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***Full Length Research Paper***

# The Occurrence and Distribution of Four Viruses on Garlic (*Allium sativum* L.) in Ethiopia

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***Abstract***

*Garlic (Allium sativum L.), an important bulb vegetable grown worldwide including Ethiopia, is infected by numerous viruses causing significant yield losses. Since there is no information available on garlic virus diseases in Ethiopia, a field survey and serological tests were conducted in the year 2009 to identify viruses infecting garlic in the major growing regions. The Survey 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 were tested by the double antibody sandwich enzyme linked immunosorbent assay for four common garlic viruses namely Onion yellow dwarf virus (OYDV) and Leek yellow stripe virus (LYSV) (Genus Potyvirus family Potyviridae) and Garlic virus B (GarV-B) and Garlic virus C (GarV-C) (genus Allexivirus, family, Flexiviridae), 119 samples (23%) were found to be infected with at least one virus. GV-B was the most frequent (17.7%), followed by OYDV (5.6%) and GarV-C (4.8%). LYSV was detected only in seven samples. Mixed infections were also very common. In this paper, information on the occurrence, identity and distribution of garlic viruses in Ethiopia is reported for the first time.*

***Key words:*** *Allium* sativum*, ELISA, Garlic virus B, Garlic virus C, Leek yellow stripe virus, Onion yellow dwarf virus.*

## Introduction

Garlic (*Allium sativum* L.) is one of the most important *Allium* plants widely cultivated throughout the world including Ethiopia for use as spice, food flavoring agent and medicinal value. Worldwide, viral diseases are among the major causes of low yield and quality in garlic (Fujisawa, 1998; Takaichi, 1998; Walkey and Antill, 1989). This is mainly due to the fact that the viruses accumulate in bulbs because of the vegetative propagation of the crop. To control these viruses effectively, it is necessary to establish their identity and determine their incidence and prevalence in a given area. Several filamentous viruses have been detected in garlic, often in complex mixtures. These include two members each of the genera *Potyvirus* (*Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV)) and *Carlavirus* (*Garlic common latent virus* (GarCLV) and *Shallot latent virus* (SLV)) all of which are aphid transmitted (Fajardo et al. 2001, Takaichi et al. 1998). In addition, several species belonging to the genus *Allexivirus* in family *Flexiviridae* which are thought to be mite transmitted including *Garlic virus* A (GarV-A) , -B (GarV-B), -C (GarV-C), and –D (GarV-D), -E (GarV-E) and –X ( GarV-X), all of which have been abundantly detected in garlic plants (Chen et al. 2004; Lee et al. 2007; Park *et al.*, 2005; Dovas and Vovlas, 2003).

Although no systematic study has been conducted on garlic viruses in Ethiopia, circumstantial evidence suggested that virus-like symptoms have been commonly observed in garlic. As a result, some attempts are being made to obtain virus-free planting materials of garlic by meristem culture by local researchers (Abraham, 2009). However, the complete lack of information on the identity, relative importance and distribution of viruses in garlic has become an obstacle in such efforts. Therefore, the aims of this study are to survey the status of garlic virus diseases in the major growing areas of Ethiopia and identify the associated viruses.

## Materials and Methods

### *Field survey and sample collection*

Field survey for garlic virus disease was conducted in major garlic growing areas of Ethiopia; 13 districts in Oromia region, eight districts in Southern Nations and Nationalities People region, one district in Amhara region and one sub-city in Addis Ababa city. 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 by stopping at an interval of at least 5km whenever the crop is encountered. A total of 54 farmer's fields and two research fields (Debre Zeit 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 crops studied ranges from one to four months. Plants were visually inspected for typical symptoms of virus infection such as yellow mosaic, stripe, and whole leaf yellowing or stunt. 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.

To determine the types of viruses present, symptomatic garlic leaf samples and random asymptomatic leaf samples were collected from farmer's fields and research centers and labeled with place of origin. In total, 220 symptomatic and 300 asymptomatic garlic leaf samples were collected from the farmers' fields. Large numbers of samples (up to 10) were collected from large fields and small numbers of samples were collected from small fields. Leaf samples were cut and put in plastic bags or vials containing silica gel or $CaCl_2$ and stored at room temperature until used.

***Serological identification of garlic viruses***

In order to confirm that the symptoms observed on garlic were indeed due to viruses, virus-specific serological tests were conducted. The double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) was used for the identification of four viruses commonly infecting garlic and for which antisera were available. Both the symptomatic and asymptomatic garlic leaf samples collected were tested for the presence of the four viruses. These were OYDV, LYSV, *GarV-B* and *GarV-C*. The polyclonal IgG and their enzyme conjugated used in this test were obtained by purchasing from the German Collection of Microorganisms and Cell Culture, Germany. Plates were visually observed after appropriate incubation period followed also reading with a HUMAREADER PLUS Plate reader with a 405 nm absorbance filter. An absorbance reading was considered positive if visually it turned yellow and its absorbency value was at least two times that of negative control wells. The laboratory tests were done in Biotechnology Laboratory of the Holetta Agricultural Research Center of the Ethiopian Institute of Agricultural Research, Ethiopia.

## Results

***Incidence of virus-like symptoms in the fields surveyed***

The virus disease symptoms most commonly observed were yellowing of the whole leaves, yellow stripes on leaves and mosaic. The symptoms were more pronounced in the leaves of adult (3 to 4 months) garlic plants than the young leaves. The highest incidence of virus disease-like symptoms were recorded in the fields from Arsi and West Shoa zones both in Oromia region, with the incidence range of 58-93% and 49-89% respectively (Table. 1). 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 of 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 Southern Nations and Nationalities People region with incidence of 0-3%. The mean incidence of all the other zones in Southern Nations and Nationalities People 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 Southern Nations and Nationalities People region (7% mean incidence).

**Table 1.** Estimated Incidence of garlic virus diseases in various regions in the country.

| Region/Zone | No. of field | No. of samples | Highest incidence observed | No of fields with incidence (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | <1 | 1-5 | 5-20 | 21-50 | >50% |
| **Arsi** | 8 | 80 | 93% | 0 | 0 | 0 | 0 | 8 |
| **West Shoa** | 11 | 110 | 89% | 0 | 0 | 0 | 1 | 10 |
| **East Shoa** | 2 | 20 | 70% | 0 | 0 | 0 | 0 | 2 |
| **North Shoa** | 15 | 150 | 27% | 0 | 0 | 0 | 4 | 11 |
| **South West Shoa** | 2 | 20 | 30% | 0 | 1 | 0 | 1 | 0 |
| **Jimma** | 2 | 10 | 11% | 1 | 0 | 1 | 0 | 0 |
| **East Hararge** | 2 | 10 | 74% | 0 | 0 | 0 | 0 | 2 |
| **Gurage** | 2 | 20 | 12% | 1 | 1 | 0 | 0 | 0 |
| **Kembata-Tembaro** | 4 | 38 | 6% | 2 | 2 | 0 | 0 | 0 |
| **Konta Liyu Wereda** | 2 | 15 | 6% | 0 | 0 | 2 | 0 | 0 |
| **Silte** | 2 | 12 | 3% | 1 | 1 | 0 | 0 | 0 |
| **Walyta** | 1 | 5 | 4% | 0 | 1 | 0 | 0 | 0 |
| **Hadiya** | 1 | 10 | 3% | 0 | 1 | 0 | 0 | 0 |
| **Addis Ababa** | 2 | 20 | 40% | 0 | 0 | 1 | 1 | 0 |
| **Total** | 56 | 520 | 93% | 5 | 7 | 4 | 7 | 33 |

***Identification of garlic viruses by DAS-ELISA***

The DAS-ELISA test indicated the presence of the four garlic viruses (OYDV, LYSV, *GarV*-B and *GarV*-C) in the leaf samples of garlic plants collected from some garlic growing regions of Ethiopia (Table 2 , Fig. 1). Twenty three percent (119 samples) of the total 520 samples (both symptomatic and asymptomatic) 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 at least one 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. 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%). All viruses were absent in samples from South West Shoa and Jimma zones of

Oromia region and all zones in SNNP region except Konta Liyu Woreda (13%). 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%).

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

**Table 2**. 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; GarV-B, garlic virus B; GarV-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 |
| | 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 | 13 | 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%) | |

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 1).

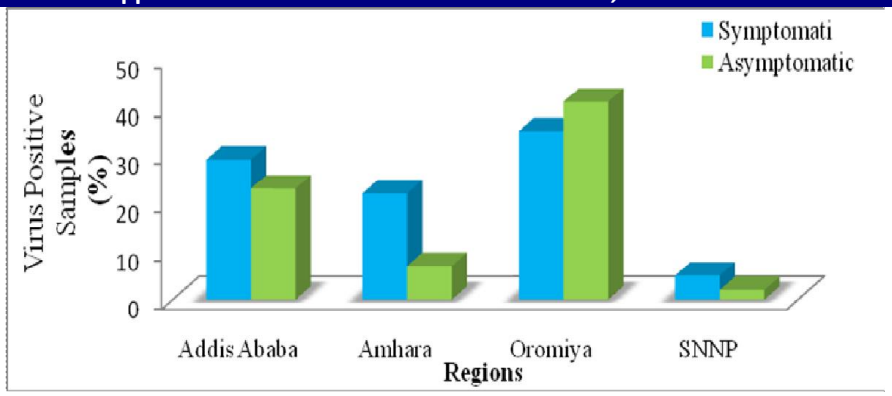


**Figure 1.** Virus infection incidence of Symptomatic and Asymptomatic samples from four regions of Ethiopia.

***Distribution and prevalence of garlic viruses***

As indicated in Table 3, the occurrence of the four virus species differed from locality to locality, but GarV-B was prevalent in all localities. GarV-B occurred very frequently in samples from Arsi, East Shoa, West Shoa and East Hararge zones of Oromia region. The frequency of occurrence of GarV-B in Amhara region was low (11%) but it was the only virus occurred there. OYDV which was the second most frequent virus was highly prevalent in samples from Arsi and West Shoa zones compared to other zones. Moreover, GarV-B and GarV-C were found in mixed infection in most districts. GarV-C occurred in West Shoa, Arsi, East Shoa and East Hararge zones in Oromia region only, with high frequency in West Shoa zone. Generally, GarV-B occurred very frequently in all districts except districts in SNNP region. The occurrence of GarV-C was low (4.8%) whereas LYSV was the least (1.3%) (Table 2).

**Table 3.** The percentage of fields having a given virus in a given zones

| Zones | OYDV | LYSV | GarV-B | GarV-C |
|---|---|---|---|---|
| **Arsi** | 62.5 | 25 | 100 | 25 |
| **West Shoa** | 45.5 | 18 | 82 | 73 |
| **East Shoa** | 0 | 0 | 50 | 50 |
| **Jimma** | 50 | 50 | 50 | 0 |
| **East Hararge** | 50 | 0 | 100 | 50 |
| **Gurage** | 50 | 0 | 0 | 0 |
| **Kambata Tambaro** | 25 | 0 | 0 | 0 |
| **Konta Liyu Wereda** | 0 | 100 | 100 | 0 |
| **North Shoa** | 0 | 0 | 100 | 0 |
| **Arada Sub city** | 0 | 0 | 100 | 50 |

***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. 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).

GarV-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 GarV-B infection. The second most prevalent single virus infection was OYDV (6% symptomatic and 5.3% asymptomatic samples), followed by GarV-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).

**Table 4.** Percentage and number (in brackets) of samples with single or mixed virus infections detected in symptomatic and asymptomatic samples collected from 16 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+GVC** | 0.9(2) | 0(0) | 0.4(2) | 0.4(2) |

*OYDV; onion yellow dwarf virus; LYSV, Leek yellow strip virus; GarV-B, garlic virus B; GarV-C, garlic virus C.*

Six different viral disease complexes were detected in the assayed garlic leaf samples (Figure 2). The most common detected multiple virus infection combination was a dual infection with GV-B and GarV-C that was present without any other viruses in 2.5 % of all samples (Table 4;Fig. 2) 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 SNNPR 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 + GarV-B, LYSV + GarV-B and OYDV + GarV-C were principally detected in both symptomatic and asymptomatic plants. However, a mixed infection of OYDV + GarV-C was rare, being detected in only one asymptomatic sample. Mixed dual infections of LYSV + OYDV and LYSV + GarV-C were never detected in any sample (Table 4; Figure 2).

Triple infection involving OYDV+ GarV-B + GarV-C was observed in two samples (one symptomatic and one asymptomatic) and triple infection of OYDV+ LYSV + GarVC 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.

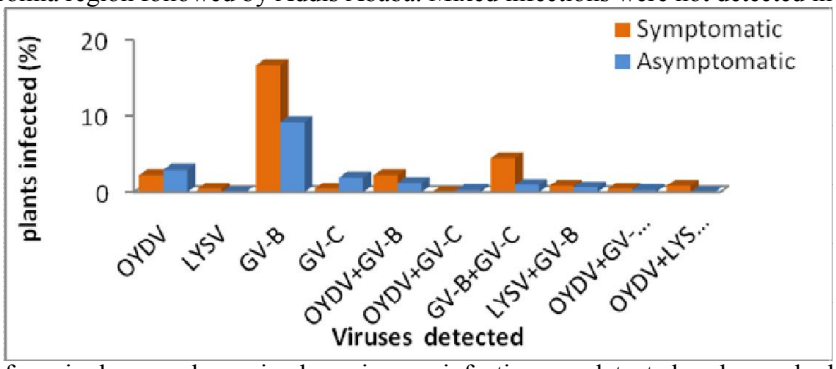


**Figure2.** Proportion of single and mixed virus infections detected by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) in symptomatic and asymptomatic garlic plants in Ethiopia. (OYDV, onion yellow dwarf virus; LYSV, leak yellow strip virus; GarV-B, garlic virus B; GarV-C, garlic virus C).

## Discussion

In this work, unprecedented attempt was made to understand the identity and distribution of viruses infecting garlic in Ethiopia. Hence, we for the first time report the presence of high virus disease incidence in garlic fields and the occurrence of four viruses which in many cases exist in mixed infection. Yellow mosaic, stripe, yellowing and stunting were the most commonly observed symptoms with incidence varying from high to low in various parts of the country. Among the identified viruses, *GarV-B* was the most common and widespread virus. Of about six different garlic allexiviruses known to infect garlic worldwide, two (*GarV-B* and *GarV-C*) were tested and identified in this study. On the other hand, OYDV and LYSV, which are the most important poyviruses affecting garlic in many countries in terms of yield loss (Barg et al. 1994, Takaichi et al. 1998) are encountered at lower frequency.
Sixty five samples (almost 30%) 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 however did not react with any antisera used although the symptoms resembled those caused by viruses. It is possible that viruses other than the four viruses detected in this study infect garlic samples. In particular, some of the allexiviruses not tested in this study due to lack of specific antibodies are very likely to occur since they are transmitted by the same species of eriophyid mites which are believed to be the natural vectors of GarV-B and -C. Alternatively, the symptoms may also be caused by non-viral factors (e.g. fungi). Conversely, 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 or early infection. In future works therefore, the use of more set of virus-specific antibodies as well as more sensitive molecular are believed to further reveal the diversity of viruses infecting Ethiopian garlic plants.

The widespread virus-infection of garlic clones is similar to situation reported elsewhere. Studies with such garlic virus diseases have shown that virus-infection causes serious yield loss in bulb yield and quality (Fujisawa, 1998, Takaichi et al, 1998, Walkey and Antill, 1989). In nature, allexiviruses including GarV-B and GarV-C are horizontally transmitted by eriophyid mites whereas potyviruses like OYDV and LYSV are transmitted by aphids. However, it is believed that the main and economically important means of vertical transmission in vegetatively propagated crops like garlic is the use of virus-infected bulbs. Experience from other countries has shown that meristem tip-culture combined with thermotherapy has been successfully used to obtain virus-free bulbs from infected plants (Walkey et al. 1989, Takaichi et al, 1998). Since Ethiopian farmers traditionally produce their own garlic bulb saved from previous season for planting the following season, it is likely that the observed high incidence of viral infection is due to virus-infected bulbs and that results in reduction in yield and quality of the crop. Taking note of this problem, scientists at the Ethiopian Institute of Agricultural Research have initiated investigation on the use of virus-free bulbs generated via meristem culture to eliminate garlic viruses (Abraham, 2009). As part of that endeavor, the knowledge of accurate identity of garlic viruses and their distribution in Ethiopia would facilitate introduction of appropriate virus indexing procedures which is a prerequisite for large scale production of virus-free garlic bulbs.

This study also showed that viruses that infect garlic plants are more common in some areas than others. Hence, further studies are required to understand whether this is due to differences in resistance of garlic varieties used, inadvertent use of virus-free or the absence or low population of virus vectors in these areas. In addition, the information on the occurrence, relative importance and distribution virus diseases in areas uncovered in this study as well as on related crops like onion which are propagated by true seeds with more set of virus antibodies will not only give more complete picture on the diversity of *Allium* viruses in the country but also shed light on the epidemiology of viruses. More sensitive method such as reverse transcriptase polymerase chain reaction as used by various authors working on garlic viruses (Parker et al. 2005, Takaichi et al. 198, Chen et al. 2004) can significantly improve detection and characterization of viruses of *Allium* crops in Ethiopia.

## 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.

## References

Abraham, A. (2009). Agricultural biotechnology research and development in Ethiopia. *Afr J. Biotechnol* 8(25):7196-7204.

Barg, E., Lesemann, D. E., Vetten, H. J. and Green, S. K. (1994). Identification, partial characterization and distribution of viruses infecting *Allium* crops in South and Southeast

Chen, J., Zheng, H. Y., Antoniw, J. F., Adams, M. J., Chen, J. P. and Lin, L. (2004). Detection and classification of *Allexiviruses* from garlic in China. *Arch. Virol.* 149:435-445.

Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme- linked immunosorbent assay for the detection of plant viruses. *J. gen. Viro.* 34: 475–83.

Dovas C.I and Vovlas, C. (2003). Viruses infecting *Alliums spp.* in Southern Italy. *J. Plant. pathol.* 85:135.

Fajardo,T.V.M., Nishijima, M. Buso, J.A., TorresA.C, Avila, A.C.and Resende, R.O.(2001). Garlic viral complex: identification of Potyviruses and Carlavirus in Central Brazil. *Fitopatol Brasil* 26: 619-626.

Fujisawa, J. (1989). Loss of garlic yield by double infection of garlic viruses. *Agric Hortic* 64:737-741.

Lee, E.T., Koo, B.J., Jung, J.H., Chang, M.U. and Kang, S.G.(2007). Detection of Allexiviruses in the Garlic Plants in Korea. *Plant Pathol. J.* 23(4):266-271.

Park, K. S., Bae, Y. J., Jung, E. J. and Kang, S. J. (2005). RT-PCR-based detection of six garlic viruses and their phylogenetic relationships. *J. Microbiol. Biotechnol*. 15: 1110-1114.

Takaichi, M., Yamamoto, M., Nagakubo, T. and Oeda, K. (1998). Four garlic viruses identified by reverse transcription polymerase chain reaction and their regional distribution in Northern Japan. *Plant Dise.* 82:694-698.

Walkey, D. G. A. and Antil, D. N. (1989). Agronomic evaluation of virus-free and virus infected garlic (*Allium sativum* L.). *J. Hortic. Sci.* 64:53-60

Walkey D.G.A., Ebb MJW, Ballooned CJ, Millar A. (1987) Production of virus free garlic (*Allium sativum* L.) and shallot (*A. ascalonicum* L.) by meristem-tip culture. J Hortic Sci 62:211–220.