



## Incidence of apical leaf curl disease (ToLCNDV), and economics and reaction of potato (*Solanum tuberosum*) cultivars against whitefly, *Bemisia tabaci* in northern India

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### ABSTRACT

Whitefly transmissible Gemini virus, *Tomato leaf curl New Delhi virus*-potato (ToLCNDV-potato) causing potato apical leaf curl disease has been posing serious threat to both seed and ware potato production in northern India. Two sets of field and laboratory experiments were conducted to assess the incidence of *Bemisia tabaci* and ToLNDV-potato under sprayed and unsprayed conditions of nine most commonly cultivated potato varieties during two consecutive years. Besides, that varietal response to *B. tabaci* and ToLCNDV-potato was also evaluated. The whitefly population significantly varied on potato cultivars sprayed and unsprayed conditions. Minimum incidence of *B. tabaci* was recorded in Kufri Bahar (5.0/5 plant) and Kufri Khyati (7.0/ 5 plant) when crop was sprayed by three insecticide sprays. The maximum incidence of whitefly (15.33/5 plant) was recorded in Kufri Pukhraj followed by Kufri Chipsona-1 (15.0/5 plant) when crop remain unsprayed. The higher population of whitefly was recorded in the year 2013-14 as compared to 2014-15. The maximum yield and highest cost: benefit ratio was found in the three sprays treatment of cv. Kufri Khyati followed by Kufri Garima. PCR results revealed that most common 22 potato cultivars grown in the country acquired ToLNDV-potato in due course of time, however some of potato cultivars like Kufri Anand, Kufri Chandramukhi, Kufri Chipsona 1, Kufri Chipsona 2, Kufri Chipsona 4, Kufri Gaurav, Kufri Himalini, Kufri Khyati, Kufri Pukhraj and Kufri Satlej acquired ToLNDV-potato very quickly and showed positive reaction in of October. The sample of whitefly drawn from the respective potato cultivars in of October also exhibited positive reaction as per retaining the virus with almost same trend of potato cultivars. Thus, it is concluded that potato cultivars must be well protected using Imidacloprid followed by Thiamethoxam and Imidacloprid insecticides, first spray on 20 days after planting followed by second and third sprays on 35 and 42 days after planting, respectively for suppression of whitefly population and effective management of apical leaf curl disease, especially crop grown for seed purpose.

**Key words:** Cultivars, Insecticides, PCR, Potato, ToLCNDV, Whitefly

Potato (*Solanum tuberosum* L.) is a major world food crop which is consumed by over a billion people globally. India is the second largest potato producer in the world, only after China. During the year, 2014-15, country produced 44.9 million tonnes of potato from 2.10 million ha area with an average productivity of 21.4 tonnes/ha (Anonymous 2016). Uttar Pradesh is one of the important potatoes producing state in the country and its area under potato cultivation has increased by three folds as compared to other crops since country independence (Venkatasalam *et al.* 2011). The potatoes in India are cultivated under diversified agro climatic conditions varying from sea level to snowline

and up to three crops are raised per year. In northern plains, potato is grown mainly in two seasons, i.e. autumn and spring (Bhatnagar 2008, 2013). Whitefly, *Bemisia tabaci* (Gennadius) is a sap-sucking insect, belonging to family: Aleyrodidae and order: Hemiptera. Whitefly has a very wide host range. There may be more than 500 host worldwide (Dhawan *et al.* 2007). Sweet potato, cucumber, water melon, squash, egg plant, pepper, tomato, potato, lettuce and many other crops are hosts, but their suitability varies. Various weeds and field crops may favor survival of whiteflies during the vegetables-free period. This pest is reported to be an efficient vector of Gemini virus (Shrestha *et al.* 1997). Garg *et al.* (2001) reported that whitefly act as a vector which transmits potato apical leaf curl virus which causes potato apical leaf curl disease. This insect damages the crop by extracting large quantities of phloem sap, which can result in yield reduction (Muniyappa 1980). Lakra (2003) observed that due to leaf curl transmitted by whitefly in early sown potato (1<sup>st</sup> week of October) severe yield losses were recorded at Hisar. The peak period of

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activity of whitefly in western Uttar Pradesh was from October-November (Raj 2003).

The yield loss by whitefly is mainly due to transmission of *Tomato leaf curl New Delhi virus*-potato ToLCNDV (Bhatnagar 2007). Whitefly, *Bemisia tabaci* is already established as one of the important sucking pest, not only sucks the sap from tender parts of potato but also transfer of ToLCNDV, resulting potato apical leaf curl disease (Chandel *et al.* 2010). It was previously unknown on potato in India and the first report of ToLCNDV was made in Hisar around 2000 (Garg *et al.* 2001). Now, the whitefly is the main threat to potato seed production and causing substantial losses in healthy potato seed production (Bhatnagar 2007). Recently, 40-75% infection was recorded in cultivars grown in the Indo-Gangetic plains of India (Venkatasalam *et al.* 2011). Whitefly transmits viruses of the genus Begomovirus (Family: Geminiviridae) mostly in the tropics and subtropics. Detection of this virus is important in potato to check the degeneration of potato. Now PCR technique is standardize for detection of this virus (Sridhar *et al.* 2016). This virus is responsible for causing heavy losses in potato crop specially susceptible varieties (Chandel *et al.* 2010).

Several conventional and synthetic insecticides have been used against whitefly on various crops in the past (Thakur *et al.* 1991, Malik *et al.* 2005). The high concentration of chemicals and more number of sprays are required to suppress the population of whitefly on potato. The problem is further aggravated due to the development of resistance against these conventional and synthetic insecticides being used by the farmers. Therefore, the combination of Neonicotinonids- Imidacloprid and Thiamethoxam needs to be evaluated for managing the whitefly. In view of that, it was thought to detect the incidence and time of ToLCNDV acquiring by potato cultivars and also to evaluate the reaction of promising potato cultivars against whitefly and incidence of ToLCNDV under sprayed and unsprayed conditions along with its economics.

#### MATERIALS AND METHODS

Two sets of field and laboratory experiments were conducted to study the incidence of whitefly, yield, cost benefit ratio of promising potato cultivars under sprayed and unsprayed conditions during main crop seasons of 2013-14 and 2014-15 followed by recording the incidence of whitefly in potato cultivars, detection of *Tomato leaf curl New Delhi virus*-potato (ToLCNDV) in whitefly and potato cultivars through PCR during the year 2014, respectively at ICAR-Central Potato Research Institute-Regional Station, Modipuram, Meerut. In first set of experiment nine potatoes cv. Kufri Pukhraj, Kufri Anand, Kufri Sadabahar, Kufri Bahar, Kufri Chipsona-1, Kufri Khyati, Kufri Chipsona-3, Kufri Surya and Kufri Garima were planted in the first week of November following all recommended agronomic practices. All the nine potato cultivars were planned in RBD design, replicated thrice in a plot size of 3 × 2 m. Each plot was separated by a gap of 1.75m for reducing drift of sprays in unprotected plots of each potato cultivars. A total

of three applications (Imidacloprid 17.5% SL (4ml/10 l), Thiamethoxam 25WG (5g/10 l) followed by Imidacloprid 17.5% SL (4ml/10l) were given during crop growth in protected plot. The whitefly incidence was recorded before spraying as pre-treatment count and after spraying as post treatment counts at weekly intervals in protected and unprotected plots. The whitefly population of both nymphs and adults were counted during early morning hours on upper, middle and lower leaves from 5 selected and tagged plants.

The yield data was recorded from net plot after removing haulm in the last week of February in the respective years. The plant growth parameters like size of tubers, yield, increase and percent increase in yield were calculated for calculating cost benefit of each plant protection treatments in comparison to unsprayed plots. For benefit cost analysis, record of costs incurred in each treatment and that of control were maintained. Similarly, the price of the harvested tubers of each protected potato cultivars and that of unprotected were calculated at market rate. Benefit-cost analysis was calculated by using the following formula

$$\text{Cost: benefit ratio} = \frac{\text{Net return (₹/ha)}}{\text{Cost of treatment (₹/ha)}}$$

In the second set of experiment, 22 potato cultivars were planted with out replication in a plot size of 3 × 2 m in September during the year 2014 without protection. The details of potato cultivars is as follows K. Anand, K. Arun, K. Badshah, K. Bahar, K. Chandramukhi, K. Chipsona – 1, K. Chipsona – 2, K. Chipsona -3, K. Chipsona -4, K. Frysona, K. Garima, K. Gaurav, K. Himalini, K. Himsona, K. Jyoti, K. Khyati, K. Pukhraj, K. Puskar, K. Sadabahar, K. Satlej, K. Sinduri, K. Surya . The whitefly incidence was recorded at 10 days intervals and 10 numbers of adult and nymphs were used for detection of ToLCNDV. The whitefly population of both nymphs and adults were counted during early morning hours on upper, middle and lower leaves from 5 selected and tagged plants. The incidence of ToLCNDV was detected in potato cultivars at weekly interval using PCR.

Each leaf sample (100 mg) was washed thoroughly and dried and ground in 700 µl of CTAB buffer (2.0 % w/v) CTAB, 20 mM EDTA (pH-8.0), 1.4 mM NaCl, 100 mM Tris-Cl (pH-8.0) and 0.2 % (v/v) 2-Mercaptoethanol. The extract was incubated at 65°C for 30 min and centrifuged at 12000 rpm for 1 min. 700 µl of chloroform: isoamy alcohol (24:1 µl) was added to the supernatant and centrifuged at 12000 rpm for 20 min at room temperature. The aqueous phase was collected and added 2/3<sup>rd</sup> vol. of isopropanol and mixed gently by inversion and centrifuged at 12000 rpm for 10 min to spin down the DNA pellet. The pellet was washed with 70% alcohol by spinning it at 10,000 rpm for 10 min at 4°C. The pellet was dried and dissolved in 30 µl of sterile water or TE buffer. Finally, 2.0 µl of RNase (10.0 mg/ml) was added to the purified DNA and incubated at 37°C for 30 min. The concentration and quality of DNA was checked by Nanodrop -2000 Spectrophotometer.

PCR was performed using virus specific primers designed from CP sequences available in GenBank. The forward and reverse primers designed were LCVCPF1-5' AAAGTCCATGTGTGTTAGTGATGTTACC-3' and LVCPR1- 5' TAGAAATAGATCCGGATTTCAAAGTA-3' (Jeevalatha *et al.* 2013). The coat protein gene of total isolated DNA of each sample was amplified. The PCR amplification was conducted in 20.0 µl of reaction volume containing 2.0 µl of template DNA (50 ng of total DNA), 2.0 µl of 10 × PCR buffer (100 mM Tris-HCl pH-9.0, 500 mM KCl, 15 mM MgCl<sub>2</sub>), 0.5 µl of 2 mM dNTPs, 0.5 µl of 10 µM forward and reverse primers respectively and 1.0 µl of (1.0 U/ µl) Red *Taq* DNA polymerase and 14.0 µl of sterile double distilled water. The thermal cycler was programmed for initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min and final extension of 10 min at 72°C. The PCR cycles were carried in Gene Amp PCR system 9700 (Applied Biosystem). The amplified product was subjected to electrophoresis in 1% Agarose gel prepared in 1 × TAE (0.04M M Tris-acetate, 1 mM EDTA, pH-8.0) buffer staining with (0.5 mg/ml) ethidium bromide. Electrophoresis was carried out at 90 volt for 1-2 h. An aliquot of (500 ng) 1 Kb DNA ladder (MBI Fermentas) is used as molecular weight marker. The gel was visualized in UV trans-illuminator in gel documentation unit (Syngene G: Box) and scanned.

## RESULTS AND DISCUSSION

The mean data of whitefly incidence was recorded on nine promising potato cultivars (Kufri Pukhraj, Kufri Anand, Kufri Sadabahar, Kufri Bahar, Kufri Chipsona-1, Kufri Khyati, Kufri Chipsona-3, Kufri Surya and Kufri Garima) under protected (three spray schedule of insecticide – Imidacloprid 17.8 SL (4 ml/10 l), Thiamethoxam 25 WG (5g/10 l) and Imidacloprid 17.8 SL and unsprayed conditions (Fig 1 and 2). The data revealed that the trend of whitefly population buildup on potato cultivars was almost similar during both the years. The population of *Bemisia tabaci* was active on potato crop throughout the crop growth. The incidence of whitefly was recorded on all the potato cultivars. However, the population of whitefly remains low in protected condition due to three sprays schedule of insecticides on 04.12, 24.12 and 13.01 during both the years. However, the population of whitefly varied significantly on potato cultivars due to their different tolerance level against whitefly.

The whitefly population was low and significantly varied in protected potato cultivars as compared to unprotected cultivars. The maximum incidence of whitefly (15.33/5 plant) was recorded in Kufri Pukhraj followed by K. Chipsona-1 (15.0/5 plant) when crop remains unsprayed. In protected potato cultivars, the minimum incidence was recorded in Kufri Bahar (5.0/5 plant) and Kufri Khyati (7.0/ 5 plant) when crop was protected by three sprays (Fig 1 and 2). The population of whitefly varied (1.0-5.0/5 plant) in sprayed treatments on potato cultivars. Subsequently, the population of whitefly declined quickly

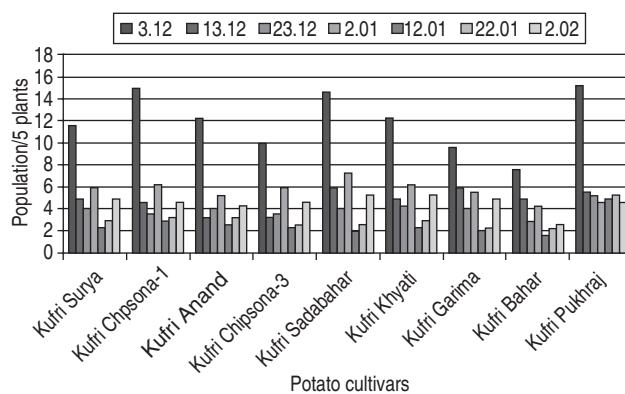


Fig 1 Mean whitefly population on potato cultivars under unsprayed condition

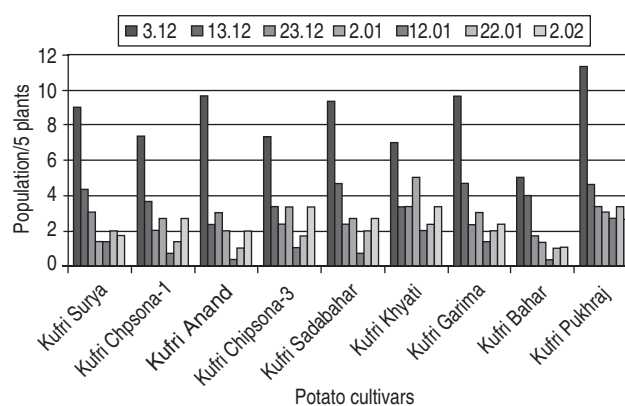


Fig 2 Mean whitefly population on potato cultivars under sprayed condition

on all the potato cultivars due to change of environmental condition, i.e. decrease of temperature, higher RH and maturity of potato cultivars in February. Almost same trend of whitefly incidence was recorded on potato cultivars under sprayed condition but the population of whitefly was comparatively low. The higher population of whitefly was recorded in the year 2013-14 in comparison of 2014-15. Ram Kishore *et al.* (2005) recorded the highest build up of whitefly in the second fortnight of October on early potato crop in the Western zone of Uttar Pradesh. In the main crop season, the whitefly population was low in comparison of early crop season. Uthama Swamy (2001) observed that high temperature boosted growth and development of whitefly which confirmed the present finding, i.e. decrease in temperature and increased relative humidity from 1<sup>st</sup> week of November did not favor the buildup of whitefly.

Number of oversize tubers statistically differed among the nine potato cultivars under sprayed and unsprayed condition while seed size and undersize tubers did not showed significant difference among potato cultivars. (Table 1). The maximum number of oversize tubers (13%) was recorded in potato cultivar K. Khyati followed by K. Garima (11.93%) under protected condition. However, maximum number of seed size tubers (68.5%) was recorded in potato cultivar. K. Bahar followed by K. Pukhraj (65.1%) and K. Anand (64.07%) under protected condition, while



least number of undersize tubers (26.9%) was recorded in K. Bahar followed by K. Pukhraj (29.0%) under same condition (Table 1). Almost similar type of grading trend was recorded when crop was unsprayed.

Mean total tuber yield differed significantly among potato cultivars under sprayed and unsprayed conditions. Table data revealed that all the potato cultivars exhibited increase in total tuber yields under protected condition as compared to unprotected condition (Table 1).

Potato cultivars – K. Garima, K. Khyati, K. Pukhraj, and K. Anand recorded the highest mean tuber yield (32.52 t/ha) (32.07 tonnes/ha), (30.2 tonnes/ha) and (30.17 tonnes/ha), respectively under sprayed condition, similarly these cultivars were also recorded maximum yield even (Table 1) under unsprayed condition. The maximum percent increase in tuber yield of protected cultivars (2.13 tonnes) was recorded in Kufri Khyati followed by Kufri Garima (1.94 tonnes) and Kufri Bahar (1.83 tonnes) as compared to unsprayed cultivars (Table 1). The cost: benefit ratio was found highest in the three sprays treatment of cv. K. Khyati (2.64) followed by Kufri Garima (2.40) and Kufri Bahar (2.27). Therefore in the light of above results and cost: benefit ratio, three sprays of insecticides (imidacloprid, thimethoxgam and imidacl prid) first at emergence, second and third at 30 and 42 days after emergence may be recommended to the farmers for main crop to get potential yield under protection umbrella.

The data of whitefly incidence was recorded on early planted twenty two potato cultivars revealed that all the potato cultivars were exhibited high incidence of whitefly population. Initially in October, the population of whitefly showed upward trend (48-31.3/plant) Subsequently, the population of whitefly declined quickly on all the potato cultivars due to change of environmental condition, i.e. decrease of temperature, high RH and maturity of potato cultivars in December (Table 2). The viruliferous nature of whiteflies and potato cultivars with respect to ToLCNDV-potato was determined using virus specific primers designed from CP sequences available in GenBank along with internal control. The PCR results revealed all the 22 potato cultivars acquired PALCV in due course of time, however some of potato cultivars K. Anand, K. Chandramukhi, K. Chipsona 1, K. Chipsona 2, K. Chipsona 4, K. Gaurav, K. Himalini, K. Khyati, K. Pukhraj and K. Satlej showed positive reaction very quickly itself in October (Table 2). This showed these cultivars are highly susceptible and quickly acquired virus under the favorable environmental condition. The sample of whitefly drawn from potato cultivars in October also showed positive reaction as per the retaining of virus with almost same trend. The virus acquired by this viruliferous whitefly further spread in other potato cultivars. The above results revealed host plant resistance is less applicable in most of the popular potato cultivars. They did not differ considerably in susceptibility to ToLCNDV and whiteflies. A perusal of whitefly incidence data revealed that whitefly population showed different order of preference on potato cultivars. Host-plant resistance

Table 1 Yield, tuber characters of potato under protected and unprotected condition (pooled data 2013-14 and 2014-15)

Potato cultivar	Yield and tuber characters of potato under unsprayed condition						Yield and tuber characters of potato under sprayed condition						Increase in yield in protected treatments and B: C ratio		
	Over size tubers (%)	Seed size tubers (%)	Small size tubers (%)	Total tuber Yield (t/ha)	Over size tubers (%)	Seed size tubers (%)	Small size tubers (%)	Total tuber Yield (t/ha)	Yield (t/ha)	Increase in tuber yield over control (t)	Cost* of potato (₹)	Cost of treatment** (₹)	Net profit (₹)	Cost: benefit ratio	
Kufri Pukhraj	4.71	63.29	33.27	28.73	5.90	65.10	29.00	30.20	1.43	8580	4836	3744	1:1.77		
Kufri Anand	5.38	62.00	31.21	28.67	5.93	64.07	30.00	30.17	1.43	8580	4836	3744	1:1.77		
Kufri Sadabahar	10.35	48.14	41.43	21.80	11.90	49.40	38.70	23.33	1.00	6000	4836	1164	1:1.24		
Kufri Bahar	2.87	67.53	30.39	27.76	4.60	68.50	26.90	29.53	1.83	10980	4836	6144	1:2.27		
Kufri Chipsona-1	2.83	52.76	44.37	23.90	4.07	54.13	41.80	25.40	1.33	7980	4836	3144	1:1.65		
Kufri Khyati	11.80	44.19	40.57	29.27	13.00	45.53	41.47	32.07	2.13	12780	4836	7944	1:2.64		
Kufri Chipsona-3	7.36	51.03	41.57	25.42	9.33	51.73	39.10	26.87	1.23	7380	4836	2544	1:1.52		
Kufri Surya	9.40	46.53	38.17	20.65	10.10	47.70	42.20	22.10	0.97	5828	4836	984	1:1.20		
Kufri Garima	10.38	50.47	41.65	29.91	11.93	51.60	36.47	32.52	1.94	11640	4836	6804	1:2.40		
SEm+1	1.84	5.73	5.32	1.85	1.85	5.70	5.63	1.42							
CD (P=0.05)	5.58	NS	NS	5.62	5.60	NS	NS	4.30							

Three sprays of insecticides (imidacloprid, thimethoxgam and imidaclprid) on 25.11, 09.12 and 16.12. \*Mean tuber rate-700/q, \*\* Cost of chemical +spraying cost + labour

Table 2 Whitefly population on early planted potato cultivars and detection of ToLCNDV in potato cultivars and whitefly

Potato cultivars	Average whitefly population/plant				PCR results of ToLCNDV detected from				
					Whitefly	Potato cultivars			
	21.10	31.10	12.11	22.11		21.10	31.10	12.11	22.11
K. Anand	43.7	30.00	14.33	7.33	+	+	+	+	+
K. Arun	48.0	23.67	13.33	7.33	-	-	-	+	+
K. Badshah	41.7	20.00	24.67	5.33	-	-	+	+	+
K. Bahar	43.3	29.67	17.33	8.67	-	-	-	-	+
K. Chandramukhi	42.0	21.33	14.00	6.67	+	+	+	+	+
K. Chipsona-1	44.0	27.00	15.00	10.33	+	+	+	+	+
K. Chipsona-2	43.0	17.67	11.00	4.67	-	-	-	-	+
K. Chipsona-3	41.7	23.00	21.33	9.33	+	+	+	+	+
K. Chipsona-4	41.7	29.67	20.67	11.33	+	+	+	+	+
K. Frysona	45.3	19.67	19.67	10.00	-	-	-	+	+
K. Garima	31.3	22.67	17.67	10.00	-	-	+	-	+
K. Gaurav	35.3	24.00	21.67	8.33	+	+	+	+	+
K. Himalini	43.3	22.00	10.33	7.00	+	+	+	+	+
K. Himsona	46.0	23.67	18.00	10.67	-	-	-	+	+
K. Jyoti	45.7	19.67	14.00	7.67	-	-	-	-	+
K. Khyati	46.3	26.67	17.00	11.00	+	+	+	+	+
K. Pukhraj	40.3	36.00	22.00	8.00	+	+	+	+	+
K. Pushkar	44.3	20.67	19.67	10.33	-	-	+	-	+
K. Sadabahar	42.0	26.67	20.33	10.33	-	-	-	+	+
K. Sindhuri	43.3	25.00	13.00	9.00	-	-	-	-	+
K. Surya	43.3	26.33	15.67	10.00	-	-	-	+	+
K. Sutlej	47.0	30.33	9.00	8.67	+	+	+	+	+

is less applicable at present because most of the popular potato cultivars do not differ considerably in susceptibility to ToLCNDV and whiteflies. However, cv. Kufri Bahar is tolerant to ToLCNDV. Whiteflies feed on the leaves of this cultivar, but virus infection has not been recorded even under epidemic conditions (Lakra 2003). Now, cv. Kufri Bahar is more tolerant to ToLCNDV. The buildup of whitefly on potato cultivars was also influenced by weather factors (Bhatnagar 2007). Presently, the whitefly has become a severe threat to potato crop by sucking the sap directly from the tender parts of potato and transmitting ToLCNDV (Bhatnagar 2007 and Lakra 2003). The yield loss by whitefly is mainly due to transmission of potato apical leaf curl virus (Bhatnagar 2007). The problem has been further aggravated due to the development of resistance against conventional pesticides being used by the farmers. Bhatnagar *et al* (2013) reported that Imidacloprid as a seed treatment and spray has effectively suppressed the sap feeder population in potato crop.

Our finding further confirmed of earlier finding of Lakra *et al*. (2005) who reported that late sowing of autumn potato crop reduce whitefly incidence. The maximum yield and highest cost: benefit ratio was found in the three sprays treatment of cv. K. Khyati followed by Kufri Garima. Therefore, present study revealed that neonicotinoids is

still an important component of effective whitefly IPM programme of potato and potato crop should be well protected by insecticides and when there will be any buildup of whitefly on crop, especially crop grown for seed purpose.

Thus, it is concluded that the PCR reaction confirmed the presence of ToLCNDV in all the 22 potato cultivars in due course of time, however some of potato cultivars K. Anand, K. Chandramukhi, K. Chipsona 1, K. Chipsona 2, K. Chipsona 4, K. Gaurav, K. Himalini, K. Khyati, K. Pukhraj and K. Satlej showed positive reaction very quickly itself in October. These cultivars are highly susceptible and quickly acquired virus under the favorable environmental condition. Therefore, these cultivars should not be grown in whitefly endemic area otherwise these potato cultivars must be well protected using Imidacloprid followed by Thiamethoxam and Imidacloprid insecticides from germination to 40-45 days after planting as per the buildup of whitefly population and effective management of apical leaf curl disease, especially potato crop grown for seed purpose.

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