OCCURANCE AND DISTRIBUTION OF POTY VIRUSES INFECTING GARLIC IN PAKISTAN

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ABSTRACT:- The study was designed to detect and determine the prevalence, incidence and distribution of the poty viruses causing diseases in garlic (*Allium sativum*) from major garlic growing areas of Pakistan. The yellow stripes, mosaic and chlorotic spot symptoms of the disease resemble the viral infection in garlic reported to occur worldwide. Altogether 690 samples were collected from 29 locations of Punjab and 40 locations of Khyber Pukhtunkhwa to determine the prevalence of Onion Yellow Dwarf Virus (OYDV) and Leek Yellow Stripe Virus (LYSV). Serological testing DAS-ELISA technique was used to test the samples collected from the farmer fields. Based on the DAS-ELISA poty viruses OYDV and LYSV were detected from both provinces although the percentage incidence varied from location to location. Few areas of district Punjab were found free of LYSV but OYDV was prevalent in all locations irrespective of the varieties cultivated. Maximum disease incidence was detected in Swabi (KPK) where OYDV was 90% and LYSV was 38% while in Punjab major disease incidence of OYDV (87.14%) and LYSV (91.44%) was found in Sialkot.

Key Words: Allium sativum; Prevalence; Incidence; Onion Yellow Dwarf Virus; Leek Yellow Stripe Virus; Double Antibody Sandwich ELISA; Pakistan.

INTRODUCTION

Garlic, *Allium sativum*, is the second most widely cultivated and used crop after onion in Pakistan. Garlic as a perennial herb is best known flavoring agent for food. It also has importance in the world of medicine to prevent or treat many diseases as well. It is a rich source of carbohydrates, protein and phosphorus. It has originated in the mountainous Central Asian region from where it has spread all over the temperate and subtropical regions around the world. Etoh and Simon (2002) concluded that diversity of Central Asian garlic supports the idea that this region is the primary center of origin of garlic. Production of garlic in Pakistan was 55300 t ha^{-1} with provincial share of Punjab, Sindh, Khyber Pukhtunkhwa and Balochistan as 24300, 4600, 19500 and 6900 t ha^{-1}, respectively (GoP, 2010-11). Close relatives of garlic family include onion, shallot, leek, chive and rakkyo.

Garlic is prone to many diseases which include: basal rot (*Fusarium culmorum*), white rot (*Sclerotium cepivorum*), downy mildew(*Peronospora destructor*), botrytis rot (*Botrytis porri*), penicillium decay (*Penicillium hirsutum*) and many others. Mohibullah (1991) reported certain yield limiting diseases of garlic including purple blotch, white rot, pink rot and penicillium decay from Pakistan. The strict asexual propagation of garlic brings with it several viruses, nematodes and other pests which lower garlic yield. Viral diseases of garlic are worldwide in nature creating a serious problem of yield and quality loss. Dovas et al. (2001) reported that virus infections are transmitted from one

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crop cycle to another through infected cloves which accounts for the heavy viral infection and most of the plants are infected with a mixture up to five different viruses. Such practices facilitate the accumulation of viruses in the reproductive materials and their dissemination and yield loss.

Viruses may not kill the plant but can reduce yield up to 50% over the time (Lot et al., 1998; Conci et al., 2003). Due to the use of distinct levels of technology and seeds with a highly variable quality by the farmers, large differences in virus species distribution and virus infection rates under major cultivation systems can be expected. Bos (1976) observed that non colonizing species of aphids transmit LYSV and OYDV in a non-persistent manner and are mainly responsible for their spread. As there is no effective chemical control method against viruses directly so virus-free plants could have significance of practical application which increase yield with better quality of crop for the local farmers. Transmission of poty viruses by aphids (common pests in bulbs), make it easier to re-infect the crops. It is, therefore, important to evaluate all garlic accessions for the potential impact of these viruses on garlic germplasm conservation and distribution. In the recent years, OYDV has emerged as an important virus infecting garlic crop in India (Arya et al., 2006). Allium viruses cause reductions in plant vigor induce mosaics, yellow stripes, and deformations in leaves, stunting in plant, which result in drastic yield losses in infected plants (Ahlawat and Varma, 1997; Arya etal., 2006). For instance, only OYDV can significantly reduce yields in onion and garlic, and yield losses as high as 25-54% have been reported (Barg et al., 1994; Dovas et al., 2001; Lot et al., 1998).

The objective of the present study was to identify the viruses causing diseases of garlic and to determine the prevalence, incidence and distribution of the poty viruses infecting garlic from major garlic growing areas of Pakistan.

MATERIALS AND METHOD

Survey of Major Garlic Growing Areas

Surveys of farmers' fields were carried out to assess the viral disease incidence and severity under natural condition.

Sample Collection

Leaves of the young fully developed garlic plants were collected from the farmers' fields. Ten random samples were collected from plants irrespective of symptoms moving in Z shape scheme within the field at equal distance. Samples were placed in labeled polythene sterile bags kept in ice box and stored at 4°C in the laboratory till use.

Serological Detection Through DAS-ELISA

The presence of the pathogens poty viruses (OYDV and LYSV) in collected samples was checked at Plant Virology Lab NARC using commercially available antisera purchased from Bioreba. Analysis of samples was done by using DAS-ELISA (double antibody sandwitch enzyme linked immuno sorbant assay) serological testing according to the method described by Clark and Adams (1977).

The polystyrene plates used for virus detection were coated with the immunoglobulin G of the Kit at dilution 1:1000 ml prepared in a coating buffer. 150 µl of coating buffer was dispensed to each well of the microtiter plate and incubated at 4°C overnight. After incubation the plates were washed thrice with the wash-

ing buffer (PBS-T). Samples were extracted in extraction buffer in ratio 1:5 w/v. After homogenization in extraction buffer, 150 µl of each leaf sample sap was placed in duplication in a single micro-titer plate well. Positive and negative controls (obtained in DAS-ELISA kit) were used for comparison. Sample (antigen) loaded plates were again incubated at 4°C overnight. On completion of incubation the washing step with PBST was repeated thrice at 3 min interval for the removal of the excess amount of antigen. Next step was loading of 150 µl of conjugate buffer containing IgG attached with alkaline phosphatase at dilution @ 1:1000 ml to the wells of the plate overnight. Plates were re-incubated at 4°C. On completion of incubation the washing step with PBST was repeated thrice. Finally 150 µl substrate buffer was loaded to the plates along with p-nitro-phenyle phosphate (0.75%). Plates were kept under dark at 37°C for 30-60 minutes. The intensity of the color is the indication of virus concentration so optical density values were measured at 405 nm wavelength by spectrophotometer/ ELISA reader (THERMO MULTISKAN EX REF 5118170). Readings with an $A_{(405)}$ more than three times the mean of the negative control were considered positive.

The percentage incidence of the virus was calculated from the following formula.

$$\% \text{ age incidence} = \frac{\text{DAS-ELISA confirmed sample} * 100}{\text{Total sample tested}}$$

RESULTS AND DISCUSSION

Survey of 29 locations from four districts of Punjab (Table 1) and 40 locations from 8 districts of Khyber Pukhtunkhwa (Table 2) was carried out to determine the incidence and percentage of infection by OYDV and LYSV at farmers' fields. Collected samples were subjected to serological test DAS-ELISA (Clark and Adams, 1977) to check the presence of suspected poty viruses that is infecting garlic. It was found that OYDV was prevalent in all districts of Punjab with the incidence range of 8.75- 87.14%. The maximum infection was recorded in Sialkot (87.14%) and minimum in Kasur (8.75%) (Figure 1). Incidence of LYSV, another member of the poty virus group was also recorded with the maximum infection of 91.44% in Sialkot and minimum 5% in Kasur but LYSV was not found in 14 locations of 2 districts (Khanewal and Gujranwala) of Punjab (Figure 1).

Survey of 40 locations from 8 districts of Khyber Pukhtunkhwa was carried out and 400 collected samples were subjected to serological test DAS-ELISA which showed the prevalence of both the poty viruses (OYDV and LYSV) from all districts although the intensity of the disease varied in all locations. Incidence of OYDV infection ranged from 20% in district Deer to 90% in district Swabi (Table 2). The infection incidence of LYSV was recorded as maximum of 38% in Swabi and minimum 3% from Deer (Table 2) but the disease was prevalent in all locations of the province (Figure 2).

Table 1. Prevalence of potyviruses of garlic in Punjab

S.No.	District	Location	No. of samples	Infected OYDV	% infection OYDV	Infected YSV	% infection LYSV
1	Khanewal	11	110	25	22.72	0	0
2	Gujranwala	3	30	4	13.30	0	0
3	Sialkot	7	70	61	87.14	64	91.44
4	Kasur	8	80	7	8.75	4	5

Table 2. Prevalence of poty viruses of garlic in Khyber Pukhtunkhwa

S.No.	District	Location	No. of samples	Infected OYDV	% Infection OYDV	Infected LYSV	% Infection LYSV
1	Peshawar	8	80	39	49	8	10
2	Swabi	10	100	90	90	38	38
3	Malakand	2	20	4	20	2	10
4	Swat	4	40	16	40	12	30
5	Nowshera	2	20	4	20	3	14
6	Mardan	6	60	34	56	7	12
7	Deer	4	40	16	40	1	3
8	Charsada	4	40	16	39	6	16

It is concluded on the basis of serological test (DAS-ELISA) that out of 290 samples collected from different locations of Punjab, 97 samples were infected with OYDV showing 33% incidence and 68 samples were infected with LYSV showing 23.44% incidence in Punjab province. Similarly out of 400 samples collected from Khyber Pukhtunkhwa, 219 samples were positive for OYDV showing 54.75% incidence and 77 samples were positive for LYSV showing 19.25% incidence in the province. Two districts of Punjab were found free from LYSV whereas OYDV was prevalent in all districts of Punjab and Khyber Pukhtunkhwa. Maximum percentage incidence of both viruses was at Sialkot from Punjab and at Swabi from Khyber Pukhtunkhwa. It was also observed that although both poty viruses OYDV and LYSV were present in both provinces but the percentage infection of OYDV was higher than LYSV.

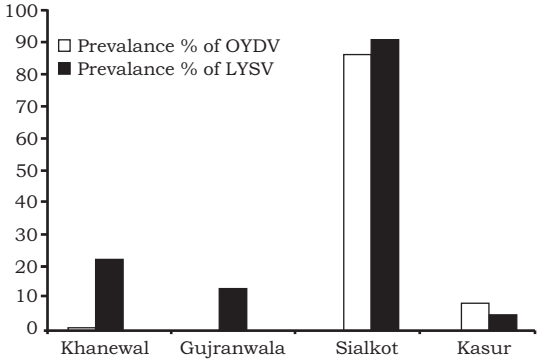

Figure 1. Incidence of OYDV and LYSV in Districts of Punjab

Van Dijik (1993) reported that among all garlic viruses, OYDV and LYSV are the most important viruses of garlic and occur worldwide Fidan et al.(2009) reported that these two viruses together affect the plant and result in a yield loss up to 78%. Barg et al. (1994) analyzed 290 garlic samples from seven Asian countries and detected the presence of poty virus spp. in all of them. Both of these poty viruses infecting garlic are reported for the first time from Pakistan on the basis of serological test of DAS-ELISA. Over all incidence of LYSV was comparatively less than OYDV. High rate of incidence of both the viruses in some areas showed that if proper management practices to control the viral diseases will not be applied the disease can cause an alarming situation in future and the synergistic effect of both the viruses will badly affect the crop yield. It is important for the control of these pathogens to obtain virus free

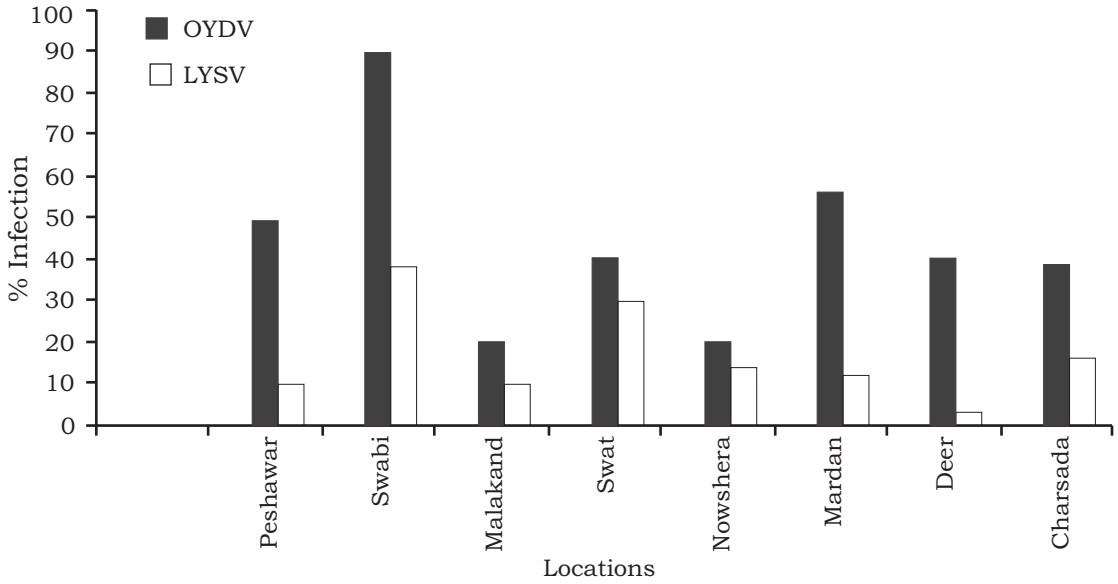

Figure 2. Incidence of OYDV and LYSV in Districts of KPK

material, and to develop programs for producing certified virus-free stocks of popular garlic cultivars. This analysis can also be made with bulbs to know the health status of seed bulbs immediately before planting. It has been reported that there is no difference in virus content between bulbs and bulbils, therefore, there is no advantage on using bulbils over cloves for the production of virus-free propagation material (Verbeek et al., 1995).

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No.	Author Name	Contribution to the paper
1.	Ms S.Talat Gilani	Wrote abstract, Methodology, Did SPSS analysis, Conclusion, Technical input at every step, Overall management of the article, Data entry and analysis, Result and discussion, Introduction, References.
2.	Dr. Shahid Hameed	Conceived and surveyed for data collection.
3.	Dr. Hussain Shah	Survey for data collection from KPK.

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