

Incidence of virus Y Strains and Effect of Infection on the Productivity of Potato Tubers.

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Abstract: Field survey studies were conducted during three growing seasons to study the prevalence and distribution of PVY strains infecting potato in different locations at Behairah, Sharkia and Menoufia governorates (Egypt). The most common symptoms were different types of mosaic, leaf crinkling, necrosis and leaf drop streak. Four virus strains namely: tobacco vein necrosis strain (PVY^N), common strain (PVY^O), stipple streak strain (PVY^C) and potato tuber necrotic ring spot (PVY^{NTN}) were detected using DAS-ELISA. At the Nile growing season PVY^O was the most prevalent virus (63.5%) followed by PVY^N (25%). PVY^N had the highest frequency (67%), followed by PVY^O (25.7%) at the winter growing season. While at summer growing season, PVY^N and PVY^O had the most prevalent viruses (36.7%). Agar double diffusion test was used to confirm the identity of the isolated PVY strains. The Egyptian PVY strains reacted strongly with the specific antiserum for each strain forming a prominent precipitin line. Productivity of potato cultivar Spunta to infect with virus strains was conducted in the farm of Benha, Qalubia decreased by 22.6% to 50.5% according to the strain as compared with control.

I. Introduction

PVY is the type member species of the family potyviridae (Galis *et al.*, 2000). PVY is considered one of the most damaging potato viruses causing significant yield losses because the infection with PVY spread easily and the yield reduction reached to 80% in potato and 39-75% in tobacco (DE Bokx and Huttinga, 1981) depending on the PVY strains, cultivars and time of infection. Primary symptoms of PVY are necrosis, mottling or yellowing of leaflets, leaf dropping or sometimes premature death. These viruses display a monopartite genome constituted of positive sense single stranded RNA bordered by two distal non-translated regions (Hollings and Brunt, 1981) and translated into a polyprotein which is processed by three viral proteases into nine functional proteins (Riechmann *et al.*, 1992). PVY is wide spread all over the world and causes economically important disease not only to solanaceous crops but also to many solanaceous weeds (Glais *et al.*, 2002; Quintero-Ferrer and Karasev, 2013).

The PVY isolates have classically been divided into three main strains (DE Bokx and Huttinga, 1981). The common strain or ordinary strain (PVY^O) induced mainly severe systemic crinkle symptoms, rugosity of leaf drop, streak on potato, systemic necrosis on *Physalis floridana*, and systemic mottling on tobacco plants. The tobacco vein necrosis strain (PVY^N) induced severe systemic vein necrosis on tobacco, systemic mottling on *Ph. floridana* and very mild mottling on almost all potato cultivars. The stipple streak strain (PVY^C) isolate evoke systemic mosaic or stipple streak symptoms in susceptible cultivars bearing the Nc resistance gene, tobacco plants reacted with vein banding symptoms while the symptoms on *Ph. floridana* were systemic necrosis (Blanco-Urgoiti *et al.*, 1998; Chikh *et al.*, 2013).

Emergence of new particular PVY isolates was detected. Inducing new diseases in potato crop. Such as: PVY^{NTN} first detected in Hungary in 1984 (Beczner *et al.*, 1984). Since then it has been detected in potato tubers from tubers (Le Romancer and Kerlan, 1997; Gorsane *et al.*, 2001). Now considered more aggressive isolate of PVY^N, it is the causal agent of the potato tuber necrotic ring spot disease (PTNRD) which is characterized by superficial bows, first protruding then later becoming sunken and necrotic (Kus, 1990). PVY^N – Wi, found in Poland in 1984 was described as differing in virulence and aggressiveness from the older PVY isolates (Chrzanowska, 1991) and was shown to be serologically related to PVY^O isolates (Chrzanowska, 1994). PVY^Z was found in Britain in 1984, differing from PVY^O and PVY^C by its ability to overcome the hypersensitive genes Nytr and Nc and furthermore targeting an hypothetical Nz gene (Kerlan *et al.*, 1999).

Surveys of commercial seed potato yields for virus diseases in Manitoba established that potato virus Y is of concern in seed potato production, the samples collected (2000 to 2001) contained PVY. Further analysis of the PVY – positive samples by a duplex RT-PCR indicated that 37.5% contained PVY^O and 63.5% contained PVY^N (Singh *et al.*, 2003).

The potato virus Y strains incidence during 2001 to 2003 in Washington and Oregon, as evidenced by more infected cultivars, sites of seed origin, and number of seed growers providing infected seed lots. Totals of 960 and 286 certified potato lots, there was a dramatic increase in seed lots with the PVY (N:O) strain from

2002 to 2003, in contrast to PVY^O, which causes yield reduction, also causes internal and external damage to tubers, making them unmarketable (Crosslin *et al.*, 2006).

Agar gel double diffusion can be used for detection of elongated viruses in purified preparations and in plant extracts (Ouchterlony, 1962).

The objective of the present work is to determine the distribution and prevalence of PVY strains in different locations at 3 governorates in Egypt based on external symptoms and using DAS-ELISA. Agar double diffusion test was identity of the isolated PVY strains. Productivity of potato PVY infected cultivar Spunta was conducted also.

II. Materials And Methods

Incidence of PVY strains affecting potato plants in some governorates

Field survey studies were conducted during three growing seasons to study the prevalence and distribution of PVY strains infecting potato in different locations at Behairah, Sharkia and Menoufia governorates (Egypt). (*Solanum tuberosum*) cultivars Nicola and Spunta. The most common symptoms detected in the field were different types of mosaic, leaf crinkling, necrosis and leaf drop streak. Evaluation of disease incidence was based on external symptoms and serological diagnosis was carried out using DAS-ELISA.

The virus strains were detected in naturally infected potato plants using specific monoclonal antibodies for PVY^N, PVY^O, PVY^C, PVY^{NTN} and polyclonal antibodies of PVY for all isolates by DAS-ELISA (Kindly provided by Prof. Dr. Robert Burns, Edinburgh University, Scotland).

During three growing season, 232, 237 and 79 samples were collected during Nile, winter and summer seasons respectively. Different types of virus like symptoms were collected in plastic bags and stored at 4°C until used for viral detection. The samples were indexed by DAS-ELISA against PVY strains.

Serological detection of PVY strains

A). Direct double antibody sandwich (DAS-ELISA):

The direct double antibody sandwich (DAS-ELISA) was used for virus detection as mentioned by Clark and Adams (1977). Four monoclonal antibodies were used: MAb1 specific to PVY^O strain, MAb2 specific to PVY^N strain, MAb3 specific to PVY^C strain, MAb4 specific to PVY^{NTN} strain and polyclonal antibody to all PVY strains. Positive samples and all antibodies were kindly provided by Dr. Robert Bruns, Edinburgh University, Scotland.

Polyethylene microtitre plates (flat bottom type) were coated with specific immunoglobulin 200µl/well IgG at the rate 1 µg/ml in coating buffer pH 9.6, then plates were incubated at 37°C for 2hr. Plates were washed three times with PBS-Tween 20 buffer pH 7.4, 3 min. The tested samples were prepared by grinding one gram of leaf and petiole tissues in 4 ml phosphate-buffer saline PBS pH 7.4, then 200 µl were added per well and 2 wells from healthy plants as a negative control and from diseased plants as a positive control followed by incubation at 4°C overnight. Plates were washed 3 times as mentioned above. Alkaline phosphatase conjugated IgG was added with rate of 200 µl /well at a dilution of 1:1000 in conjugate buffer and then incubated at 37°C for 3hr. Plates were washed 3 times as mentioned above and then 200 µl of the substrate (P-nitrophenyl phosphate) prepared immediately before use in substrate buffer with a final concentration of 1 µg/ml were added for each well. The plates were incubated at room temperature for one hour or as long as it necessary to develop reaction. The reaction was stopped by adding 50 µl 3M NaOH to each well. Plates were examined to measure the optical density (O.D.) at 405nm-wave length with a spectrophotometer (Dynatech laboratory MR7000). The positive results were recorded over to 2 time fold a negative control and less than that is considered as a negative reaction.

B). Agar double diffusion test:

Serological detection and distinction of the virus strains was done by Ouchterlony double-diffusion test according to Purcifull and Batchelor (1977). 4 g agar was added to 300 ml of distilled water in a beaker and autoclaved for 5 min at 121°C, then 5.0 g sodium azide was added to the molten agar and mixed well. 2.5 g SDS was added to 100 ml of hot distilled water and added to the agar-azide mixture. Bring up to a volume of 500 ml with blot distilled water and mixed well. Petri dishes were placed on a level surface and added 12 ml/ dish and allowed medium to gel, then were stored plates in closed plastic bags at 4°C.

Preparations of crude sap obtained from tobacco plants (cv. White Burley) infected leaves of the four virus strains were separately tested against five antiserum PVY^O, PVY^N, PVY^C, PVY^{NTN} and universal antiserum for all PVY strains. Crude sap preparation were placed in the peripheral wells after extracted in 3% SDS and each test antiserum was placed in the control well in a separate plate. Petri dishes were placed in covered plastic boxes to maintain humidity and incubated for 2 days at 25 °C.

Productivity of potato cultivar Spunta to infected with virus strains

This work was conducted in open field in the farm of Meit-Kenana, Benha, Qalubia, Egypt to assess the productivity of potato cv. Spunta as infected by virus strains. Field inspections and the collection tubers were done. The tubers which used in this experiment were checked for the freeing from virus infection using DAS-ELISA, 50 tubers were planted separately in open field on 5 lines (each line contains 10 plants). Ten plants of growing potato plants were mechanically inoculated five weeks after planting with each strain and ten healthy plants were left without inoculation as a control. The inoculation process were carried under natural conditions and the potato plants were covered with a transparent plastic sheets, After 120 days the tubers from each potato plant were collected and the weight of tubers were estimated. The reduction of productivity of potato yield as compared to control was calculated as follows:

$$\frac{(\text{The weight of healthy tuber} - \text{The weight of infected tuber})}{\text{The weight of healthy tuber}} \times 100$$

The inoculated plants were tested using DAS-ELISA.

III. Results

Incidence of PVY strains affecting potato plants in some governorates

Field survey studies were conducted during three growing season to study the prevalence and distribution of PVY strains infecting potato in different locations at Behairah, Sharkia and Menoufia Governorates (Egypt) using ELISA test.

Data in Table (3, 4 & 5) showed that incidence of PVY strains were from (6%-63.5%), (4.2%-67%) and from (2.5%-36.7%) during the Nile, winter and summer growing season respectively.

232 samples of suspected infected potato plants were collected at the Nile growing season. Obtained data presented in Table (3) and Fig. (4) showed that PVY^O was the most prevalent virus being detected in 147 samples followed by PVY^N in 58 samples and then PVY^C in 23 samples and finally PVY^{NTN} in 14 samples representing 63.5%, 25%, 9.9% and 6% respectively from the whole previously inspected samples.

At the winter growing season, 237 samples showed virus like symptoms were collected. The data presented in table (4) and Fig. (5) showed that PVY^N had the highest frequency (159 samples, 67%), followed by PVY^O (61 samples, 25.7%), then PVY^C (23 samples, 9.7%) and finally PVY^{NTN} (10 samples, 4.2%).

Out of 79 samples were collected at summer growing season, data presented in Table (5) and Fig. (6) showed that PVY^N and PVY^O had the most prevalent viruses (29 samples, 36.7%), followed by PVY^C (25 samples, 31.6%) and finally PVY^{NTN} (2 samples, 2.5%).

Table (1): Occurrence and relative prevalence of PVY strains infecting potato in some producing governorates during the Nile growing season.

Governorates	No. of tested samples	No. of infected samples							
		PVY ^N	%	PVY ^C	%	PVY ^O	%	PVY ^{NTN}	%
Behairah	65	46	70.7	12	18.5	12	18.5	1	1.5
Sharkia	68	9	13.2	7	10.5	50	73.5	6	8.8
Menoufia	99	3	3	4	4	85	85.8	7	7
Total	232	58	25.0	23	9.9	147	63.5	14	6.0

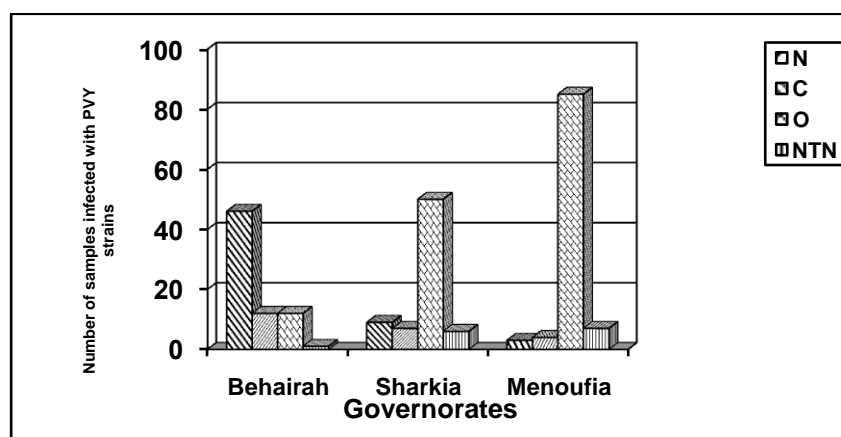


Fig. (1): Diagram illustrated virus strains incidence during the Nile growing seas

Table (2): Occurrence and relative prevalence of PVY strains infecting potato in some producing governorates during the winter growing season.

Governorates	No. of tested samples	No. of infected samples							
		PVY ^N	%	PVY ^C	%	PVY ^O	%	PVY ^{NTN}	%
Behairah	58	40	68.9	4	6.8	13	22.4	1	1.7
Sharkia	60	20	33.3	13	21.6	32	53.3	5	8.3
Menoufia	119	99	83.5	6	5	16	13.4	4	3.5
Total	237	159	67	23	9.7	61	25.7	10	4.2

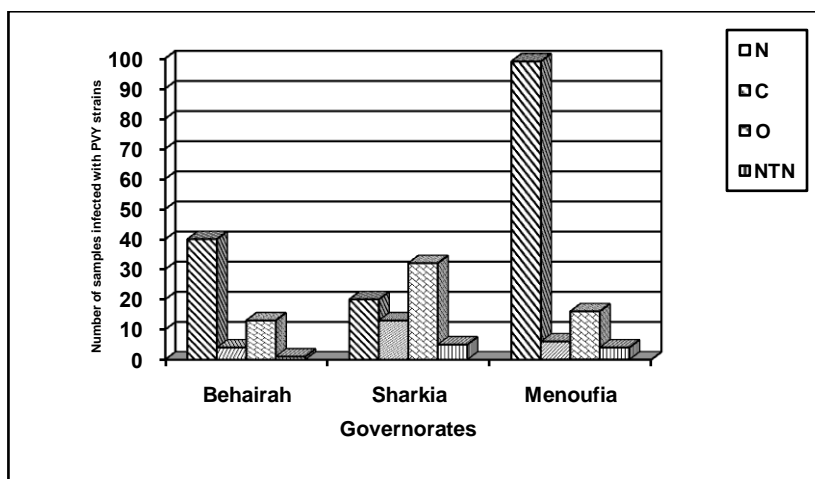


Fig. (2): Diagram illustrated virus strains incidence during the winter growing season.

Table (3): Occurrence and relative prevalence of PVY strains infecting potato in some producing governorates during the summer growing season.

Governorates	No. of tested samples	No. of infected samples							
		PVY ^N	%	PVY ^C	%	PVY ^O	%	PVY ^{NTN}	%
Behairah	28	20	71.4	-	-	8	28.5	-	-
Sharkia	21	5	23.8	10	47.6	11	52.5	1	4.7
Menoufia	30	4	13.3	15	50	10	33.3	1	3.3
Total	79	29	36.7	25	31.6	29	36.7	2	2.5

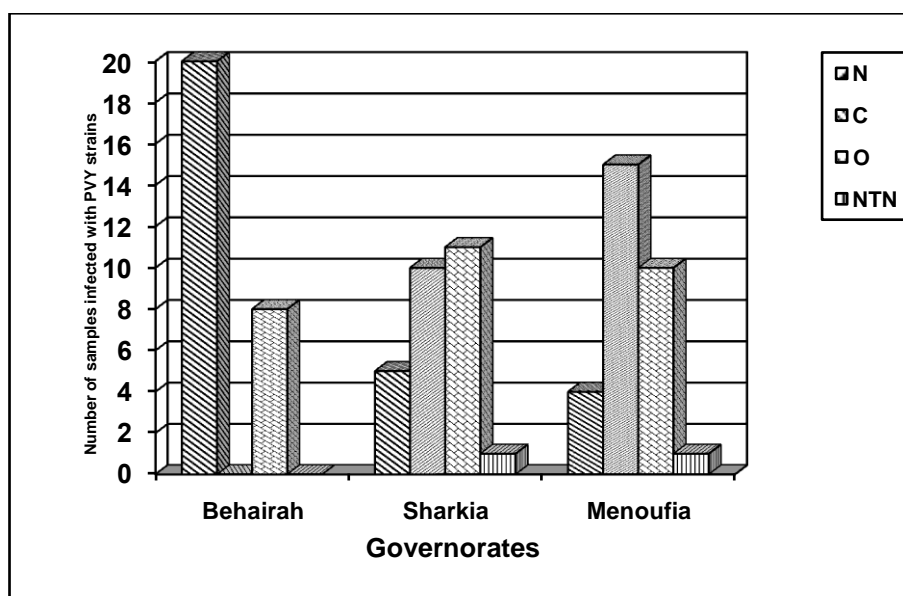


Fig. (3): Diagram illustrated virus strains incidence during the summer growing season.

Serological detection of PVY strains

Enzyme linked immunosorbent assay was successfully conducted using monoclonal antibodies (MAbs) were applied to identify PVY strains (PVY^O, PVY^N, PVY^C and PVY^{NTN}). The results showed that each strain reacted positively with its specific monoclonal antibodies and reacted negatively with other strains antibody. Agar double diffusion test was used to confirm the identity of the isolated PVY strains. The Egyptian PVY strains (PVY^O, PVY^N, PVY^{NTN} and PVY^C) reacted strongly with the specific antiserum for each strain forming a prominent precipitin line in SDS-double diffusion test (Fig. 4). Also, clear positive reaction was obtained when specific PVY^N antiserum was checked with the sap of PVY^{NTN} and PVY^N. Fig. (4) Showed that antiserum for all PVY strains (universal) reacted strongly with the four tested PVY strains.

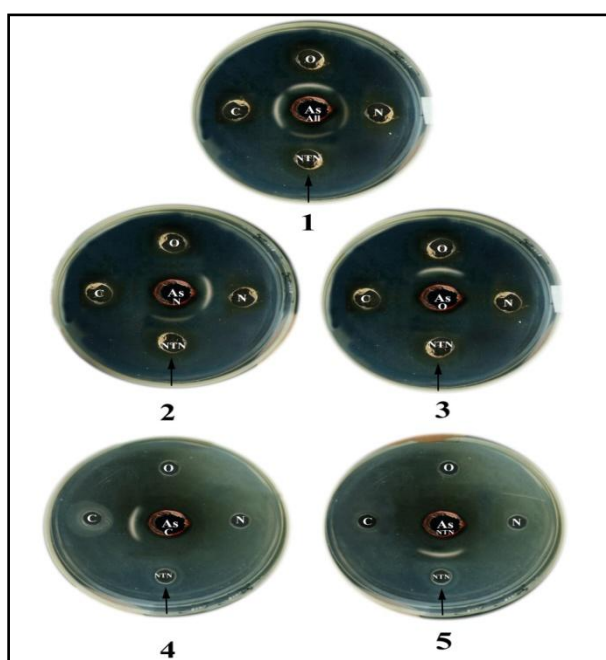


Fig. (4): Serological reaction of PVY strains by the immunodiffusion test; central well contains antiserum of all PVY strains (universal) (1), antiserum of N strain (2), antiserum of O strain (3), antiserum of C strain (4) and the antiserum of NTN strain (5). A positive reaction for PVY^N, PVY^{NTN}, PVY^C (react strongly) and PVY^O (also react strongly) (1), also positively in PVY^N (react strongly) and PVY^{NTN} (react moderately) (2). And a positive reaction for PVY^O (3), gave a positive reaction with PVY^C (4) and PVY^{NTN} (5).

Productivity of potato (*S. tuberosum* cv. Spunta) infected with virus strains

Under field condition, the effect of PVY strains infection on the productivity of potato tubers was carried out in Qalubia governorate.

The data showed that, PVY^{NTN}, PVY^O, PVY^N and PVY^C – infection decreased productivity of potato tubers by 32.8%, 38.5%, 50.5% and 22.6% respectively compared to control [(Table 4) and (Fig5)].

Table (4): Productivity of potato (*S. tuberosum* cv. Spunta) infected with PVY strains.

No. of sample	Productivity of healthy potato tubers (gram)	Productivity of infected potato tubers with (gram)			
		PVY ^{NTN}	PVY ^O	PVY ^N	PVY ^C
1	900	650	650	430	800
2	920	675	575	400	700
3	975	650	675	500	730
4	950	630	650	450	700
5	1000	660	560	500	750
6	950	650	530	525	825
7	900	625	550	500	725
8	980	650	570	525	750
9	950	725	650	525	750
10	1250	650	625	475	830
Total	9775	6565	6035	4830	7560
Productivity reduction %		32.8%	38.5%	50.5%	22.6%

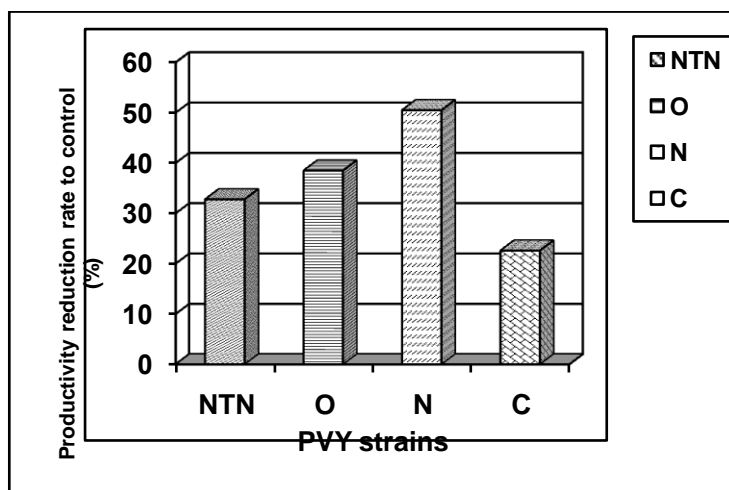


Fig. (5): Effect of PVY strains-infection on the productivity of potato tubers yield.

IV. Discussion

Field surveys were conducted during three growing seasons to study the prevalence and distribution of PVY strains infecting potato in some locations along three governorates (Behairah, Sharkia and Menoufia) in Egypt. Four strains namely: PVY^N, PVY^C, PVY^O and PVY^{NTN} were detected using direct ELISA. These strains were reported in other countries (Blanco-Urgoiti *et al.*, 1998; Takacs *et al.*, 2001; Crosslin *et al.*, 2006) and in Egypt.

The incidence of PVY strains in the collected samples showed virus like symptoms ranged from (2.5% to 67%) during three growing season. The data showed that, the most prevalent strains were PVY^O (63.5%); PVY^N (67.0%) and PVY^O PVY^N (36.7%) in Nile, winter and summer growing seasons, respectively. Sigh *et al.* (2003) in surveys of commercial and seed potato fields in Manitoba (Canada) found that, of the samples collected and tested by RT-PCR, 4.0% contained PVY and further analysis of tested PVY-positive samples by a duplex RT-PCR facilitating the simultaneous detection of PVY^O and (PVY^{N/NTN}) indicated that 37.5% and 63.5% contained PVY^O and PVY^N type isolate, respectively. The obtained results also indicated the high incidence of PVY in Nile and winter growing seasons compared with summer growing season. The high incidence in Nile and winter growing seasons could be attributed to the fact that farmers during these seasons used their own stored potato tubers, while the low incidence in the farms during the summer growing season may be due to the use of imported and certified potato tubers which are virus-free. The incidence of PVY in summer growing season may be due to the presence of aphids which transmit the PVY strains and also mechanical transmission from susceptible hosts which act as additional reservoirs for these strains.

Potato virus Y is one of the most important viral diseases of potato worldwide, causing up to 80% yield losses in several Solanaceous crops (DE Bokx and Huttinga; and Nilesen *et al.*, 1999). The disease is especially serious in areas and in years where large numbers of aphids cause the spread virus infection.

The identity of PVY strains using agar double diffusion test showed that Positive reactions were obtained when specific PVY strains antiserum was checked individually with the sap of infected-PVY strains tobacco leaves.

Results of the effect of infection with PVY different strains on the productivity of potato (*S. tuberosum* cv. Spunta) were carried out under field condition. The results found that potato plants inoculated with PVY^{NTN}, PVY^O, PVY^N and PVY^C showed reduction of productivity reached 32.8%, 38.26% 50.5% and 22.6% respectively when compared to control. Several authors working on PVY indicated that, the infection causes yield reduction of potato reach (50-90%) by Spaar and Hamann, 1977, (33-77%) by Sutic *et al.*, 1994 and (10-100%) by Nielsen *et al.*, 1999.

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