

Mixed Infection of Garlic Viruses, Detected by Das –Elisa Method, in Mongolia

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ABSTRACT

*Garlic (*Allium sativum*) is one of the important crops, but yield and quality of garlic is decreasing due to the infection by mixture of viruses.*

The aim of this study was conducted to detect for genus Potyvirus Onion yellow dwarf virus (OYDV) and Leek yellow stripe virus (LYSV); Carlavirus (Garlic common latent virus) (GarCLV) and Allxivirus (GV-B, GV-C) in two local garlic cultivars using the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

Samples collected from garlic planted fields of 3 different regions and the viral disease incidence recorded 22.9% in the Central zone, 5% in the Eastern zone and Western zone is showing most low 2%.

Leaf and bulb samples were tested. Virus infection rate was recorded in average - 41,7% and percentage of healthy plants - 58,3 % . The appearance of 2-3 types of viral infections in one plants indicates, that this is one of the reasons for decrease the garlic yield. Generally, the highest mixed infection was observed in dual infections OYDV+ LYSV.

Keywords: leaf, diagnose, disease incidence, DAS-ELISA, infection

INTRODUCTION

Due to the global warming and climate change in Mongolia, it is important to provide the population with healthy and safe food, grow vegetables of high therapeutic importance, improve the garlic cultivation technology and increase crop yields.

Garlic is valuable food and medicinal crop. It has antibiotic properties, health, and therapeutic benefits.

According to the statistics of the Ministry of Agriculture 80 % of all garlic is imported from China and garlic planting area contributes 8,7% to total vegetable production areas. In 2017 year 17775 tonnes of onion and garlic were imported, it is increased in 2018 by 8,8tonnes compared with previous years and covered to 26,228 tonnes.

The National Vegetable Program for 2018-2022 approved by the Mongolian Government Resolution № 278/2017 and plans to increase the supply onion and garlic seeds to 100 tonnes by 2022. However, garlic planting area is increased in last years, but yield of garlic in Mongolia are still low compared to other countries and well below the potential.

Approximately, average yield per year is 0.6 tonnes/ha. At present, mainly two varieties of garlic are cultivated in Mongolia. Those varieties were developed by clonal selection method and realized in 1996 in the country.

When garlic is multiplied conventionally using bulbs for many years, the varieties and seeds degenerated because of progress accumulation of virus and other pathogens. One of the reasons for decreasing yield of garlic is viral infections. Therefore, it is need to conduct detailed scientific studies on diagnostics, identification of garlic viruses, and selection of healthy speeds to increase the yields.

MATERIAL AND METHODS

Plant Materials

In this study two Mongolian varieties (Uliastai and Khovd) were used for the detection of viruses.

Variety of Uliastai: Early maturity. The color of skin is red.

Variety of Khovd: Mid- early maturity and it has purple striped.

The survey was conducted by walking though garlic fields and visually inspecting garlic plants

for presence of typical virus disease symptoms. The fields were examined using an “X” shaped sampling path. Plants were evaluated by symptoms of virus infection such as yellow mosaic, strip, and whole leaf yellowing or stunt, which major symptoms are caused by virus diseases.

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.

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

Sample Collection of Garlic Plants

The samples collected were designated according to their collection number and place of origin. Leaf samples were collected in first week of August from garlic planted fields of farmers. 2-3 leaves from each plant collected into bag and numbered the samples.

Samples of seed bulb was collected in May before planting it in the open field.

Serological Analysis

Plants were analyzed by DAS-ELISA. For each plant, about 2 cm of the apical portion of youngest leaf was assayed.

Bulbs also were analyzed. One clove from each bulb was assayed. The sample consisted of a cube (1.0 by 0.5 by 0.5 cm) taken from the basal portion of the clove.

Antiserum Immunoglobulin (IgG) and the (IgG+AP) conjugate with alkaline phosphatase for 5 types of garlic viruses such as *GarV-B*, *GarV-C*, *GarCLV*, *OYDV* and *LYSV* was used for the DAS-ELISA.

The IgG was diluted 1:1000 in coating buffer (0.05 M sodium carbonate, pH 9.6), and alkaline phosphatase conjugated IgG was diluted 1:1000 in conjugate buffer (PBS + 0.05% Tween-20 + 2% polyvinylpyrrolidone + 0.2% egg albumin + 2% nonfat dried milk). The substrate was used p-nitrophenyl phosphate disodium at 0.8 mg/ml of substrate buffer.

RESULT

A survey conducted in the 2016 - 2020 year to identify virus diseases infecting garlic in different growing areas in Mongolia.

That survey was including varieties such as Khovd and Uliastai, planted in Darkhan-Uul, Tuv, Selenge, Zavkhan and, Khentii aimags (Fig1). Visually, the viral disease incidence recorded 22.9% in the Central zone, 5% in the Eastern zone and Western zone is showing most low 2%.



Pic 1. Location of garlic planting and Sample collected areas

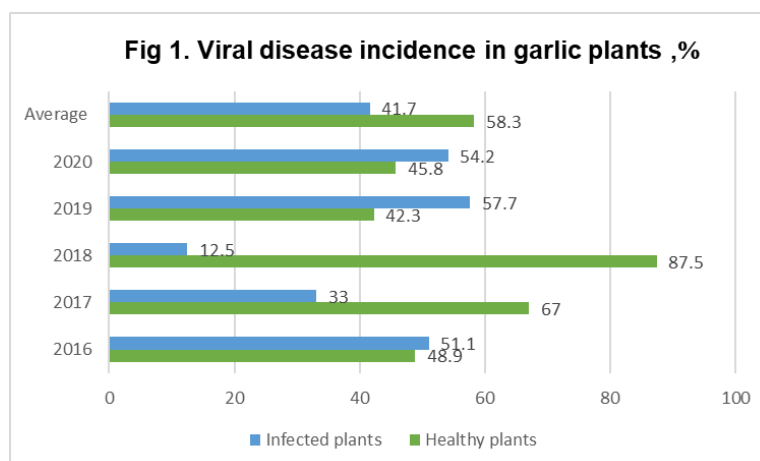


Pic 2. Healthy and viral symptomatic garlic leaves

The most common symptoms of garlic viruses observed are yellow mosaic and stripe of the plants (Pic 2).

In 2016-2020 years were taken 288 leaf samples per year from garlic planted fields of Darkhan-Uul, Zavkhan, Dornod, Khentii provinces and 5 types of garlic virus diagnosed by DAS-ELISA test.

Virus infection rate was recorded in average 41,7% and percentage of healthy plants -58,3% (Fig1). The appearance of 2-3 types of viral infections in one plants indicates, that this is one of the reasons for decrease the garlic yield.



Virus disease of garlic usually induced by mixed infection of several viruses belonging to different taxonomic groups, such as genus *Potyvirus* (Onion yellow dwarf virus(OYDV)

and Leek yellow stripe virus (*LYSV*); *Carlavirus* (Garlic common latent virus) (*GarCLV*) and *Allexivirus* (*GV-B*, *GV-C*) et.

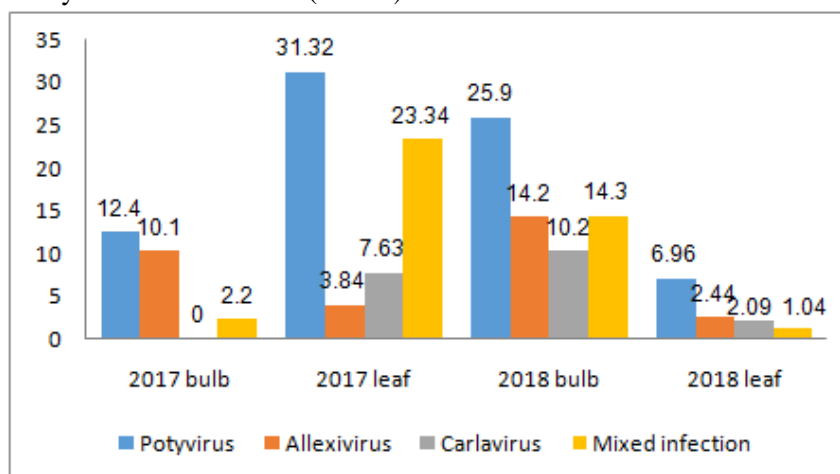
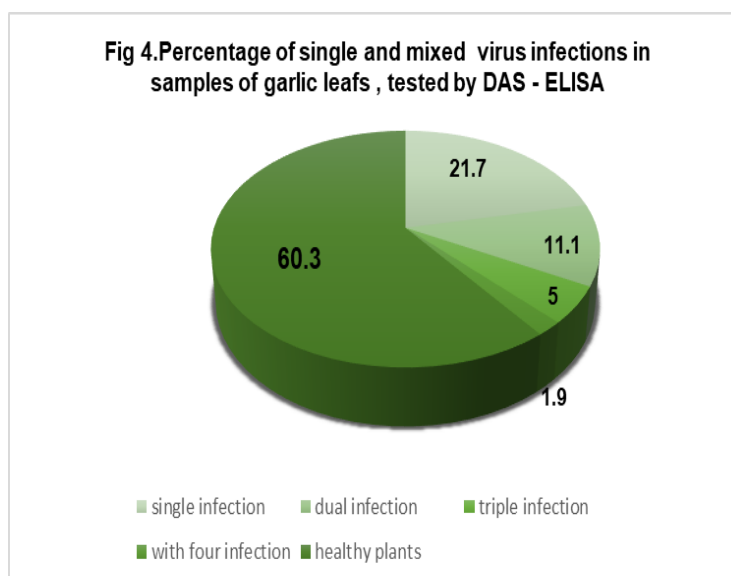
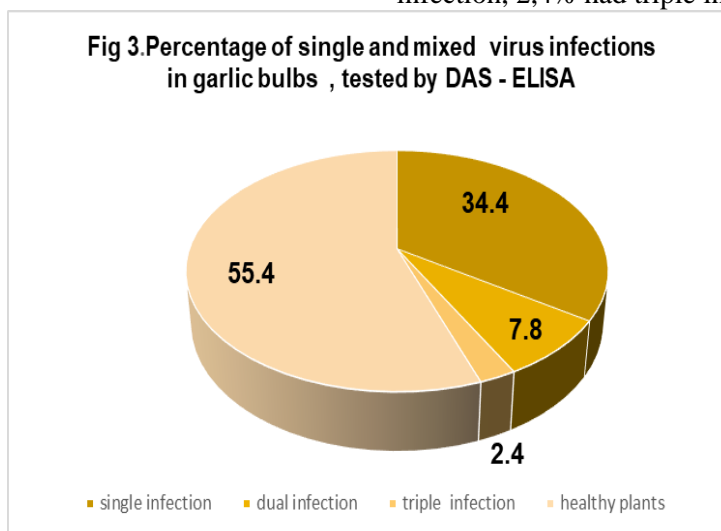


Fig2. Infection rate of garlic viruses belonging to different taxonomic groups, recorded in the samples of garlic leaves, %

OYDV and *LYSV*, members of genus *Potyvirus* were the most frequent (6.96 - 31,32%) followed by *GV-C*, *GV-B* member of *Allexivirus* (2,44 - 14,2%) and *GarCLV* member of *Carlavirus* (2.09- 10,2 %) .

By the result of viral diagnosis in the average of 2017-2018 years single infections in samples of garlic bulbs were detected 34,4 % and mixed infections were also very common. Among the mixed infections of samples 7,8% had dual infection, 2,4% had triple infections (Fig.3).



The same ratio was observed in samples of garlic leaves. Among the mixed infections of the samples 21,7% had single infection ,11,1 % - dual infection, 5 % - triple infections and additionally 1,9 % (Fig.4) found with four virus infections in one plants.

Generally, the highest mixed infection was observed in dual infections *OYDV*+ *LYSV*.

DISCUSSION

Ethiopian researchers KeroJumal and TillioFessia have discovered a garlic virus using the DAS-ELISA test. *OYDV*, *LYSV*, *GarV-B*, and *GarV-C* virus had 23% of all 119 samples (Thor, 2001) (Barg, 1996). There has unusual mono virus and also 2-3 mixed pathogenic viruses.

In many countries such as Argentina, Japan and Greece, *OYDV* virus has disease symptoms such as yellow mosaic, strip, yellowing in garlic crop and B, C, X, A, and D have significantly reduced the size and quality of crops (Clark, 1977). Although the greatest economic losses in garlic caused by viruses have traditionally been attributed to potyviruses such as *Onion yellow dwarf virus (OYDV)* (virus, that are transmitted by mites (allexiviruses) also may have a significant economic impact on the crop (6).

Our study result have been principally based on the study of foreign scientists in detecting *OYDV*, *GarV-B*, *GarV-C* and *LYSV*, *GarCLV* viruses by the DAS-ELISA test. The most percentage of viral disease is *OYDV* compared to other viruses.

CONCLUSION

According to the visual assessment of external symptoms of the viruses, the incidence of virus in garlic crops was recorded to low or 2-5 % in Eastern and Western Agricultural zone and moderate or 22.9% in the Central zone of Mongolia.

The result shows, that onion yellow dwarf virus (OYDV) is a more common *Potyvirus*, than other garlic viruses in the country. Therefore, the need more research work understanding the role of aphids and mites as virus vectors of *Potyvirus* and control to them.

The bulb and leaves of garlic infected not only one virus, but also 2-3 mixed pathogenic viruses recorded per plant. This causes by decreasing the size and quality of the garlic. It represents an important step for establishment of virus free garlic seed program in the country.

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