elevating HDL (High density lipoproteins or good cholesterol) levels, reducing blood pressure and decreasing platelet aggregation. Alliin, one of garlic's sulfur containing compounds, apparently has an inhibitory effect upon key enzymes involved in cholesterol biosynthesis, such as HMG CoA reductase or 3-hydroxy-3-methyl-glutaryl-CoA reductase or HMGR (Schulz *et al*., 2004). HMG CoA reductase is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol. LDL (Low density lipoproteins or bad cholesterol) synthesis is suppressed by garlic. By lowering lipids in the blood (such as cholesterol and triglycerides) it benefits the heart. It not only lowers low density lipoprotein in the blood, but shifts the ratio of low density lipoproteins in favor of high density lipoproteins, so called good cholesterol, which helps the liver, metabolize fat substances in the blood, rather than allow them to be deposited in tissues. It also increases flow of blood to the capillaries, helping to reduce blood pressure (Koch and Lawson, 1995). In Europe, garlic has been registered as a drug for the prevention of cardiovascular diseases (Grunwald, 1993).

Garlic helps with anticlotting of platelets (prevents an excessive tendency of platelets to group, forming clots), and fibrinolytic (disintegrates fibrin, the protein that forms blood clots). In other words, it helps reduce the stickiness of blood platelets and their ability to aggregate (or produce blockage) (Foster, 1996). Hence, garlic can reduce chances of stroke and heart attack. Compounds alliin and ajoene, which have fibrinolytic activity, are responsible for the anticlotting property of garlic. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes (Schulz *et al*., 2004; Duke *et al*., 2003; Bruneton, 1999).This makes garlic highly recommended for people suffering from thrombosis, embolism, or vascular accidents due to the lack of blood flow.

Garlic contains the classic antioxidant vitamins ascorbic acid (Vitamin C) and tocopherols (Vitamin E) but also other very potent antioxidants such as phenols, thiols (as sulphur compounds) and carotenoids. As an antioxidant all these compounds, slow down, stop or reverse oxidation process by scavenging oxidizing agents such as reactive oxygen species and recycling oxidized lipids, proteins and nucleic acids. Allicin in garlic has been shown to act as an antioxidant by scavenging reactive oxygen species and preventing lipid oxidation (Banerjee *et*

al., 2003). It reduces damage to liver cells by inhibiting the formation of free radicals and preventing the oxidation of lipid peroxides. It has been shown to prevent lysis of blood cells *in vitro* due to toxic levels of metals such as lead, aluminum, mercury and copper (Lewis, 1977; Koch and Lawson, 1996).

One of the most exciting aspects of the therapeutic value of garlic lies in its potential use as an anti-cancer agent. Several animal experiments have suggested that garlic can inhibit or even reverse the growth of certain tumors. Garlic contains several potentially important agents that possess antitumor and anticarcinogenic properties (Agarwal, 1996). Many studies provide compelling evidence that garlic and its organic allyl sulphur component are effective inhibitors of the cancer process (Milner, 2001). Furthermore, garlic is seleniferous plant, accumulating selenium from the soil against a concentration gradient. Selenium has many anticancer actions, particularly in control of genes involved in carcinogenesis (Galeone *et al*., 2006).

2.4 Garlic virus diseases that limit production

Although, Garlic (*Allium sativum* L.) is one of the most important *Allium* plants widely cultivated throughout the world and used as food and medicine, a significant reduction in yield and quality due to virus infection is now a serious economic problem (Fujisawa, 1989; Hwang *et al.*, 1986; Ogawa *et al.*, 1976; Walkey and Antil, 1989). There are many viruses infecting garlic plant and cause reduction in yield and quality. More than eight viruses have been detected in garlic, the most common viruses belong to three genera: *Potyvirus* (Onion yellow dwarf virus (OYDV) and Leek yellow stripe virus (LYSV)), *Carlavirus* (Garlic common latent virus (GarCLV) and Shallot latent virus (SLV)) and *Allexiviruse* (Garlic viruses A, B, C, D, E, and X) (Koo *et al*., 2002; Chen and Adams, 2001; Park *et al*., 2005). In many cases, garlic plants are usually infected by a mixture of these viruses forming viral complexes that cause disease known as Garlic mosaic (Dovas and Vovlas, 2003; Van Dijk, 1993). Elimination of these viruses is difficult because this crop is propagated through bulbs and the viruses accumulate in the plant.

The genus Potyvirus (named for its prototypical member, potato virus Y (PVY)) is the largest and economically most important group of plant viruses (Ward and Shukla, 1991). In this genus there are nearly 200 definite and tentative species of viruses (30% of all known plant viruses)

which cause significant losses in crops (Ward and Shukla, 1991). Potyvirus virions are non-enveloped (Langenberg and Zhang, 1997) filamentous particles, 680 to 900nm long and 11 to 15nm wide (Dougherty and Carrington, 1988; Riechmann, *et al*., 1992). The definitive morphological structure is composed of approximately 2000 copies of capsid protein (CP) (Martin and Gelie, 1997) which encapsidates a single stranded, positive sense RNA genome approximately 10kb in length which has a 5' terminal linked protein(viral protein genome-linked) (VPg) (Murphy *et al*., 1990), and a 3' poly-A tail (Hari, *et al*., 1979). The ORF encodes a single, large polyprotein that is subsequently processed into ten functional proteins (Adams *et al*., 2005). Potyviruses are mainly transmitted by aphids in a non-persistent manner and infect a wide range of crops including garlic in which they cause significant losses. Although worldwide in distribution they are most prevalent in tropical and subtropical countries (Shukla *et al*., 1998).

Members of the genus Potyvirus cause a significant loss in garlic (Lot *et al*., 1998). Among these, *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) were the first two viruses described and characterized in garlic plants (Bos, 1981). They are considered to be the major garlic potyviruses. Both viruses were detected in South and Southeast Asia (Barg *et al*., 1994), Italy (Bellardi *et al*., 1995), Israel (Koch, M., and Salomon, R., 1994), Venezuela (Marys *et al*., 1994), France (Messiaen, 1994), Java (Sutarya, 1994), and Australia (Sward and Brennan, 1994) . Several estimates have proposed that garlic mosaic caused by Potyvirus genera can generate up to 88% losses in bulb weight (Wlkey, 1989; Lot *et al*., 1998). *Onion yellow dwarf virus* (OYDV) was found at higher frequency than LYSV and being the main cause of losses.

Onion yellow dwarf virus (OYDV), an aphid-borne Potyvirus, is one of the major viral pathogens of onion and garlic. OYDV described by Bos (1976) causes, mosaic and yellow streak symptoms, striping, curling and impairment of seed quality. In garlic, OYDV produces symptoms of mild chlorotic stripes to bright yellow stripes depending on virus isolate and cultivars. Reduction in growth and bulb size also occurs. Infection by other viruses such as *Leek yellow stripe virus*, *Garlic common latent virus* and *Shallot latent virus* also occurs and may aggravate the symptoms further. However, OYDV is recognized as a major element of the virus disease complex in garlic (Takaichi *et al*., 2001; Herve *et al*., 1998).

Limited data is available in the literature for *Carlaviruses* (Van Dijk, 1993). Carlavirus infecting garlic plants in the world include *Garlic common latent virus* (GCLV) and *Shallot latent virus* (SLV). Carlaviruse detected in diseased garlic plants were not considered to cause severe symptoms in the case of single infection (Walkey and Antil, 1989). However, there was extensive damage when carlaviruses and potyviruses were mix-infected in the garlic plants (Abiko *et al*., 1980).

Carlavirus Virions are flexuous filaments about 610–700 nm in length and 12–15 nm in diameter with helical symmetry. The genome is a ssRNA 7.4–7.9 kb in size and comprises six ORFs, encoding, in order, the replication-related proteins, the putative movement proteins or triple gene block (TGB), the coat protein (CP) and a putative nucleic acid-binding regulatory protein. Coat protein subunits are of one type, and 31–36 kDa in size. The natural host range of individual species is restricted to one or a few species. Those viruses that infect vegetatively-propagated hosts persist in the host propagative material. Most species are mechanically transmissible to a wide range of hosts. Many of the viruses have a restricted geographical distribution but those that infect vegetatively-propagated hosts like garlic are more widely-distributed (Adams *et al*., 2004).

Allexivirus, mite-borne mosaic viruses, which have rod-shaped particles similar to potyviruses, also cause weak symptoms in garlic plants (van Dijk *et al*., 1991; Yamashita, 1992). Interestingly, the gene organizations of these viruses are closely related to those of *Potyvirus* and *Carlavirus* (Sumi *et al*., 1993). Virions are very flexuous filaments about 800 nm in length and 12 nm in diameter, have helical symmetry and a surface pattern of cross-banding. The genome is a ssRNA(positive sense polyadenylated RNA) about 9.0 kb in size and comprises six ORFs, encoding, in order, the replication-related proteins, the first two proteins of a triple gene block (TGB), a serine-rich protein of unknown function, the coat protein (CP) and a putative nucleic acid-binding regulatory protein (Chen *et al*., 2004; Koo *et al*., 2002). The viruses are transmitted by mites and also persist in the host propagative material. The viruses have a wide geographical distribution (Adams *et al*., 2004). To date, six variants of *Allexivirus* family have been reported in garlic plants and are made up of GarV-A (Koo et al., 2002; Sumi *et al*., 1999), GarV-B (Kang *et al*., 2007; Sumi *et al*., 1993), GarV-C (Sumi *et al*., 1999), GarV-D (Koo *et al*., 2002; Sumi *et al*., 1993), GarV-E (Chen *et al*., 2001) and GarV-X (Chen *et al*., 2004). *Allexiviruses* cause light

green stripes on garlic leaves (Barg *et al.,* 1997; Takaichi *et al.,* 1998; Daniels 1999). Their genome organization differs from carlaviruses and potyviruses by the presence of an extra gene (ORF 4 = 42K) with unknown function, absent in these two virus genera (Van Regenmortel *et al*., 2000).

Both potyviruses and carlaviruses have been previously detected in garlic plants from many countries. Details of the occurrence of OYDV and LYSV have been described in Greece (Dovas *et al*., 2001), Italy (Dovas and Vovlas 2003), Brazil (Daniels, 1999; Fajardo *et al*., 2001), and Japan (Takaichi *et al*., 2001). Conci *et al.,* (2002) studied LYSV in Argentina. GarCLV has been detected in Brazil (Daniels, 1999), Italy (Dovas and Vovlas, 2003) and, along with SLV, in Greece (Dovas *et al*. 2001). Dovas *et al*. (2001) detected Allexiviruses in Greece and Takaichi *et al*., (1998) in Japan. In addition to *Potyvirus* and *Carlavirus*, garlic plants have often been infected by *Allexivirus* (Barg *et al*. 1997; Takaichi *et al*., 1998; Daniels 1999).

Viruses in garlic (*Allium*) plants are widespread, causing economic loss around the world (Fujisawa, 1989; van Dijk, 1994). The greatest economic losses in garlic have been attributed to OYDV, and to a lesser degree, to LYSV (Lot, *et al.,* 1998). Because garlic plants (*Allium sativum*) propagated vegetatively, the viruses accumulated and evolved over generations, resulting in the evolution of a diversity of garlic viruses (Conci *et al*., 1992; Sumi *et al*., 1993; Koo *et al*., 2002). Almost all commercial varieties of garlic are sexually sterile and the crops are vegetatively propagated by cloves. Therefore, the viruses in cloves of infected garlic plants are inevitably transferred from one generation to the next. This cause gradual reduction in quality of the crops and leads to the sever decrease in yield.

Since, the above-mentioned viruses appear to be economically important, causing garlic quality and yield reduction; there should be prevention and control procedures and methods. The first basic step to suggest prevention and control measures is to detect the presence of the viruses and characterization of these viruses in garlic plant. Different methods can be used to detect the presence of viruses in plants from which serological (ELISA) and molecular methods (RT-PCR) are the common.

2.5 Methods for the detection of plant virus diseases

Viruses infect many different plant species. Unfortunately, there are no economically feasible chemical agents similar to fungicides and bactericides that are effective against plant viruses. Strategies aimed at plant virus disease management are largely directed at preventing virus infection by: (i) eradicating the source of infection to prevent the virus from reaching the crop, (ii) minimizing the spread of the disease by controlling its vector, (iii) utilizing virus-free planting material, and (iv) incorporating host-plant resistance to the virus. An essential precursor of the implementation of control measures is an accurate diagnosis (detection) of a virus disease. Virus detection is the establishment of the presence of a particular target virus within a sample (Lopez *et al*., 2003). Once the virus particles get into the host plant, they move to the whole parts of the plant body by short distance and long distance movements. Virus particles use movement proteins to move through the whole plant body. However, to carry out virus assay, choosing appropriate tissue is of critical importance because of uneven distribution of virus particles and seasonal changes in virus (Di Terlizzi, 2000).

Many methods have been developed for the detection and identification of plant viruses, each of which has its own advantages and limitations. Diagnostic techniques for plant viruses fall into two broad categories: biological properties related to the interaction of the virus with its host and/or vector (e.g., symptomatology and transmission tests) intrinsic properties of the virus itself (coat protein and nucleic acid). Detection methods based on coat protein include precipitation/agglutination tests, enzyme-linked immunosorbent assays (ELISA and its variants), and immunoblotting. Viral nucleic acid-based techniques like dot-blot hybridization assays and polymerase chain reaction (PCR and RT-PCR) are more sensitive than other methods. A single diagnostic test or assay may provide adequate information on the identity of a virus, but a combination of methods is generally needed for unequivocal diagnosis.

The enzyme-linked immunosorbent assay (ELISA) has been very popular for detection of viruses in plant material, insect vectors, seeds, and vegetative propagules since it was introduced to plant virology by Clark and Adams (1977). ELISA is a solid phase heterogeneous immunoassay and usually done in microtiter plates made up of either polystyrene (inflexible “rigid” plates) or polyvinyl chloride (PVC, flexible plates). Virus detection is most commonly carried out using

ELISA which is the use of polyclonal antibodies or monoclonal antibodies elicited against known strain of virus. This method is found to be effective to detect plant viruses and other pathogens (Mukasa *et al.,* 2003). Due to its adaptability, sensitivity, and economy in use of reagents, ELISA is used in a wide range of situations, especially to test a large number of samples in a relatively short period of time. Many variations of ELISA have been developed (Clark and Bar-Joseph 1984; Cooper and Edwards, 1986; Van Regenmortel and Dubs, 1993) and fall into two broad categories: "direct" and "indirect" ELISA procedures.

In "direct" ELISA procedures, the antibodies (usually as an immuno-γ- globulin or IgG fraction of the antiserum) bound to the well surface of the microtiter plate capture the virus in the test sample. The captured virus is then detected by incubation with an antibody-enzyme conjugate followed by addition of color development reagents (substrate or substrate/dye combination). The capturing and detecting antibodies can be the same or from different sources. Since the virus is sandwiched between two antibody molecules, this method is called the double antibody sandwich (DAS) ELISA. In practice, DAS-ELISA is highly strain-specific and requires each detecting antibody to be conjugated to an enzyme.

There are several alternative "indirect" forms of ELISA available for virus detection. A widely used approach is triple antibody sandwich (TAS) ELISA. This is similar to DAS-ELISA, except that an additional step is involved before adding detecting antibody–enzyme conjugate. In this step, a monoclonal antibody (MAb), produced in another animal (usually mice) different from the trapping antibody, is used. This MAb is then detected by adding an enzyme-conjugated species-specific antibody (e.g., rabbit antimouse IgG), that does not react with the trapping antibody, followed by color development reagents.

Although ELISA is versatile and individual steps are simple, the assay is complex in that several steps with different reagents are involved. Many factors can therefore influence the sensitivity and reliability of the assay that include quality of antibodies, preparation and storage of reagents, incubation time and temperature, selection of appropriate parts of plant samples, and use of suitable extraction buffer. Generally a sample is regarded as positive if the absorbance value exceeds the mean value of a negative control by 2-3 standard deviations. In some cases, the simple arithmetic cut-off of twice the absorbance value of the average of the negative controls is

used. ELISA is used to detect virus coat proteins while the presence of viral genetic material is detected by PCR (and its variants like RT-PCR and real time PCR) and viral genome hybridization (Val Verde *et al*., 1998).

Recently, molecular techniques have been developed to identify viruses. Polymerase Chain Reaction (PCR) is one of these techniques (Duncan and Torrance, 1992). It is based on differences between viral nucleic acid, and is very efficient, as well as accurate depending on the type of primers used (specific or general) (Lewis, 1997). However, it is an expensive technique using high cost equipment and reagents (Maniatis *et al*., 1982). PCR amplification of coat protein genes or conserved sequences of the viral genome is the most powerful, sensitive and reliable method. PCR is an in-vitro method for amplifying target nucleic acid sequences. The speed, specificity, sensitivity, and versatility of PCR made it suitable in many areas of research in biology. Since PCR has the power to amplify the target nucleic acid present at an extremely low level and form a complex mixture of heterologous sequences, it has become an attractive technique for the diagnosis of plant virus diseases (Candresse *et al*. 1998; Henson and French, 1993).

3. Objectives

3.1 General objective

- To survey the distribution and incidence of virus infections in garlic fields of some of the major garlic growing areas of Ethiopia and detection and identification of some of the viruses infecting garlic plants in Ethiopia.

3.2 Specific objectives

- To detect the occurrence of different virus species (OYDV, LYSV, GV-B and GV-C) infecting garlic in Ethiopia using serological method (DAS-ELISA).
- To study the distribution and incidence of viruses infecting garlic plant in different parts of Ethiopia.
- To assess the incidence of virus like symptoms in the garlic fields of some garlic growing regions of Ethiopia.
- To generate information for further improvement of garlic and production of virus free garlic.

4. Materials and Methods

4.1 Field Survey of Garlic virus disease in Ethiopia

Field survey for garlic virus disease was conducted in major garlic growing (23 districts or weredas) of Ethiopia; 13 districts in Oromia region, 8 districts in SNNP (Southern Nations and Nationalities People) region, one district in Amhara region and one sub-city in Addis Ababa city (Table 2 and Figure 1). The Survey was carried out in the year 2009 cropping season in the months of October, November and December when garlic crops were grown using rain and irrigation. The studied fields were selected randomly by travelling along main and rural roads. Total of 54 farmer's fields and two research fields (Debrezeit Agricultural Research Center, Ethiopia) were inspected. Most fields owned by farmers were small in size and found near farmers houses. The age of the garlic studied ranges from one to four months. Plants were evaluated for symptoms of virus infection such as yellow mosaic, strip, and whole leaf yellowing or stunt, which major symptoms are caused by virus diseases.

The survey was conducted by walking through garlic fields and visually inspecting garlic plants for presence of typical virus disease symptoms as described above. The fields were examined using an "X" shaped sampling path. Virus disease symptom incidence was calculated according to James (1974) as the percentage of plants showing garlic virus symptoms to the total number of plants observed in the field.

$$\text{Disease incidence (\%)} = \frac{\text{Number of symptomatic (infected) plants}}{\text{Number of plants in the field}} \times 100$$

The virus disease incidence calculated was recorded and estimated as percentage infection, whereby 1-20% =low incidence; 21-49% moderate incidence; and 50-100 % =high incidence.

4.2 Collection of garlic samples

To determine the types of viruses present in garlic, garlic leaf samples showing characteristic symptoms of virus infection including mosaic, yellow strip, yellowing and stunting and leaf samples without virus infection symptoms were randomly collected from farmers fields during field survey. The samples collected were designated according to their collection number and place of origin. Leaf samples were collected during October-December, 2009.

A total of 56 fields in major garlic growing areas of Ethiopia were visited (Table 2 ; Figure 1) and with a 1 to 4 months old garlic plants were selected at random along rural paths at an interval of at least 5km. Samples were collected along two diagonals in each field. In total, 220 symptomatic and 300 asymptomatic garlic leaf samples were collected from the farmer's fields. Large number of samples (up to 10) was collected from large fields and small number of samples were collected small fields.

Table 2. Locations where survey of garlic fields carried out and garlic samples were obtained.

Region	Zone	District or Wereda	Sample Type		Total No of samples collected
			Symptomatic	Asymptomatic	
Oromia	Arsi	Bele Gasgar	5	3	8
		Robe	11	7	18
		Ticho	8	2	10
		Lode Hetosa	30	10	40
	West Shoa	Walmera	43	17	60
		Burayu	4	6	10
		Ambo	22	8	30
		Ejere	5	5	10
	East Shoa	Bushoftu	10	10	20
	Jimma	Gido	1	9	10
		Dilbi	0	5	5
	South West Shoa	Tere Sodo	7	13	20
	East Hararge	Diretiyara	5	5	10
SNNP	Gurage	Abashag	5	15	20
	Tonta Liyu wareda	Tonta Liyu	0	10	10
	Kambata Tambaro	Doyogana	11	26	37
	Silte	Silti	3	7	10
		Yubarak	0	2	2
	Wolayita	Kindokosha	0	5	5
	Hadiya	Semen Beles	0	10	10
	Konta Liyu Wareda	Konta Liyu	2	3	5
Addis Ababa	Arada sub city	Arada	7	13	20
Amhara	North Shoa	Basuna Warana	41	109	150
		Total	**220**	**300**	**520**

- SNNP**:** South Nations and Nationalities People Region

Leaf samples were cut and put in plastic bags or bottles containing silica gel (1 gram sample in 5 grams of silica gel). In the case of $CaCl_2$, first 5 grams $CaCl_2$ was added to the plastic bottle and then covered by cotton to prevent direct contact with the sample, 1gram of leaf samples were put

on the cotton and the bottles were closed. Samples were dried under silica gel or Calcium Chloride ($CaCl_2$) and stored at room temperature until used.

Figure 1. Map of Ethiopia showing locations of the surveyed districts and where samples were collected (shown with ■).

4.3 Identification of Garlic Viruses

In order to confirm the fact that symptoms observed on garlic were indeed due to viruses, serological tests were conducted. Both the symptomatic and asymptomatic garlic leaf samples collected were tested for the presence of four viruses namely Onion Yellow Dwarf Virus (OYDV), Leak Yellow Strip Virus (LYSV), Garlic Virus B (GV-B) and Garlic Virus C (GV-C). There are a number of serological techniques available to detect plant viruses. In this context a serological method called direct double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) was used for virus detection. Virus disease infection incidence was calculated according to James (1974).

4.3.1 Serological identification of garlic viruses

Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA), as described by Clark and Adams (1977), was selected because it is efficient and cheap. Therefore, DAS-ELISA was used to confirm that virus disease symptoms of garlic seen in the field were actually due to virus causal organisms. This test was carried out using a polyclonal antibody (DSMZ, Germany) specific for each virus, ELISA micorplates and different buffers important for DAS-ELISA.

Buffers Used in DAS-ELISA Experiments

1. **Coating buffer (pH 9.6)**
   - 1.59 g sodium carbonate (Na_2CO_3) and 2.93 g sodium bicarbonate ($NaHCO_3$) dissolved in 900 ml double distilled H_2O, the pH was adjusted to 9.6 with HCl and filled to final volume of 1 liter.
2. **PBS (pH 7.4) phosphate buffer saline**
   - 8.0 g sodium chloride (NaCl)**,** 0.2 g monobasic potassium phosphate (KH_2PO_4)**,** 1.15 g dibasic sodium phosphate (Na_2HPO_4) and 0.2 g potassium chloride (KCl) dissolved in 900 ml double distilled H_2O, the pH was adjusted to 7.4 with NaOH or HCl and filled to final volume of 1 liter.
3. **PBS-Tween (PBST)(Washing buffer)**
   - PBS + 0.5 ml Tween 20 per liter.
4. **Sample extraction buffer (pH 7.4)**
   - PBST + 2% PVP (Sigma PVP-40 polyvinyl pyrrolidone).
5. **Conjugate buffer**
   - PBST + 2% PVP + 0.2% egg albumin (Sigma A-5253).
6. **Substrate buffer**
   - 97 ml diethanolamine dissolved in 600 ml double distilled H_2O, the pH was adjusted to 9.8 with *HCl* and made up to 1 liter with double distilled H_2O.

-10X stock buffers were prepared and stored at 4 °C for up to one month. The buffers were warmed to room temperature before use.

7. **Substrate.** The substrate used was p-Nitrophenyl phosphate prepared as tablets.

Garlic leaf samples, which were collected, dried and preserved over silica gel or calcium chloride, were weighed and put in polythene bags, with 20 mg per bag. Dry samples were then

crushed to powder. A total of 1.5 ml of sample extraction buffer pH 7.4 was added to each bag. The mixture was ground with a pestle to extract sap, which was used for ELISA tests.

Using DAS-ELISA, garlic leaf sample extracts were tested for four viruses commonly infecting garlic and for which antisera were available. These were Onion Yellow Dwarf Virus (OYDV, Leak Yellow Strip Virus (LYSV), Garlic Virus B (GV-B) and Garlic Virus C (GV-C). Polyclonal antisera and conjugates for these viruses and related chemicals of ELISA were kindly provided by Ethiopian Institute of Agricultural Research, Holetta Agricultural Research Center. The whole laboratory works were done in Holetta Agricultural Research Center, Molecular Biotechnology Laboratory, Ethiopia.

ELISA micorplates (NUNC IMMUNO PLATES, 8 ∇ 12 flat-bottom wells of 400µl/well, nunc^{Tm}) were coated with polyclonal antibodies or immunoglobulin G (IgG) of the four viruses diluted in coating buffer based on standard dilution recommendations (1:1000). One hundred microlitres of coating buffer and immunoglobulins (IgG) solution was put in each well and incubated for 4 hours at 37 ºC to achieve maximum detection of target viruses. Plates were then washed four times at 5 minutes interval with phosphate buffered saline (PBS-Tween -20) buffer, and blotted by tapping upside down on tissue paper.

The micorplate wells were filled with 100 µl of garlic samples extract ground in sample extraction buffer, positive control (as available) and negative control (extraction buffer). Sample extracts were prepared by grinding the leaf samples at a ratio of 1:10 (w/v) in extraction buffer. Each sample, positive control (available) and negative control were added in duplicate wells. The plates were incubated at 4 °C overnight. After the plates were washed four times at 5 minutes interval and blotted on tissue paper, 100 µl of conjugated IgGs (antibodies) diluted (OYDV, 1:1000; LYSV,1:500;GV-B,1:1000; GV-C,1:500) in conjugate buffer were added per well and incubated at 37ºC for 4 hours. Plates were again washed four times with PBS-Tween-20 and blotted as described above. Then 100 µl substrate p-nitrophenyl phosphate (10mg p- nitrophenyl phosphate dissolved in 20ml of substrate buffer) solution was added to each microtiter plate wells.

Microtiter plates, which had the substrate added, were incubated for 1-2 hours at room temperature until clear reactions were obtained. Positive samples were visually recognized from wells that turned yellow, while healthy control remained colorless. Plates were also read with a HUMAREADER PLUS, Plate reader with a 405 nm absorbance filter. A well reading was considered positive if visually it turned yellow and its absorbency value was at least two times that of negative control wells.

4.3.2 Data Analysis

From the respective plates, yellow color developed wells having ELISA reader reading two times and more of that of negative control were recorded as positives for virus and clear wells were recorded as negatives for the tested virus. The raw data was subjected to SPSS (Statistical Package for Social Sciences) software version 15 to determine the frequency of virus detection.

5. Results

5.1 Incidence of Virus-like Symptoms in the Fields Surveyed

The virus disease symptoms observed in the field were yellow mosaic, strip, yellowing and dwarfism (stunt). The virus like symptoms became more pronounced in the leaves of adult (3 to 4 months) garlic plants than the young leaves. In most investigated sites, the viral diseases on the garlic plants have been identified (Figure 2; Figure 3). Figure 3 depicted the virus infected garlic plants, which have retarded growth, yellowing, yellow mosaic and strip on leaves. The most commonly observed symptoms were yellowing of the whole leaves, yellow stripes on leaves and mosaic.

The highest incidence of virus disease-like symptoms were recorded in Arsi and West Shoa zones both in Oromia region, with the incidence range of 58-93% and 49-89% respectively. The mean incidences of the two zones were found to be 75.25% and 72.3% respectively. East Hararge in Oromia region was in the third position with the incidence of 74%, followed by East Shoa in Oromia region, Arada Sub City in Addis Ababa and North Shoa in Amhara region, with the incidence ranges of 64-70%, 12-40%, and 21-27% respectively. The zone with the lowest incidence is Silte zone in SNNP region with incidence of 0-3%. The mean incidence of all the other zones in SNNP region ranges 3-19%. Generally, Oromia region showed the highest incidence of virus like symptom of garlic (57.1% mean incidence) followed by Amhara region (41.13% mean incidence), Addis Ababa (26% mean incidence) and SNNP (7% mean incidence) (Appendix III).

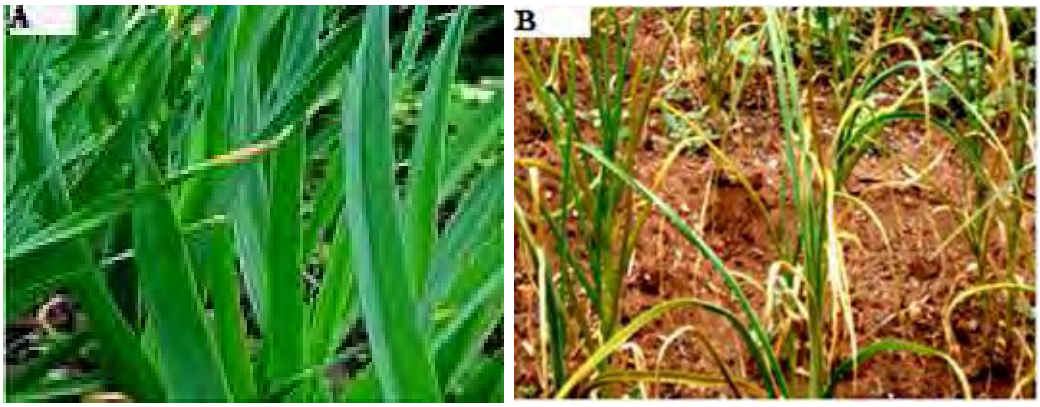

Figure 2. Healthy and symptomatic garlic plants. A) Healthy garlic leaves (Asymptomatic)
B) Symptomatic garlic leaves.

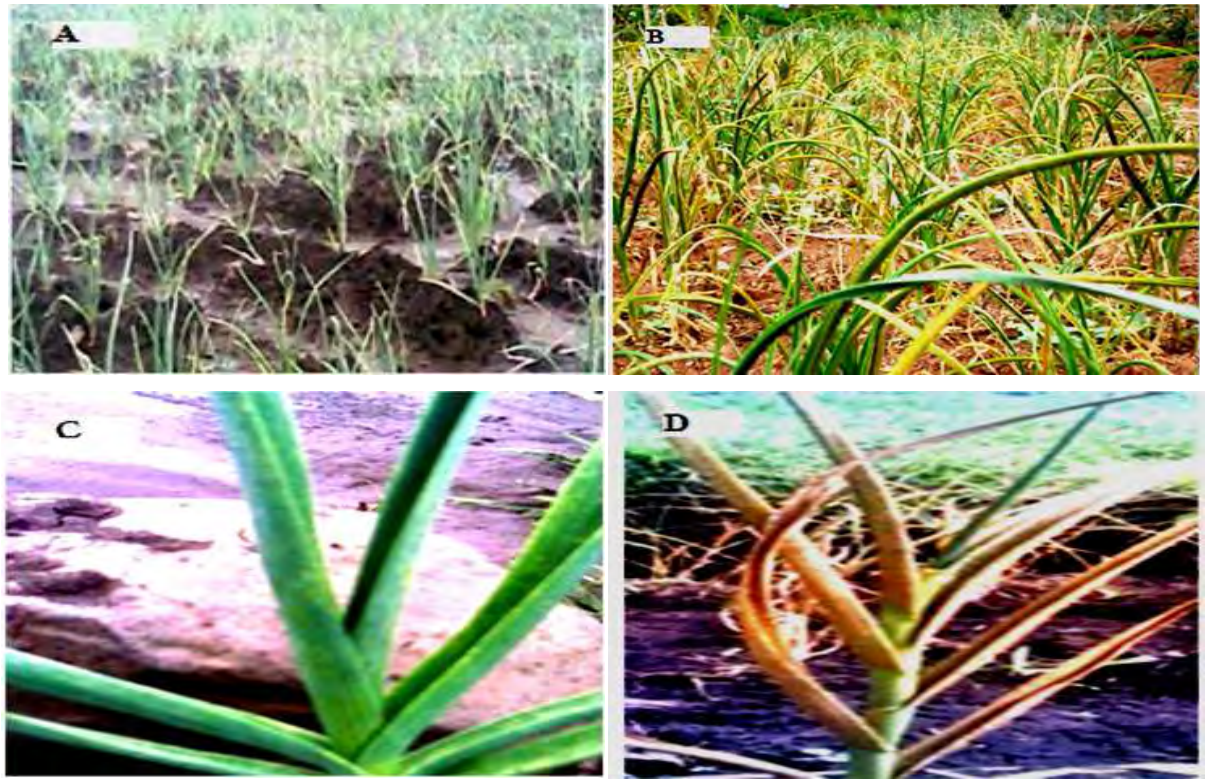

Figure 3.Virus diseasesed Garlic Plants. A) Field of garlic plant 2 months old B) Field of garlic plant 4 months old C) Leaves of symptomatic plant (2 months old) D) Leaves of symptomatic garlic plant 4 months old. The virus infection symptoms include Yellow stripes on the leaves, Stunting of the plant, mosaic and yellowing of the leaves.

5.2 Identification of garlic viruses by DAS-ELISA

The DAS-ELISA test indicated the presence of the four garlic viruses (OYDV, LYSV, GV-B and GV-C) in the leaf samples of garlic plants collected from some garlic growing regions of Ethiopia (Figure 3). The antisera reaction for GV-B was strong (deep yellow color developed when substrate added quickly) and ELISA plate's readings were high (greater than two times that of negative control) compared to other viruses. The antisera reaction of OYDV, GV- C and LYSV was weak (not deep yellow) but yellower than that of negative control (Figure 4).

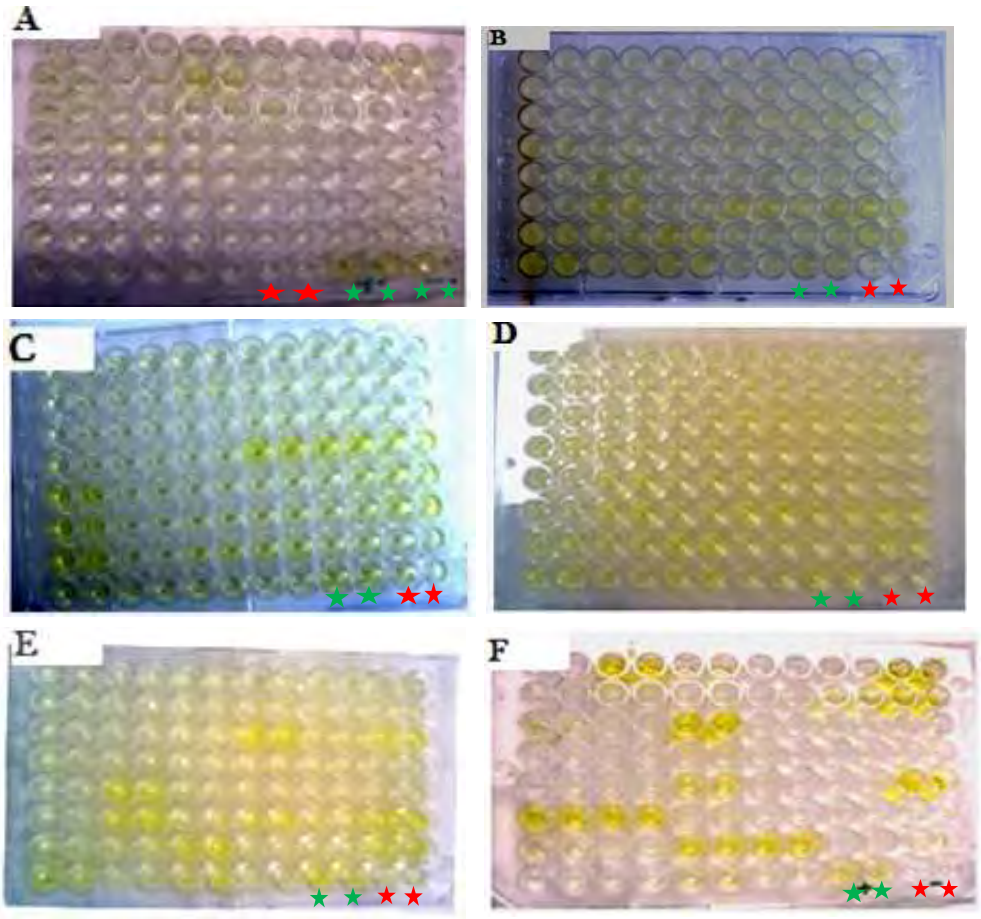

Figure 4. DAS-ELISA test results. Yellow cavities indicate the presence of virus and clear cavities indicate the absence of virus. Plates A and B: treated samples for OYDV test, Plate C: treated samples for GV-C, Plate D: treated samples for LYSV, Plates E and F: treated samples for GV-B (★ indicate positive controls and ★ indicate negative controls).

5.3 Incidence of Virus Infection

Twenty three percent (119 sample) of the 520 samples tested by DAS-ELISA for the four garlic viruses show positive result for at least one virus. Of the 220 symptomatic plant samples 30% (65 samples) reacted with antisera to one or more viruses, with the highest frequency of detection being samples obtained from East Shoa zone (70%) and the neighboring Arsi zone (48%) both in Oromia region (Table 3). Moderate incidence was observed in Arada sub city (29%), West Showa (26%) and North Showa (22%). In other zones the frequency of detection was low (East Hararge (20%). The frequency of detection of all viruses was absent in South West Showa and Jimma zones of Oromia region and all zones in SNNP region except Konta Liyu wareda (50%).

Of the 300 asymptomatic plant samples collected and assayed only 18 % (54 samples) reacted with antisera for at least one virus, with the highest frequency of detection being samples obtained from East Shoa (70%), East Hararge (60%), West Shoa (50%) and Arsi Zones (50%) all in Oromia region. Again in South West Showa zone of Oromia region and all zones in SNNP region, zero frequencies of detection were observed except Gurage Zone (13%) (Table 3).

Table 3. Proportion of symptomatic and asymptomatic garlic plant samples, which tested positive for at least one virus when assayed serologically by DAS-ELISA.

Region	**Zone**	**Symptomatic samples**		**Asymptomatic Samples**	
		Number of Plants Assayed	Positive for one or more viruses (%)	Plants Assayed	Positive for one or more viruses (%)
Oromia	Arsi	54	26(48%)	22	11 (50%)
	West Shoa	74	19 (26%)	36	18(50%)
	East Shoa	10	7(70%)	10	7(70%)
	South West Shoa	7	0%	13	0%
	Jimma	1	0%	14	2(14%)
	East Hararge	5	1(20%)	5	3(60%)
SNNP	Gurage	5	0%	15	2(13%)
	Tonta Liyu Wereda	0	0%	10	0%
	Kambata Tambaro	11	0%	26	0%
	Silte	3	0%	9	0%
	Wolayita	0	0%	5	0%
	Hadiya	0	0%	10	0%
	Konta liyu Wereda	2	1(50%)	3	0%
Amhara	North Shoa	41	9(22%)	109	8(7%)
Addis Ababa	Arada Sub city	7	2(29%)	13	3(23%)
	Total	**220**	65 (30%)	**300**	54(18%)

Virus diseases were wide spread in most of the districts surveyed with frequencies of detection ranging from 20 to70% and from 7 to 70% in the symptomatic and asymptomatic plant samples, respectively (Table3).

All the four viruses (OYDV, LYSV, GV-B and GV-C) were detected in both symptomatic and asymptomatic garlic leaf samples. The frequency of detection was higher in symptom bearing samples than in asymptomatic samples. GV-B was detected in samples from all districts surveyed except all districts in SNNP region and two districts in Oromia. A total of 58(26.4%) symptomatic and 34 (11.3%) asymptomatic samples reacted with the GV-B antibodies, making it the most frequently detected virus(Appendix I).

Generally from the four regions surveyed, the highest incidence of virus infection was observed in Oromia region (35% of symptomatic and 41% of asymptomatic samples were positive for at least one virus). Total 38% of samples from this region were reacted with antisera to one or more virus. The second highest incidence was recorded in Addis Ababa City (29% symptomatic and 23% asymptomatic samples were positive for at least one virus) with total infection incidence of 25%. Amhara region had infection incidence of 11% totally (22% symptomatic and 7% asymptomatic). The lowest infection incidence was recorded in SNNP region (5% symptomatic and 2% asymptomatic) and in total only 3% of the samples were reacted with antisera (Figure 5; Appendix II).

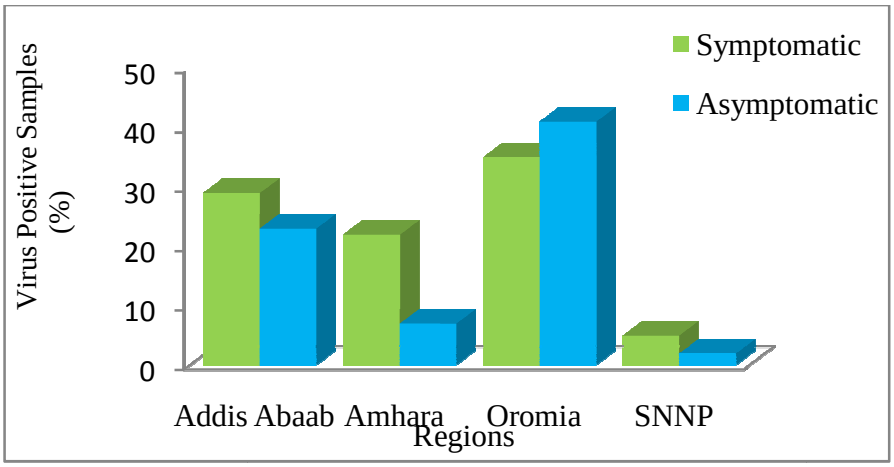

Figure 5. Virus infection incidence of Symptomatic and Asymptomatic samples from four regions of Ethiopia.

5.4 Distribution and prevalence of garlic viruses

The occurrence of the four virus species differed from locality to locality, but GV-B was prevailed in all localities. GV-B occurred very frequently in samples from Arsi, East Shoa, West Shoa and East Hararge zones of Oromia region. The frequency of occurrence of GV-B in Amhara region was low (11%) but the only virus occurred there. OYDV was highly prevalent in samples from Arsi and West Shoa zones compared to other zones. GV-C occurred in West Shoa, Arsi, East Shoa and East Hararge zones in Oromia region only, with high frequency in West Shoa zone. Generally, GV-B prevailed very frequently in all districts except districts in SNNP region. OYDV was the second frequent virus. Moreover, the two viruses were found in mixed infection in most districts. The occurrence of GV-C was low (4.8%) whereas LYSV was the least (1.3%) (Figure 6; Appendix I).

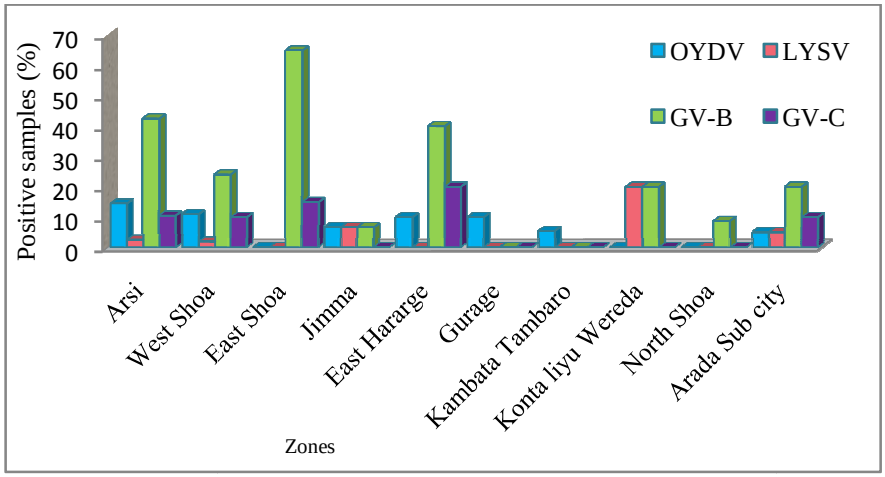

Figure 6. Prevalence of garlic viruses in 10 zones in Ethiopia. Samples of garlic were analyzed by DAS-ELISA and prevalence was calculated as percentage of positive samples per total plants tested for a given virus.

5.5 Single and multiple infections

Single infections were detected in 44 (20 %) of the symptom bearing samples and in 43 (14%) of the asymptomatic samples. About 20 samples (31 %) of the symptomatic samples that tested positive had multiple infections. Seventeen (17) samples had dual infection and only three samples had triple infections. On the contrary, only 11(20%) asymptomatic samples that tested positive had mixed infections (ten samples had dual infections and one sample had triple

infection). There was no sample with four virus infections detected in both symptomatic and asymptomatic samples (Table 4).

GV-B was the most common single infection in both symptomatic (26.4%) and asymptomatic (11.3%) samples, leading to 17.7% of the plants tested showing single GV-B infection. The second most prevalent single virus infection was OYDV (6% symptomatic and 5.3% asymptomatic samples), followed by GV-C (6.8% in symptomatic and 3.3% in asymptomatic samples). Single infection with LYSV was rare with only 1.3% of samples showing such infections (Table 4; Appendix I).

Table 4. Percentage and number (in brackets) of samples with single or mixed virus infections detected in symptomatic and asymptomatic samples collected from 15 zones of Ethiopia.

Virus/Viruses Detected	**Single and mixed infections Detected**			**Total Infection**
	Symptomatic Samples (%) (n=220)	Asymptomatic Samples (%) (n=300)	Total Samples (n=520)	Presence in Single and Mixed Infections
OYDV	2.3(5)	3(9)	2.7 (14)	5.6(29)
LYSV	0.5(1)	0(0)	0.2(1)	1.3(7)
GV-B	16.8(37)	9.3(28)	12.5(65)	17.7(92)
GV-C	0.5(1)	2(6)	1.3(7)	4.8(25)
OYDV+GV-B	2.3(5)	1.3(4)	1.7(9)	2.1(11)
OYDV+GV-C	0(0)	0.3(1)	0.2(1)	0.9(5)
GV-B+GV-C	4.5(10)	1(3)	2.5(13)	2.9(15)
OYDV +LYSV	0(0)	0(0)	0(0)	0.4(2)
LYSV+GV-B	0.9(2)	0.7(2)	0.8(4)	0.8(4)
LYSV+GV-C	0(0)	0(0)	0(0)	0.4(2)
OYDV+GV-B+GV-C	0.5(1)	0.3(1)	0.4(2)	0.4(2)
OYDV+LYSV+GVB	0(0)	0(0)	0(0)	0(0)
OYDV+LYSV+GVC	0.9(2)	0(0)	0.4(2)	0.4(2)
LYSV+GVB+GV-C	0(0)	0(0)	0(0)	0(0)
OYDV+LYSV+ GV-B+ GV-C	0(0)	0(0)	0(0)	0(0)

OYDV, onion yellow dwarf virus; LYSV, leak yellow strip virus; GV-B, garlic virus B; GV-C, garlic virus C.

Six different viral disease complexes were detected in the assayed garlic leaf samples (Figure 5). The most common detected multiple virus infection combination was a dual infection with GV-B and GV-C that was present without any other viruses in 2.5 % of all samples (Table 4; Figure 7) and was detected in samples from all zones in Oromia region except Jimma and South West Shoa zones. This dual infection was also detected in Addis Ababa but never detected in SNNP

and Amhara regions. It was noted that in 10 samples that possessed these viruses, disease symptoms were generally observed and only 3 samples containing these viruses were asymptomatic. Similarly, dual infections with OYDV + GV-B, LYSV + GV-B and OYDV + GV-C were principally detected in both symptomatic and asymptomatic plants. However, a mixed infection of OYDV + GV-C was rare, being detected in only one asymptomatic sample. Mixed dual infections of LYSV + OYDV and LYSV + GV-C were never detected in any sample (Table 4; Figure 7).

Triple infection involving OYDV+ GV-B + GV-C was observed in two samples (one symptomatic and one asymptomatic) and triple infection of OYDV+ LYSV + GVC also observed in only two samples (both were symptomatic). Generally, the highest mixed infection was observed in Oromia region followed by Addis Ababa. Mixed infections were not detected in Amhara and SNNP regions (Appendix I).

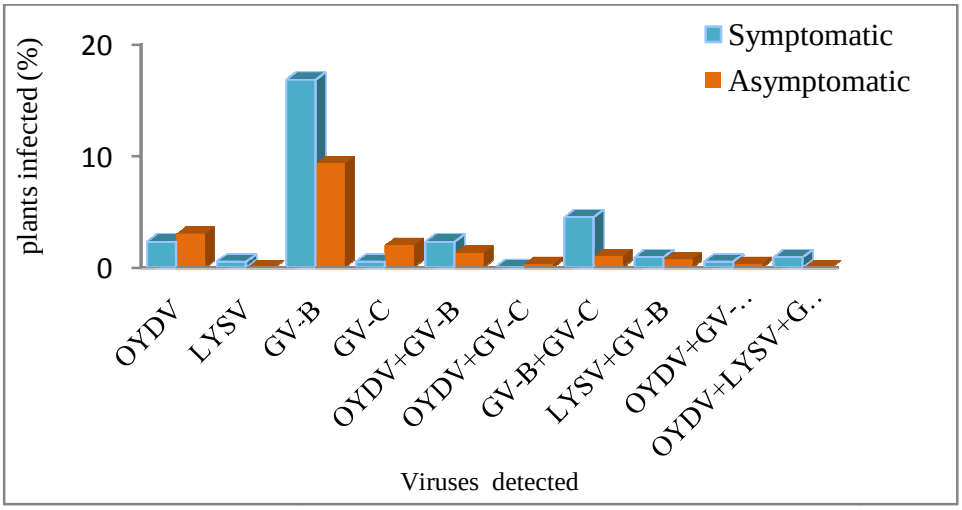

Figure 7. Proportion of single and mixed virus infections detected by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) in symptomatic and asymptomatic garlic plants in Ethiopia. (OYDV, onion yellow dwarf virus; LYSV, leak yellow strip virus; GV-B, garlic virus B; GV-C, garlic virus C).

6. Discussion

Garlic is an economically and medically important crop for several Ethiopian agricultural regions. There are reports on occurrence of garlic viruses which decreases the quality and yield of garlic plants from different countries. But in Ethiopia no any research report is yet available on garlic viruses. Therefore this work determines the incidence and identification of viruses infecting garlic plant in Ethiopia using DAS- ELISA.

In this study, various experimental activities including the survey of garlic virus diseases , studies on the incidence of virus like symptoms and virus infections of garlic , distribution and prevalence of some garlic viruses and serological identification of some garlic viruses (OYDV,LYSV,GV-B and GV-C) in 15 garlic growing zones of the country were carried out.

A survey of garlic viruses was conducted for the first time in Ethiopia. Surveys made in this study, demonstrated the presence of virus disease symptoms and high disease incidence in garlic plant fields. Almost in all inspected fields virus like symptoms were found except few fields. Garlic plants in the field displayed yellow mosaic, leaf yellow strip, leaf yellowing and plant stunting symptoms. Yellowing of the leaves and strip are the most common symptoms observed. The highest incidence of virus like symptoms were observed in the fields from Oromia region, Arsi Zone (58-93%) and West Shoa zone (49-89%). SNNP region is the region with the lowest virus like symptoms of garlic (Appendix 3). Most of the plant samples that are seropositive for the detected viruses are from Oromia region and very rare in SNNP region, which indicate that the symptoms observed are mostly due to virus infections. In symptomatology and immunological analysis it was observed that mixed virus infected garlic plants showed clear symptoms. This proved that complex infection of the viruses caused strong disease symptoms.

This is the first report of the occurrence of viruses that infect garlic in Ethiopia. Four viruses namely, OYDV, LYSV, GV-B and GV-C were detected in garlic plants collected from 54 farmer's fields in major growing areas of Ethiopia and two research fields (Debrezeit Agricultural Research Center) by DAS-ELISA. Among these identified viruses, *Allexivirus* was the most abundant, indicating that members of this virus are widespread in Ethiopia. This is in agreement with the report from Korea (Koo *et al*., 2002). Previous studies showed that, six

different garlic viruses (Gar V-A, Gar V-B, Gar V-C, Gar V-D, Gar V-E and Gar V-X have been identified as *Allexivirus* (Chen and Adams, 2001; Sumi *et al*., 1999). However, only two *Allexivirus* (GV-B and GV-C) were tested and identified in this study. The most common and widespread virus is a member of *Allexivirus*, GV-B which was detected both in single and mixed infections. The second prevalent virus was OYDV, a member of *Potyvirus*, followed by GV-C, an *Allexivirus*. LYSV which is another *Potyvirus* was very rare. This result generally indicated that *Potyvirus* (OYDV and LYSV) and *Allexivirus* (GV-B and GV-C) are viruses infecting garlic plants in Ethiopia. These viruses have previously been also detected in other countries (Barg *et al*., 1994; Koch and Salomon 1994; Marys *et al*., 1994; van Dijk *et al*., 1991). As in previous investigations, which reported that the viral diseases of garlic plants were widespread around the world (Delecolle and Lot, 1981; Fujisawa, 1989; Song *et al*., 1997; Sumi *et al*., 1993; Van Dijk, 1994; Van Dijk *et al*., 1991), form this result it can be concluded that virus diseases of garlic plants are also widespread in Ethiopia.

Garlic virus B (GV-B), an *Allexivirus* was detected at high frequency in garlic samples collected from all fields. The frequencies of disease symptoms were higher when frequencies of GV-B infection were high. Thus, GV-B, in combination with other detected and non tested garlic viruses that may occur in garlic plants is likely to be a major virus in garlic in Ethiopia and the major cause of disease symptom. But further studies are required to confirm this result.

The DAS-ELISA result for GV-B positives was strong, deep yellow color developed rapidly. This indicates that GV-B is not only the most prevalent but also highly concentrated in plant tissues. The positive results for other viruses were weak. The possible explanation for this is that, these viruses are rare (less concentrated) in the tissues of the plants.

Almost 30% (65 samples) of the 220 symptomatic plants tested positive with at least one of the virus specific antisera used, which suggests that the four viruses detected are largely responsible for the virus disease of garlic in Ethiopia. Several symptomatic samples (70%) did not react with any antisera used although the symptoms resembled those caused by viruses. It is possible that more viruses or virus like agents other than the four viruses tested in this study infect garlic in Ethiopia. The other possible explanations are the presence of phenolic compounds, latex and inhibitors in the plant tissues that adversely affect serological detection and symptoms caused by non viral factors (e.g Fungi).

Some symptomless plants (18%) also reacted positively with the antisera used, which might be due to the ability of the plant to tolerate the effects of virus infection, no observable symptoms developed. However, GV-B and GV-C, the most common combination of mixed infection was detected in 10 symptomatic samples and only 3 symptomless plant samples, confirming that these viruses are responsible for the symptoms (Lee *et al*., 2007).

In nature, simultaneous infection with two or even more viruses is not uncommon in higher plants (Hull, 2002). Similarly, garlic plants are usually infected by a mixture of viruses: *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Garlic common latent virus* (GarCLV,), *Shallot latent virus* (SLV,), and mite-borne mosaic viruses (*Allexiviruses*). In this study, of the total samples analyzed, 2.5% were doubly infected with GV-B and GV-C and there are also other double and triple multiple infections (Table 5).

The result shows that OYDV is a more common Potyvirus than LYSV in Ethiopia, which is in agreement with reports from Czech Republic (Klukackova, *et al*., 2007) and from other countries (Barg et al., 1994; Bellardi *et al*., 1995; Koch and Salomon, 1994; Sutarya, 1994), but in contrast to reports on common occurrence of LYSV in garlic, e.g from Brazil (Daniels,1999; Fajardo *et al*.,2001), Argentina (Conci *et al*.,2002), Greece (Dovas et al., 2001) and Japan (Takaichi *et al*., 1998). GV-B is more common *Allexivirus* than GV-C. But in general, *Allexivirus* are more common than *Potyvirus* in Ethiopia. To the best of our knowledge this is the first report of DAS-ELISA based detection of garlic viruses in Ethiopia.

Ethiopian farmers traditionally produce their own garlic propagative material. This fact accounts for the observed viral infection and implies reduction in yield and quality of the crop. To face this problem, a strategy is currently under development at Ethiopian Institute of Agricultural Research (EIAR). Meristem tissue culture of garlic for virus cleaning is at initial stage (Adane Abraham, 2009).

The results from this study also indicate that, this detection method (DAS-ELISA) can be successfully used to monitor a high quality program of virus free garlic production associated with an efficient program of virus eradication from garlic by tissue culture (Torres *et al*., 2000).

Generally the results from this study confidently reveal the occurrence of two *Potyvirus* (OYDV and LYSV) and two *Allexivirus* (GV-B and GV-C) in Ethiopia for the first time. As in previous investigations, which reported that the viral diseases of garlic plant were wide spread around the world (Song *et al*., 1998; Van Dijk, 1994), virus diseases in garlic plants are also wide spread in Ethiopia.

7. Conclusion

- Accurate identification and early detection of the viral diseases is the corner stone of the management of garlic virus diseases. Garlic viruses are difficult to identify using morphological criteria, which can be time consuming and challenging and requires extensive knowledge in taxonomy. Serological detection such as DAS- ELISA and molecular methods are best to detect the various viruses infecting garlic.

- This study presents the occurrence of virus diseases and virus like symptoms of garlic plants in the fields surveyed and the occurrence of four viruses infecting garlic plants in Ethiopia identified by DAS-ELISA which represents an important step for the establishment of virus free garlic seed program in the country.

- The most common symptoms observed are yellow mosaic, strip, and stunting of the plants. The occurrence of four garlic viruses (OYDV, LYSV, GV-B and GV-C) in Ethiopia is established. The most frequently occurred virus is GV-B followed by OYDV. This indicates that both *Potyvirus* and *Allexivirus* are common in Ethiopia.

- There are also mixed infections of different garlic viruses identified in this study. Dual infection of GV-B +GV-C (2.5%), both *Allexivirus*, is the most common mixed infection followed by OYDV+GV-B (1.7%). Other dual infections are also detected but the frequency of detection is low. Triple infections of OYDV+ GV-B + GV-C and OYDV+ LYSV + GVC are also detected in two samples each. No mixed infections of the four viruses occur in this study.

- The region with the highest both in virus like symptoms and virus infection is Oromia region in which all the single and mixed infections are detected (38% of all samples are positive for at least one virus), followed by Addis Ababa city (25%) and Amhara region (11%). In Amhara region only one garlic virus (GV-B) is detected. South Nations and Nationalities region is the region with lowest virus like symptoms and virus infection.

8. Recommendations

As this work, the first report of the occurrence of garlic viruses in Ethiopia is an important step in the identification of garlic viruses and production of virus free garlic plants, a lot has to be done in the future. Some of the works waiting and worth mentioning include:

- The occurrence of viruses infecting garlic plants in Ethiopia is confirmed. These results present a good starting point for garlic virus diseases diagnosis in Ethiopia and production of virus free garlic plants. Thus, training of extension workers in viral diseases diagnosis would be much helpful for farmers to select seeds. The loss of quality and yield caused by garlic viruses should also be studied in order to know the effect of viruses on garlic production which will help to take possible measures to control the diseases.

- The presence of garlic viruses in garlic plants produced in research centers and released for farmers is also confirmed. Research centers should first test the presence of viruses on garlic seeds before release to farmers. Attempts should also be made to produce virus free garlic plants using either by conventional breeding or by tissue culture techniques such as meristem culture combined with thermotherapy or chemotherapy and/or development of virus resistance varieties.

- Garlic virus B (GV-B) followed by onion Yellow Dwarf Virus (OYDV), *Allexivirus* and *Potyvirus* respectively, were found to be the major viruses detected in garlic plants in Ethiopia. Thus, when planning management strategies for virus problems of garlic in Ethiopia, attention should be paid to *Allexiviruses* and *Potyviruses*, more specifically to GV-B and OYDV. Research centers and/or other concerned bodies should also carryout studies on the identification of other viruses infecting garlic in Ethiopia not included in this study.

- This study results show that viruses that infect garlic plants are more common in some areas than others. Then further studies are required to know whether this is due to garlic variety differences, geographic differences or the presence or absence of vectors in an area.

- This study is not extensive in terms of area coverage, distribution of viruses among different geographical areas, distribution and biology of vectors and the survey and detection of viruses in related plants like onion. Hence, detection and identification of garlic virus diseases by assessing all the varieties throughout the country should be carried out to know the significant effect of each virus and its distribution.

- More specific and sensitive molecular method of virus detection such as Reverse Transcription Polymerase Chain Reaction (RT-PCR) is also required, since serology is not a very good tool for differentiation among some viruses.

- Further verification of the importance of virus strains on garlic production in Ethiopia in particular and in Africa in general is necessary.

- This study results provide a firm basis for future research on garlic viruses in Ethiopia. The complex farming system and the multiplicity of viruses identified on garlic, imply possible involvement of vectors. Hence, the need for more research geared toward understanding the role of aphids and mites as virus vectors of *Potyvirus* and *Allexivirus* respectively, and control measures, which would provide smallholder farmers with appropriate garlic virus management tools.

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Appendix I. Proportion of asymptomatic (A) and symptomatic (S) Garlic plant samples from 16 zones in 4 regions of Ethiopia reacting positive for different garlic viruses. (OYDV, onion yellow dwarf virus; LYSV, leak yellow strip virus; GV-B, garlic virus B; GV-C garlic virus C).

Region	**Zone**	**District**	**No of Samples assayed**		**Viruses Detected**							
					OYDV		**LYSV**		**GV-B**		**GV-C**	
			S	A	S	A	S	A	S	A	S	A
Oromia	Arsi	Bele Gasgar	5	3	1	0	0	0	4	1	0	0
		Robe	11	7	4	2	1	0	6	3	1	2
		Ticho	8	2	1	2	1	0	4	2	0	0
		Lode Hetosa	30	10	0	1	0	0	8	4	5	0
	West Shoa	Walmera	43	17	4	3	1	0	9	3	3	2
		Burayu	4	6	1	0	0	0	0	2	0	1
		Ambo	22	8	0	3	0	0	3	1	0	2
		Ejere	5	5	0	0	0	1	3	3	1	1
	East Shoa	Bushoftu	10	10	0	0	0	0	7	6	2	1
	Jimma	Gido	1	9	0	1	0	0	0	0	0	0
		Dilbi	0	5	0	0	0	1	0	1	0	0
	South West Shoa	Tere Sodo	7	13	0	0	0	0	0	0	0	0
	East Hararge	Diretiyara	5	5	0	1	0	0	1	3	1	1
SNNP	Gurage	Abashag	5	15	0	2	0	0	0	0	0	0
	Tonta Liyu wareda	Tonta Liyu	0	10	0	0	0	0	0	0	0	0
	Kambata Tambaro	Doyogana	11	26	1	1	0	0	0	0	0	0
	Silte	Silti	3	7	0	0	0	0	0	0	0	0
		Yubarak	0	2	0	0	0	0	0	0	0	0
	Walita	Kindokosha	0	5	0	0	0	0	0	0	0	0
	Hadiya	Semen Beles	0	10	0	0	0	0	0	0	0	0
	Konta Liyu Wareda	Konta Liyu	2	3	0	0	1	0	1	0	0	0
AA	Arada sub city	Arada	7	13	1	0	1	0	4	0	2	0
Amhara	North Shoa	Basuna Warana	41	109	0	0	0	0	8	5	0	0
Total			**220**	**300**	13 (6%)	16 (5.3%	5 (2.3 %)	2 (0.7 %)	58 (26%)	34 (11.3%)	15 (6.8%)	10 (3.3%)
			520		29 (5.6%)		7 (1.3%)		92 (17.7%)		25 (4.8%)	

Appendix II. Regional Virus Infection Incidence (Symptomatic and Asymptomatic samples) in Ethiopia.

Region	Symptomatic samples		Asymptomatic Samples		Total Samples	
	No of sample Assayed	No and %(in bracket) of positive samples	No of sample Assayed	No and %(in bracket) of positive samples	No of sample Assayed	No and %(in bracket) of positive samples
Addis Ababa	7	2(29%)	13	3(23%)	20	5(25%)
Amhara	41	9(22%)	109	8(7%)	150	17(11%)
Oromia	151	53(35%)	100	41(41%)	251	96(38%)
SNNP	21	1(5%)	78	2(2%)	99	3(3%)

Appendix III. Samples of Garlic plant (leaves) Collected from Different Garlic Growing Regions of Ethiopia for Serological Detection of Garlic Viruses (GV-B, GV-C, OYDV and LYSV), sample type, DAS-ELISA results and the incidence of virus like symptoms. Samples positive for at least one virus in DAS-ELISA are listed. (O, onion yellow dwarf virus; L, leak yellow stripe virus; B, Garlic virus B; C, Garlic virus C).

Field NO	**No**	**Sample Code**	**Region 4=Oromia 3=Amhara AA= Addis Ababa SNNP= South Nations**	**Zone**	**Wareda (District)**	**Kebele**	**Date Collected**	**Age of Garlic plant in months**	**Viruses Tested for Their Presence (+ Present - Absent) ++Strong Positives +- Weak Positives**				**Sample type X Asymptomatic ✓ Symptomatic**	**Virus like symptoms incidences**
									O	L	B	C		
1	1.	Bele-1	4	Arsi	Bele Gasgar	Teke	30-10-09	3-4	-	-	+	-	✓	
	2.	Bele-2							+	-	+	-	✓	
	3.	Bele-4							-	-	+	-	✓	
	4.	Bele-5							-	-	+	-	X	
	5.	Bele-6							-	-	++	-	✓	70%
2	6.	Chafe-1	4	Arsi	Robe	Chafe	30-10-09	3-4	-	-	+	-	X	
	7.	Chafe-4							-	-	+	+-	X	
	8.	Chafe-5							-	-	+	+-	X	67%
	9.	Chafe-7							-	+-	-	-	✓	
	10.	Chafe-8							+-	-	-	-	✓	
3	11.	Ahma-1	4	Arsi	Ticho	Ahmaraba	30-10-09	3-4	+-	-	+	-	X	
	12.	Ahma-2							+-	-	+	-	X	
	13.	Ahma-3							-	-	+	-	✓	
	14.	Ahma-4							-	-	++	-	✓	
	15.	Ahma-5							-	+-	++	-	✓	82%
	16.	Ahma-6							+-	-	-	-	✓	
	17.	Ahma-10							-	-	+	-	✓	
4	18.	Qiltu-2	4	Arsi	Lode Hetosa	Qiltu bal'a	31-10-09	3-4	-	-	+	-	X	93%
5	19.	Jimata-1	4	Arsi	Lode Hetosa	Jimata Lode	1-11-09	3	-	-	+	+-	✓	
	20.	Jimata-2							-	-	+	+-	✓	
	21.	Jimata-3							-	-	+	-	X	
	22.	Jimata-5							+	-	+	-	X	86%
	23.	Jimata-7							-	-	+	+-	✓	

	24.	Jimata-9							-	-	-	+-	✓	
6	25.	Gudelch-1	4	Arsi	Lode Hetosa	Gudelcha	1-11-09	3-4	-	-	++	+-	✓	
	26.	Gudelch-2							-	-	++	-	✓	
	27.	Gudelch-3							-	-	++	-	✓	77%
	28.	Gudelch-4							-	-	+	-	X	
	29.	Gudelch-8							-	-	+	-	✓	
7	30.	Robe-1	4	Arsi	Robe	Robe02	30-10-09	3-4	+-	-	++	-	✓	
	31.	Robe-2							+-	-	++	-	✓	
	32.	Robe-3							-	-	++	-	✓	
	33.	Robe-4							+-	-	++	-	✓	
	34.	Robe-5							+-	-	-	-	X	
	35.	Robe-7							+-	-	+		✓	69%
	36.	Robe-8							-	-	+	+-	✓	
8	37.	Arb-3	4	Arsi	Lode Hetosa	Arbgebeya	1-11-09	3-4	-	-	+	-	✓	
9	38.	AA-1	AA	Arada subsity	Arat killo	Science faculty	5-11-09	3	-	-	+	+-	✓	
	39.	AA-4							+	+-	-	+-	✓	12%
10	40.	Menagasha-1	4	W.Shoa	Walmera	Menagasha	5-11-09	3	+	-	-	+-	X	
	41.	Menagasha-2							+	-	-	-	X	56%
	42.	Menagasha-4							+	-	-	-	✓	
11	43.	Gafarsa-1	4	W.Shoa	Burayu	Malka Gafarsa	5-11-09	3	-	-	++	-	X	
	44.	Gafarsa-4							-	-	++	-	X	65%
	45.	Gafarsa-5							-	-	-	+-	X	
	46.	Gafarsa-9							+-	-	-	-	✓	
12	47.	HARC-2	4	W.Shoa	Walmera	Holeta Agri. Res.Center	6-11-09	3	-	-	++	-	✓	
	48.	HARC-3							+	-		-	✓	
	49.	HARC-4							+	-	++	+-	✓	88%
	50.	HARC-5							+	+		+	✓	

										-		-		
	51.	HARC-6							+-	-	-	-	X	
13	52.	AARC-1	4	W.Shoa	Ambo	Ambo. Agri.Res.Center	9-11-09	1	-	-	-	+-	X	
	53.	AARC-2							+-	-	-	-	X	
	54.	AARC-5							-	-	+	-	✓	63%
14	55.	AAlem-1	4	W.Shoa	Ejere	Addis Alem	9-11-09	3	-	-	++	+-	✓	
	56.	AAlem-2							-	-	++	-	✓	
	57.	AAlem-5							-	+-	++	-	X	89%
	58.	AAlem-6							-	-	+	-	X	
	59.	AAlem-7							-	-	-	+-	X	
	60.	AAlem-8							-	-	+	-	X	
	61.	AAlem-9							-	-	+	-	✓	
15	62.	Qurbo-5	4	W.Shoa	Ambo	BayoQurbo	9-11-09	3	-	-	++	-	✓	83%
16	63.	Ginchi-2	4	W.Shoa	Ambo	Ginchi	9-11-09	3	+-	-	++	+-	X	55%
	64.	Ginchi-6							-	-	+	-	✓	
17	65.	WW14-2	4	E.Shoa	Bushoftu	Debrezeit.Agr.Res.Center	23-11-09	3	-	-	+	-	X	
	66.	WW14-3							-	-	-	+-	X	
	67.	WW14-4							-	-	+	-	X	64%
	68.	WW14-6							-	-	++	-	✓	
	69.	WW14-7							-	-	++	-	✓	
	70.	WW14-9							-	-	++	+-	✓	
18	71.	G493-A	4	E.Shoa	Bushoftu	Debrezeit.Agr.Res.Center	23-11-09	3	-	-	-	+-	✓	
	72.	G493-B							-	-	+	-	X	
	73.	G493-C							-	-	+	-	X	
	74.	G493-E							-	-	++	-	✓	
	75.	G493-F							-	-	++	-	✓	
	76.	G493-G							-	-	++	-	✓	
	77.	G493-H							-	-	++	-	✓	70%
	78.	G493-I							-	-	++	-	X	
	79.	G493-J							-	-	++	-	X	
19	80.	Tawla-A1	SNNP	Gurage	Abashag	Tawla	24-11-09	3	+	-	-	-	X	12%
	81.	Tawla-A4							+	-	-	-	X	
21	82.	Matanso-4							-	-	-	-	X	11%
	83.	Matanso-	4	Jimma	Gido	Matans	24-11-	3-4	+	-	-	-	X	

		5				o	09						
23	84.	Amoch-5	Amoch-1	SNNP	Kambata tambaro	Doyogana	Amoch omoto	25-11-09	+	-	-	-	X	6%
	85.	Amoch-7							+	-	-	-	✓	
30	86.	Gudina-A2	4	W.Shoa	Wlmera	Madagudina	3-12-09	3-4	-	-	++		X	49%
	87.													
	88.	Gudina-A3							-	-	++	+-	✓	
	89.	Gudina-A4							-	-	++	-	✓	
	90.	Gudina-A5							-	-	+	-	✓	
31	91.	Gudina-B1	4	W.Shoa	Wlmera	Madagudina	3-12-09	3-4	-	-	+	-	✓	
	92.	Gudina-B6							-	-	+	-	✓	56%
	93.	Gudina-B7							-	-	+	-	✓	
	94.	Gudina-B9							-	-	+	-	X	
	95.	Gudina-B10							-	-	+	-	X	
33	96.	Sadamo-2	4	W.Shoa	Wlmera	Sadamo	8-12-09	3-4	-	-	-	+-	X	59%
	97.	Sadamo-7							-	-	+	-	✓	
34	98.	Kelemw-1	AA	AA	Arada subcity	Kelemework School	8-12-09	3	-	-	+	-	X	
	99.	Kelemw-2							-	-	+	-	X	40%
	100.	Kelemw-5							-	-	+	-	X	
35	101.	DBirhan-2	3	N.Shoa	Basunawarana	Debre Birhan city	19-12-09	2	-	-	+	-	✓	33%
36	102.	Baqelo-A2	3	N.Shoa	Basunawarana	Baqelo	19-12-09	2	-	-	+	-	X	48%
38	103.	Baqelo-C1	3	N.Shoa	Basunawarana	Baqelo	19-12-09	2	-	-	+	-	✓	
	104.	Baqelo-C2							-	-	+	-	✓	62%
	105.	Baqelo-C9							-	-	+	-	✓	
	106.	Baqelo-C10							-	-	+	-	✓	
39	107.	Baqelo-D8	3	N.Shoa	Basunawarana	Baqelo	19-12-09	2	-	-	+	-	✓	51%
40	108.	Qayito-A1	3	N.shoa	Basunawarana	Qayito	20-12-09	2	-	-	+	-	✓	
	109.	Qayito-							-	-	+	-	✓	

		A2											44%
	110.	Qayito-A7							-	-	+	-	X	
42	111.	Qayito-C2	3	N.shoa	Basunawarana	Qayito	20-12-09	2	-	-	+	-	X	61%
	112.	Qayito-C8							-	-	+		X	
43	113.	Dinbaro-A8	3	N.shoa	Basunawarana	Dinbaro	20-12-09	2	-	-	+	-	X	27%
50	114.	Awlimay-1	4	E.Hararge	Diretiyara	Awlimay	15-1-2010	3	+	-	+	-	X	
	115.	Awlimay-3							-	-	+	+	X	74%
	116.	Awlimay-5							-	-	+	-	X	
	117.	Awlimay-7							-	-	+	+	✓	
51	118.	Dido-3	4	Jimma	Dilbi	Dido	25-12-09	3	-	+	+	-	X	0%
52	119.	Senga-3	SNNP	Kontaliyu Warda	Kontaliyu Warda	Tsenga	25-12-09	3	-	+	+	-	✓	0%

DECLERATION

I, the undersigned, declare that this thesis entitled “Survey and Serological Identification of Viruses Infecting Garlic (*Allium sativum* L) in Ethiopia” comprises my original research work under the guidance of my advisor Tileye Feyissa (PhD). It has never been submitted elsewhere as thesis or as any other examination paper and that all sources of materials used for the thesis have been fully acknowledged.

Name: Kero Jemal

Signature: _____

Date: _____

This Thesis is submitted under my supervision.

Tileye Feyissa

Signature_____

Date_____