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Incidence and Severity of Tomato Yellow Leaf Curl Virus under Phytopesticidal Management

[1]S.B. Bhyan, [1]M.A.H. Chowdhury, [2]M.M. Alam and [1]M.S. Ali
[1]Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Genetics and Plant Breeding, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh

Abstract: The experimentation was made to investigate the incidence of tomato yellow leaf curl virus and its effect on the nutritional components in fruits and chlorophyll content in the leaves of *Lycopersicon esculentum* taken under phytopesticidal management. Phytopesticidal treatments used in the study were extracts of neem (*Azadiracta indica*) fruits, garlic (*Allium sativum*) bulbs, karamja (*Pongamia pinnata*) leaves and mehogoni (*Swietenia macrophylla*) seeds. Plots with no phytopesticidal treatments were used as control. Plants under no management were found to be in highest incidence of the virus. There were significant role of phytopesticides in reducing the incidence and severity of tomato yellow leaf curl virus. Among the treatments, Karamja extract performed best against TYLCV in all respect of yield and yield related parameters of tomato. Viral infection in tomato plants caused a negative effect on fruit nutrition. Though negative effect of TYLCV infection was found for chlorophyll A content in tomato leaves, but for chlorophyll B, it caused no significant effect.

Key words: Incidence, severity, phytopesticides, plant extracts, tomato and TYLCV

INTRODUCTION

Plant virus, an important biotic factor, causes severe constraints on the productivity of a wide range of economically important crops world wide (Das Gupta *et al*., 2003). There are a large number of viruses that infect plants and cause serious losses in production both in terms of yield and quality. Tomato Yellow Leaf Curl Virus (TYLCV) is one of the most economically important virus causing disease in tomato plant world-wide it is present in most Mediterranean countries and parts of sub-Shaharan Africa, Asia, Japan, Australia, the Caribbean islands and USA (Czosnek *et al*., 1990; Nakhla and Maxwell, 1998; Polston *et al*., 1999). Usually the disease causes a loss of the order of 28-92%, but may be as high as 100% (Nakhla and Maxwell, 1998; Moriones and Navas-Castillo, 2000).

For a long time, scientists have been trying to control virus diseases by using different methods. Out of several methods, protection of vector transmission by using chemicals is widely recognized. But, presently, scientists have become very much concerned about the uses of phytopesticides in controlling vectors instead of chemo pesticides. Various experiments using plant extracts in human and animal health protection, agriculture and household pest management have been particularly promising (Pascual-Villolobos and Robledo, 1999; Scott *et al*., 2004). The apparent societal hope for using plant extracts in place of more traditional pesticides has also increased the attention paid to natural products in the past decade (Duke *et al*., 2003). Plants belonging to Araliaceae, Asteraceae, Cannabaceae,

Corresponding Author: Salma Begum Bhyan, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh, Bangladesh

Caprifoliaceae, Chenopodiaceae, Lamiaceae, Poaceae and Scrophulariaceae families are known to produce monoterpenes, sesquiterpene lactones and triterpenes; all of which may have commercial applications (Heywood *et al*., 1977; Tucker and Maciarello, 1994; Barney *et al*., 2005). These chemicals are known to affect insects in various ways. The chemicals may act as antifeedants or repellents as well as pesticides (Gökçe *et al*., 2005, 2006). However, a number of virus inhibitor of plant origin has been studied by many workers, but a few of them have been recognized (Verma *et al*., 1995). Some extracts have already been selected from a number of plants to inhibit the infection and multiplication of plant viruses (Bennet, 1953). There are also few works in managing the viral diseases of plants by using plant extracts. Therefore, the present study was undertaken to determine the rate of incidence of okra mosaic virus and its severity on yield and nutrition of okra plants under the treatments of plant extracts.

MATERIALS AND METHODS

The experimentation was undertaken at the experimental fields of the Department of Plant Pathology; and laboratories of the Department of Agricultural Chemistry and Biochemistry, Bangladesh Agricultural University (BAU), Mymensingh during November, 2005 to April, 2006. Four different plant extracts *viz.* extracts of neem (*Azadiracta indica*) fruits, garlic (*Allium sativum*) bulbs, karamja (*Pongamia pinnata*) leaves and mehogoni (*Swietenia macrophylla*) seeds were used as phytopesticidal agents. Plots with no phytopesticidal treatments were used as control. The treatments were laid out in a randomized complete block design with four replications. Suspension of different plant extracts containing 50% (w/w) plant extract/water with 10% acetone sprayed individually by hand sprayer.

The research work included both field and laboratory experiments. In the field, the suspension of different plant extracts were sprayed individually by hand sprayer. First spray was done after 30 days of transplanting. The plant extracts were sprayed 3 times at 15 days interval. All exposed surface of the plants including leaves, buds, twigs, branches and fruits were sprayed. Control plots were sprayed with distilled water.

Data were recorded on individual plant basis from 10 randomly selected plants (5 healthy and 5 diseased) in each plot. Sampling was done at one stage of the plants. Virus infestation was examined carefully in the plants from top to bottom. Fruits were harvested in the morning considering the uniformity in size, shape and color at each maturity stage. The collected fruits were carried in gunny bags and then half of the collected samples were immediately transferred to the storage rooms and the rest were in the laboratory for chemical analyses. Proper care was taken while harvesting and handling to avoid any mechanical injury.

Five healthy and five diseased plants were selected randomly from each plot for data collection in such a way that the border effect could be avoided for the highest precision. Data recorded in the field experiment were percentage of infested plants plot^{-1}, percentage of leaf area infestation, plant height, number of flowers plant^{-1}, number of fruits plant^{-1}, percentage of fruit setting, individual fruit weight and yield plant^{-1}. Estimation of vitamin C, nitrogen, phosphorus, potassium, calcium, iron and protein content in the fruits and chlorophyll content in the leaves were done to evaluate the severity of the virus under phytopesticidal treatments. The amount of chemical components was analyzed using standard analytical methods.

Total nitrogen was determined by Kjeldahl method using $CuSO_4$ and K_2SO_4 mixture (1:9) as catalyst following the method of Jackson (1958). Then the percentage of protein was calculated by multiplying the per cent nitrogen of the sample with a factor of 6.25 (Jackson, 1973). Potassium content of the fruit extract was determined by Flame Photometer measuring the intensity of light emitted by potassium at 768 cm wave length as described by Jackson (1973). The fruit extract were analysed for iron by atomic absorption spectrophotometric method at 248.3 cm wave length as described by Olsen (1982). Calcium content of fruit extract was determined by atomic absorption spectrophotometric method (Issac and Kirber, 1971). For calcium content analysis, 5 mL of 3.25% $LaCl_3$ solution was added with 50 mL extract and the absorbance reading was taken at 422.7 cm wave lengths. Chlorophyll content of leaf was determined extracting with 80% acetone method as proposed by Witham *et al*. (1986). Vitamin C content was measured as followed by Ranganna (1994).

Data were analyzed for ANOVA with the help of a computer package program of MSTAT. A two way ANOVA was made by F variance test. The pair comparisons were performed by Least Significant Difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984). Regression line was prepared using MS Excel.

RESULTS AND DISCUSSION

Disease Incidence

The incidence of tomato yellow leaf curl virus as measured by the rate of infected plants plot^{-1} and the rate of leaf area infection plot^{-1} was found to be significant due to the application of phytopesticides (Table 1). The highest percentage of disease infected plants plot^{-1} was observed in those plots where no phytopesticides were applied i.e., in control treatments (Fig. 1). While, the lowest percentage of infection was found in the tomato field treated with *karamja* extract. Other phytopesticides showed more or less similar influence on the incidence of the disease caused by TYLCV in tomato plants.

Plants under control treatments showed highest leaf area infection at three different growth stages and lowest under the treatments of karamja and Garlic extracts (Fig. 1). In all treatments, higher leaf area infection was found at the later stage of growth (at 80 DAT) than previous stages. However, nearly same rate of leaf area infection was observed at 60 DAT and 80 DAT in karamja and mehogoni extract treated plants. It was thus revealed that there would have a negative interaction between phytopesticides and TYLCV for disease incidence in tomato plants.

Effect on Yield Parameters

Data on yield related characters reflected the significant effect phytopesticides on TYLCV in tomato plants (Table 2). Investigations made on healthy plants revealed that all phytopesticidal treatments showed similar influence on plant height, though control treatment gave slightly lower height. Highly significant variation was observed on infested plants. Application of phytopesticides showed to increase the height of the infected plants significantly. Experimental plots treated with karamja produced tallest plant (53.50 cm), but in control treatment infected plants were smallest in height (35.00 cm). The reduced height in control plot is due to viral infection as observed by Hasan *et al*. (1993) and Ndunguru and Rajabu (2004). Neem extract (50.25 cm) and Garlic extract (52.25 cm) showed statistically similar performance on the height of infected plants (Table 2).

There was a significant influence of plant extracts on tomato yellow leaf curl virus for flower production in tomato as the lowest number of flowers was produced under control treatments. Among

Table 1: ANOVA for incidence of TYLCV on tomato plant under different phytopesticidal treatments

Sources of variation	df	Mean sum of square: Infected plant plot^{-1}	Leaf area infection plant^{-1} (%): 40 DAT	60 DAT	80 DAT
Treatments	4	39.630***	716.000***	1273.000***	39.620***
Error	12	1.256^{ns}	2.391^{ns}	5.854^{ns}	1.256^{ns}

***: Significant at 0.1% level; ns: Non significant

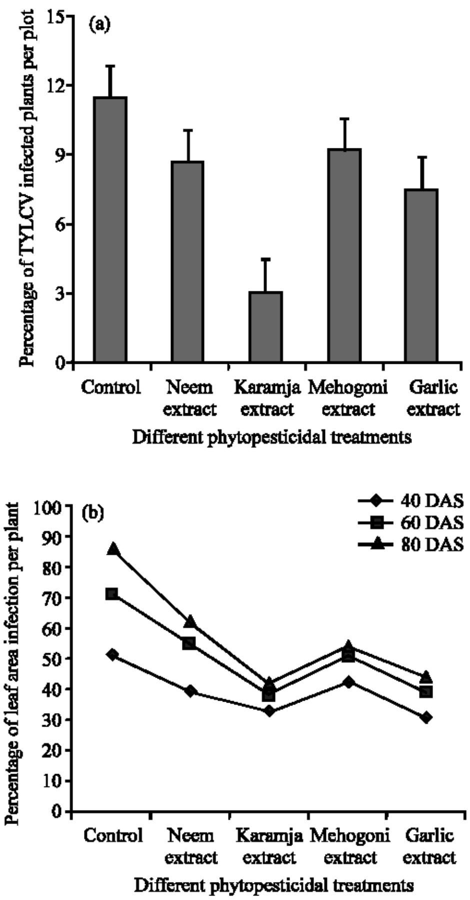

Fig. 1: Incidence (a) Plant number basis and (b) Leaf area basis) of tomato yellow leaf curl virus under different phytopesticidal treatments

the healthy plants, karamja extract (100.8) showed best performance for the production of flowers per plant; though statistically identical results were observed in case of mehogoni (87.5) and Garlic extract (93.75). In infested plants karamja extract performed best (57.75) for the production of flowers plant^{-1}, though statistically similar performance was also found on the phytopesticidal treatments of Garlic (53.75) extracts (Table 2).

The number of fruits plant^{-1} and the rate of fruit setting were increased due to the application of phytopesticides. Fruit formation in the healthy plants ranged from 12.25 to 17.25 and in the virus infected plants from 4.00 to 7.25. In each plant groups, highest number of fruits was produced in plants treated with karamja extract and lowest in control plants where no plant extracts were applied. Garlic extract also showed same performance as karamja extract in infested group of plants, while in healthy it was ranked second. Therefore, karamcha and Garlic extracts could be considered as good phytopesticidal agents against TYLCV for the production of fruits in tomato. There was no significant variation for fruit setting among the treatments in healthy plants. But virus infected plants showed significant results for this character. Percentage of fruit setting was ranged from 81.96 to 83.92 in healthy plants and 70.65 to 80.99 in infested plants. Virus infected plants treated with phytopesticides gave almost 80% fruit setting but in control plants it was near 70% (Table 2).

Table 2: Effect of phytopesticides on TYLCV for yield related traits of tomato

Treatments	Plant height (cm)		No. of flowers plant^{-1}		No. of fruits plant^{-1}		Fruit setting (%)		Yield per plant (kg)	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Control	60.80^{b}	35.00^{d}	76.30^{c}	19.50^{c}	12.25^{d}	4.00^{c}	83.92^{a}	70.65^{b}	1.040^{d}	0.160^{d}
Neem extract	63.50^{ab}	50.30^{bc}	83.80^{bc}	36.80^{b}	14.50^{c}	5.50^{b}	82.65^{a}	80.75^{a}	1.190^{c}	0.380^{c}
Karamja extract	65.50^{a}	53.50^{a}	100.80^{a}	57.80^{a}	17.30^{a}	7.25^{a}	82.50^{a}	80.99^{a}	1.280^{a}	0.540^{a}
Mehogoni extract	62.50^{ab}	49.30^{c}	87.50^{abc}	49.00^{ab}	15.00^{bc}	6.75^{a}	81.96^{a}	80.63^{a}	1.230^{bc}	0.540^{a}
Garlic extract	66.00^{a}	52.30^{ab}	93.80^{ab}	53.80^{a}	16.00^{b}	7.25^{a}	82.82^{a}	80.86^{a}	1.260^{ab}	0.470^{b}
lsd	3.75	2.41	15.41	13.27	1.20	0.92	3.24	1.72	0.048	0.048

Values with different superscripts are significantly different

Table 3: Effect of phytopesticides on TYLCV for fruit and leaf nutrition of tomato

Treatments	Vitamin C content (mg 100g^{-1})				Nitrogen (%)			
	Green		Ripe		Green		Ripe	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Control	12.32^{c}	4.70^{b}	19.74^{c}	5.06^{c}	0.36^{c}	0.07^{c}	0.37^{c}	0.12^{d}
Neem extract	13.39^{bc}	5.59^{b}	25.74^{a}	16.35^{b}	0.38^{a}	0.21^{b}	0.38^{b}	0.24^{c}
Karamja extract	14.85^{a}	8.20^{a}	22.84^{b}	16.35^{b}	0.37^{b}	0.27^{a}	0.39^{a}	0.28^{a}
Mehogoni extract	13.81^{ab}	5.46^{b}	22.63^{b}	15.68^{b}	0.38^{a}	0.26^{a}	0.38^{b}	0.26^{b}
Garlic extract	12.64^{bc}	7.19^{a}	23.45^{b}	17.55^{a}	0.36^{c}	0.21^{b}	0.37^{c}	0.24^{c}
lsd	1.33	1.32	0.91	1.09	0.008	0.02	0.005	0.002

Treatments	Phosphorus (%)				Potassium (%)			
	Green		Ripe		Green		Ripe	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Control	0.46^{b}	0.12^{d}	0.47^{a}	0.14^{e}	0.30^{a}	0.11^{c}	0.30^{b}	0.12^{e}
Neem extract	0.47^{a}	0.23^{b}	0.48^{a}	0.23^{d}	0.31^{a}	0.19^{a}	0.32^{a}	0.19^{b}
Karamja extract	0.46^{b}	0.25^{a}	0.47^{a}	0.26^{a}	0.31^{a}	0.19^{a}	0.31^{ab}	0.10^{d}
Mehogoni extract	0.47^{a}	0.23^{b}	0.48^{a}	0.25^{b}	0.31^{a}	0.18^{b}	0.32^{a}	0.19^{b}
Garlic extract	0.46^{b}	0.22^{c}	0.47^{a}	0.24^{c}	0.31^{a}	0.19^{a}	0.31^{ab}	0.20^{a}
lsd	0.005	0.005	0.015	0.002	0.015	0.005	0.015	0.002

Treatments	Iron content (ppm)				Chlorophyll content (mg/100g)			
	Green		Ripe		Chlorophyll A		Chlorophyll B	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Control	210.8^{e}	103.3^{d}	233.0^{d}	111.0^{c}	1.74^{a}	1.12^{b}	2.37^{c}	1.42^{c}
Neem extract	250.0^{b}	181.5^{c}	322.5^{b}	196.0^{b}	1.74^{a}	1.72^{a}	2.37^{c}	1.46^{bc}
Karamja extract	264.0^{a}	192.3^{a}	345.0^{a}	211.5^{a}	1.71^{a}	1.71^{a}	2.45^{a}	1.57^{a}
Mehogoni extract	231.0^{d}	186.3^{b}	312.5^{c}	194.0^{b}	1.73^{a}	1.71^{a}	2.40^{bc}	1.47^{bc}
Garlic extract	240.8^{c}	181.5^{c}	344.3^{a}	195.5^{b}	1.72^{a}	1.72^{a}	2.42^{ab}	1.47^{b}
lsd	1.828	2.424	3.541	3.479	0.05	0.03	0.05	0.05

Values with different superscripts are significantly different

Yield was increased with application of phypesticides in tomato plants. Yield was ranged from 1.036 to 1.280 kg plant^{-1} in healthy plants and 0.1597 to 0.5350 kg plant^{-1} in infested plants. In both groups, highest yield was found to be produced in karamja treated plants and lowest in plants under no use of phytopesticide.

Effect on Nutritional Components in Fruits

Vitamin C content in tomato fruits was higher in healthy plants then in that of virus infected plants. In control plot, healthy plants contained approximately three to four times higher amount of vitamin C then that of TYLCV infected plants (Table 3). But in phytopesticide treated plots the ratio of vitamin C content in the fruits of healthy and infested plants were much lower, which indicates the effect (direct or indirect) of phytopesticides on TYLCV. Among the treatments, karamja extract were best in performance for the vitamin C content in green fruit of healthy (14.85 mg 100 g^{-1}) and of TYLCV infected plants (8.195 mg 100 g^{-1}). For the vitamin C content of ripe fruit, neem extract ranked first (25.74 mg 100 g^{-1}) among the treatments in healthy plants and Garlic extract (17.55 mg 100 g^{-1}) in virus infected group (Table 3).

Irrespective of treatments, healthy plants contained more nitrogen then infected plants, which indicated that virus hampered the absorption and storage of nitrogen in tomato fruits (Table 3). Though statistically dissimilarities were observed in the nitrogen content of the healthy plants, but the range was narrower in this case. In green fruits, healthy plants showed a range of nitrogen content of 0.355 to 0.382% and in ripe fruits it was 0.372 to 0.391%. In green fruits of healthy plants, highest nitrogen content was observed in plants under mehogoni treatment which was statistically similar to neem extract. Lowest performance was found to be in control treatment. In ripe fruits best performance went to karamja treatment and least to Garlic extract (Table 3).

Interference of TYLCV on host phosphorus content had been observed as the healthy plants contained more phosphorus then infected plants in green and ripe fruits. Among the healthy plants, the range of phosphorus content in green fruits was 0.463 to 0.473% and in ripe fruits 0.465 to 0.477%. In ripe fruits of healthy plants, all the treatments showed statistically similar performance on phosphorus content, though in virus infected plants significant difference was observed. TYLCV infected plants of control plots showed lowest level of phosphorus content in both green (0.123%) and ripe fruits (0.144%). Highest phosphorus content in green and ripe fruits of infected plant was observed in plants under karamja treatment (0.245 and 0.258%, respectively) (Table 3).

Results of the virus infected plants revealed the greater importance of phytopesticidal action against TYLCV. It was observed that control treatment contained least amount of potassium both in green and ripe fruits (0.071 and 0.118%, respectively) of infected plants. While other treatments contained above 0.21% in green fruits and above 0.24% in ripe fruits. It would be due to phytopesticidal action (direct / indirect) on TYLCV for potassium content in fruits. It was indicated that fruits of TYLCV infected plants treated with karamja contained highest amount of potassium both in green (0.274%) and ripe fruits (0.281%), though mehogoni showed similar result in green fruits (0.257%) (Table 3).

In healthy plants, the range of iron content in green fruits was 210.8 to 264.0 ppm and in ripe fruits 233.0 to 345.0 ppm. In TYLCV infected plants the range was 103.3 to 192.3 ppm in green and 111 to 211.5 ppm in ripe fruits. Statistically different performance for iron content was exhibited in all cases (Table 3). In all respect, plants under control treatments showed to contain least amount of iron in fruits. While best result was observed in karamja treated plots, though Garlic extract was statistically similar in iron content of ripe fruit of healthy plants (Table 3).

Effect on Chlorophyll Contents in Leaves of Tomato

TYLCV Infected leaves contained less amount of chlorophyll than healthy leaves. No significant difference was observed between the treatments for chlorophyll A content in the leaves of healthy plants (Table 3). The range of chlorophyll A content in healthy plants was 1.711 to 1.741 mg g^{-1} and of chlorophyll B was 2.366 to 2.454 mg g^{-1}. For chlorophyll B content in the leaves, karamja extract was seemed to be the best among the treatments. Though control treatment and neem extract performed least for chlorophyll B content in the leaves of healthy plants, but they were considerably similar in performance with mehogoni extract (Table 3). The range of chlorophyll A content in TYLCV infected plants was 1.121 to 1.721 mg g^{-1} and of chlorophyll B was 1.419 to 1.574 mg g^{-1}. Chlorophyll (both type) content in the tomato yellow leaf curl virus infected plants showed that the amount was least in control plots. For chlorophyll A content, the infected plants treated with phytopesticides exhibited around similar performance, while for chlorophyll B karamja treatment was best.

Relationship Between TYLCV Infection and Plant Nutritional Status

Leaf area infection by TYLCV at all growth stages had significant negative interactions with the nutrient contents of green fruits of tomato (Fig. 2). In ripe fruits, the rates of leaf area infection at three

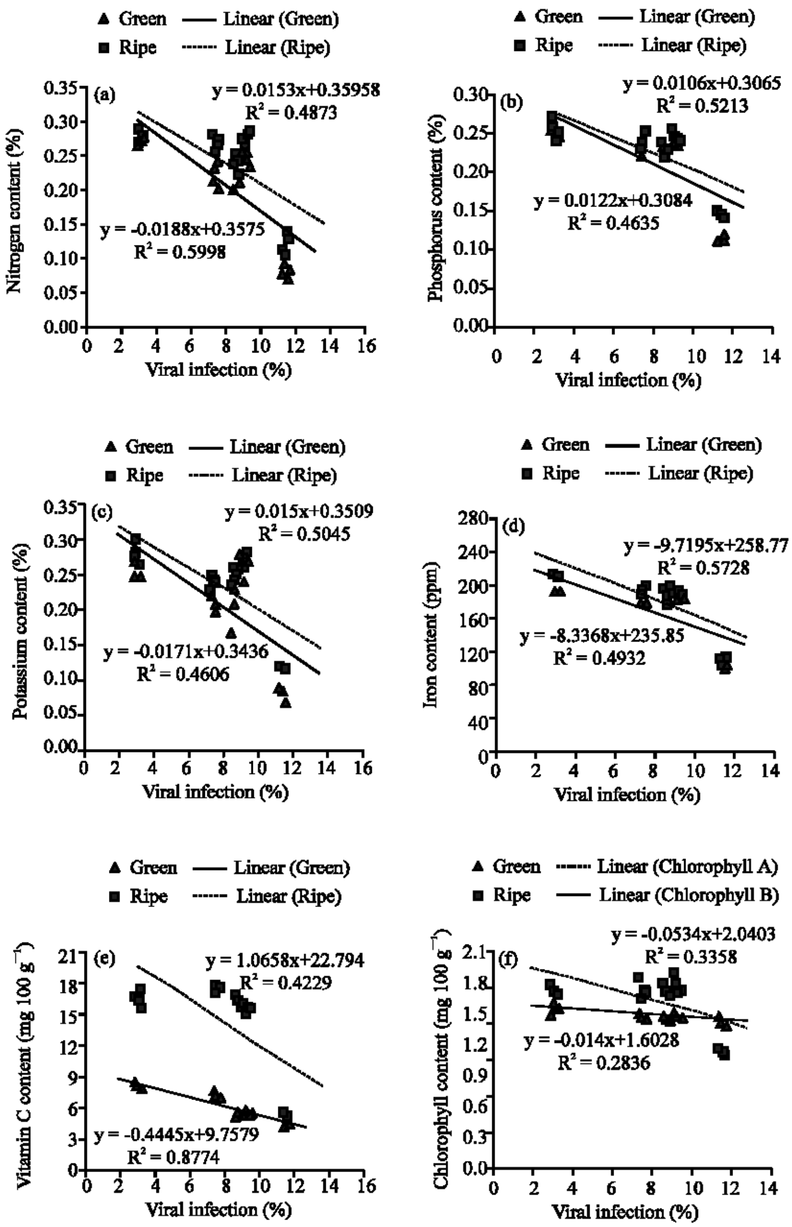

Fig. 2: (a-f) Relationship between nutrient contents of tomato as influenced by TYLCV infection (Leaf area basis)

growth stages were negatively correlated with most of the nutrient contents except potassium content. Results revealed that there was no significant relationship between tomato yellow leaf curl virus infection and potassium contents in the ripe fruits. There were also negative relationships between the rate of leaf area infection and chlorophyll contents in the leaves of tomato.

It was illustrated that TYLCV infection caused the declination of nutrient content in tomato fruits. The drop of nutrient content due to TYLCV infection was slower in case of vitamin C content in green fruits of tomato than ripe fruits (Fig. 2). For nitrogen, phosphorus, potassium and iron content, TYLCV infection showed more or less similar trend of change of nutrient contents in the fruits of tomato. But the trend was different in case of chlorophyll content in the leaves of tomato. tomato yellow leaf curl virus infection caused a steady rate of diminishing of chlorophyll A content in tomato and with no effect on chlorophyll B content (Fig. 2).

Nakhla and Maxwell (1998) and Moriones and Navas-Castillo (2000) reported that yield losses due to TYLCV infection usually ranges from 28-92%, but may be as high as 100%, making tomato production unprofitable. Similar results were also reported by Sastry and Singh (1973), Yassin (1983), Brown (1995) and Sanchez-Campos (1999). Among the phytopesticidal treatments karamcha leaf extract was best in performance in reducing the disease infection as well as in keeping the yield potentiality and optimum nutritional status. But, Tripathy and Tripathy (1982) investigated the antiviral activity of extracts of 17 plants against Bean Common Mosaic Virus (BCMV) and found neem extract to be most potent in reducing the infectivity of virus.

Thus, it can be concluded that phytopesticides played an important role in reducing the intensity and severity of tomato yellow leaf curl virus infection in tomato plants. Infection of TYLCV caused a significant loss of yield parameters and nutrient contents in the fruits and leaves of tomato. More over, disease infection caused by TYLCV had a significant negative effect on fruit nutrition and chlorophyll content in the leaves of tomato except for chlorophyll B content. But, it is not clear from the present experimentation whether the effect of phytopesticides is direct or indirect. So, to have a sound understanding about the present result, future investigation on the biochemical study of the plant extracts and of molecular response of the host due to TYLCV infection is needed.

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