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The effect of tobacco waste application on *Tobacco mosaic virus* (TMV) concentration in the soil

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Abstract

The levels of *Tobacco mosaic virus* (TMV) in a clay soil were monitored for more than two years after tobacco waste (TOW) application. It was incorporated to 15 cm soil depth at four different rates (0, 33, 67 and 100 ton ha^{-1}) in three replicates. Surface soil samples were collected 8, 16, 23 and 30 months after TOW application. The DAS-ELISA method was used to determine the level of TMV in the soil. ELISA absorbance values of TMV were influenced by soil physical properties, plant growth and climatic conditions. The ELISA values were significantly correlated with relative saturation (0.596**), total porosity (−0.502**), organic carbon content (−0.475**) and soil pH (−0.349*). According to path analysis, soil pH showed the highest direct effect (59.7%) on the ELISA values. The highest indirect effects of soil pH and relative saturation on ELISA values were mediated by total soil porosity. Increasing total soil porosity, infiltration rate and mean monthly precipitation decreased the concentration of TMV in the clay soil. Growing lettuce and beans or the presence of host plants in the field increased TMV concentration.

Key words: TMV, tobacco waste, soil properties, temporal monitoring.

Introduction

Tobacco mosaic virus (TMV), which belongs to the genus *Tobamovirus*, infects many crops and causes economic losses in crop production worldwide (Agrios, 1988). TMV is easily transmitted through seed, soil and cultural processes (Tan et al., 1997). Viruses having a stable structure like TMV persist in different soil types over a long period without losing their infection ability (Cheo, Nikoloff, 1980; Allen, 1984).

Agricultural wastes are widely used to increase the organic matter content of degraded soils (Madejon et al., 2001; Tejada, Gonzalez, 2004). Tobacco plant waste has been also used as a soil conditioner (Ic, Gülser, 2008; Candemir, Gülser, 2011). However, the presence of plant residues infected with TMV in soils is the primary source of infection of plants (Broadbent, 1976; Allen 1981). Knowing the viral concentration in soil is important in the control of viral diseases such as TMV that are transmitted through the soil. There have been limited studies on the concentration of TMV in soil resulting from tobacco waste application (Erkan, 1987; Ozgüven et al., 1999; Gülser et al., 2008). In this study, the effects of soil properties and other factors on TMV concentration were investigated for different application rates of tobacco waste.

Material and methods

A field experiment was conducted on *Vertic Haplustoll* soil in the experimental field of the Agricultural Faculty of Ondokuz Mayis University (41.3º N, 36.11º E) in Samsun, Turkey between 2001 and 2004. The total precipitation (mm) and monthly mean temperatures (°C) during the study period between November 2001 and June 2004 are given in Figure 1.

Twelve plots (1.20 m^2) were established in a randomized block design after ploughing to a depth of 15 cm depth and rototilling the soil to have almost uniform soil media. There was a 0.40 m buffer between plots and between rows. Tobacco waste, supplied by the Samsun "Tekel" tobacco processing factory, was incorporated to 15 cm depth at four different rates (0, 33, 67, and 100 tons ha^{-1}) and in three replications in December 2001. After 8 months (July 2002), 16 months (March 2003), 23 months (October 2003) and 30 months (June 2004), disturbed soil samples were taken from 0–20 cm depth in each plot. Infiltration rates were measured at the same time. Lettuce (cv. 'Yedikule') and green bean (cv. 'Atalanta') were grown in the plots in November 2002, and in May 2003 and 2004, respectively.

Bulk density (BD), total porosity (F) and percentages of water-saturated pore ratio or relative saturation (RS) were determined in undisturbed soil samples (Demiralay, 1993). In disturbed soil samples (Kacar, 1994), soil reaction (pH) 1:1 (w:v) was measured in a soil water suspension with a pH meter; electrical conductivity ($EC_{25ºC}$) was determined in the same soil suspension by EC meter; exchangeable cations were extracted with 1 M ammonia acetate; and organic matter (OM) content was calculated by using a modified Walkley-Black method. Infiltration ratio (I) in each plot was measured for the second run of a unit depth of water by using the single ring infiltrometer technique (Soil quality test kit guide, 1999). The textural class of the soil in the experimental field was clay, moderate in organic matter content, moderately alkaline, and non-saline, according to EC values (Table 1).

After 0.1 g of tobacco powder was ground in 100 mM phosphate buffer (pH 7.0), one millilitre of the extract suspension was centrifuged at 10.000 × g for 2 min for foliar rub inoculation. The supernatant was applied to carborundum-dusted leaves of *Nicotiana tabacum* cv. xanthi nc plants. Inoculated plants were maintained in a growth chamber at 25°C. According to this biological test, tobacco waste used in this study was found to be infected with TMV.

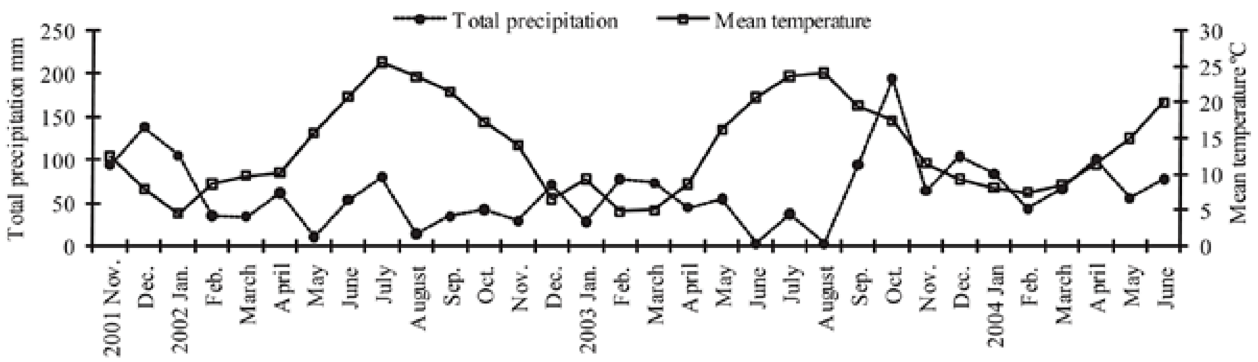


Figure 1. Climate data for Samsun province during the study period between November 2001 and June 2004

Table 1. Some physical and chemical soil properties of the field

Sand, %	19.92	OM, %	2.91
Silt, %	22.86	Exch. Ca, cmol kg^{-1}	31.00
Clay, %	57.22	Exch. Mg, cmol kg^{-1}	10.67
pH (1:1)	7.93	Exch. K, cmol kg^{-1}	1.32
EC, dS m^{-1}	0.28	Exch. Na, cmol kg^{-1}	0.43

OM – organic matter, EC – electrical conductivity

To extract TMV from the soil samples, one gram of each air-dried soil sample was suspended in 1 ml of extraction buffer in an Eppendorf tube. The buffer for TMV extraction from soil was 0.01 M phosphate buffer (PB) at pH 7.0. The suspensions were agitated on a vortex mixer for 1 min, shaken on a rotary shaker (250 rpm) for 30 min at room temperature, incubated at 4°C overnight, and then centrifuged at 10.000 × g for 2 min. The supernatants were used for DAS-ELISA analysis (Fillhart et al., 1998). The negative control for the soil was taken from a different field of the same soil series as the experimental field and with no history of agricultural crops infected by TMV. The DAS-ELISA method was performed according to Clark and Adams (1977) using an antiserum ("Loewe Biochemica", Germany) specific to TMV. The ELISA absorbance value of the negative control was 0.249. Soil samples were considered to be positive when the absorbance values exceeded the mean of the negative controls by at least a factor of two (0.249 × 2 = 0.498) (Arli-Sökmen et al., 1998).

The relationships between TMV absorbance values and soil properties were determined by using the *SPSS 12.0* program. In path analysis, the direct and indirect effects of soil properties on TMV absorbance were determined by using the *TARIST* (1994) statistics program.

Results and discussion

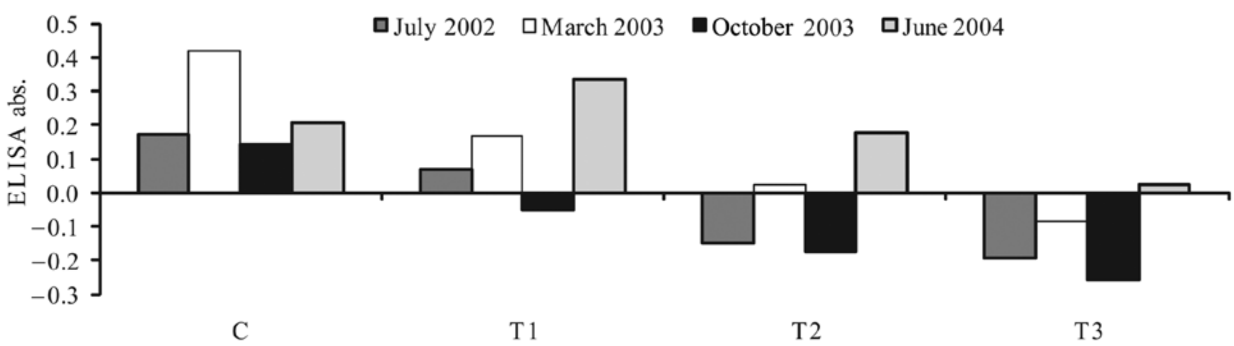
Soil properties and ELISA values determined for different rates of tobacco waste (TW) application at the different sampling times are given in Table 2. Differences between ELISA values for each treatment and the negative control ELISA value multiplied by a factor of two are given in Figure 2. The positive and negative ELISA values in Figure 2 represent soils infected and uninfected with TMV, respectively. Soil samples from the control treatment were infected with TMV at all sampling times. The presence of TMV in the control treatment may possibly be explained by contamination from the adjacent plots by surface runoff, wind or cultural practices during the experiment. Gilbert et al. (1976) reported that the virus could elute near the soil surface if heavy rainfall occurred, resulting in some virus migration.

Increasing application doses of tobacco waste decreased TMV concentration in the soil (Fig. 2). This situation most probably was due to changing soil properties with TOW application. Tobacco waste application decreased soil bulk density (BD) and increased organic carbon (OC) content, total porosity (F) and infiltration rate (I) compared to the control treatment (Table 2). Organic carbon content had a significant positive correlation with total porosity and infiltration rate (Table 3). The volume flux of water flowing into the soil profile per unit of soil surface area is defined as infiltration rate (Hillel, 1982). Increases in total porosity decreased relative saturation and infiltration rates. Relative saturation values were significantly and negatively correlated with total porosity (−0.830**) and infiltration rate (−0.337*) (Table 3). Increasing total porosity and infiltration due to waste application increased leaching of TMV through the soil profile and decreased ELISA absorbance values of the soil. Lance and Gerba (1984) reported that movement of poliovirus during unsaturated flow of sewage through soil columns was much lower than during saturated flow. In a study by Powelson and Gerba (1994), unsaturated flow of sewage effluent in a sandy alluvium soil had poliovirus removed at a three times greater rate than in saturated flow. There are a number of factors affecting virus migration in soils, such as soil pH, moisture content, cation exchange capacity, clay type and content, hydraulic conductivity, and type and strain of virus (Lipson, Stotzky, 1987; Kegler et al., 1995). In the present study, a significant negative correlation between pH and ELISA values (−0.349*) showed that increases in soil pH values decreased TMV concentration in the soil.

Table 2. Changes in ELISA absorbance values and some soil properties during the study

Treatments	Sampling date	ELISA abs.	OC %	pH (1:1)	BD g cm^{-3}	F %	RS %	I cm h^{-1}
C	July 2002	0.672	2.46	6.97	1.11	0.58	78.07	66.06
	March 2003	0.916	1.80	6.83	1.08	0.59	80.02	13.56
	October 2003	0.639	1.53	6.95	1.17	0.56	78.55	38.40
	June 2004	0.703	1.30	6.73	1.11	0.58	69.01	64.84
T1	July 2002	0.568	2.85	6.93	1.01	0.62	60.09	79.31
	March 2003	0.666	2.09	7.07	0.96	0.64	64.64	16.56
	October 2003	0.448	1.63	7.08	0.95	0.64	54.72	80.42
	June 2004	0.833	1.59	6.85	1.05	0.60	65.88	128.21
T2	July 2002	0.348	3.78	6.88	0.89	0.66	50.33	143.09
	March 2003	0.520	2.47	7.30	0.94	0.65	67.70	56.78
	October 2003	0.322	2.13	7.17	1.02	0.61	63.93	49.51
	June 2004	0.687	1.88	7.07	1.06	0.60	65.92	151.88
T3	July 2002	0.304	4.30	6.83	0.89	0.66	45.97	144.07
	March 2003	0.413	2.88	7.34	0.84	0.68	59.44	63.28
	October 2003	0.241	2.14	7.18	0.94	0.65	55.83	69.27
	June 2004	0.491	2.16	7.29	1.07	0.60	66.55	240.36

C – control, T1 – 33 tons ha^{-1}, T2 – 67 tons ha^{-1}, T3 – 100 tons ha^{-1}; OC – organic carbon, BD – bulk density, F – total porosity, RS – relative saturation, I – infiltration rate



C – control, T1 – 33 tons ha^{-1}, T2 – 67 tons ha^{-1}, T3 – 100 tons ha^{-1}

Figure 2. Soils in the treatments having positive ELISA abs. values considered to be infected with TMV in the different sampling times

Table 3. The correlation matrix among ELISA values and some soil properties

	OC	pH	F	RS	I
ELISA abs.	−0.474**	−0.349*	−0.502**	0.596**	−0.201
OC		0.003	0.559**	−0.531**	0.289*
pH			0.331*	−0.108	0.068
F				−0.830**	0.086
RS					−0.337*

** – correlation significant at 0.01 probability level, * – correlation significant at 0.05 probability level; OC – organic carbon, F – total porosity, RS – relative saturation, I – infiltration rate

The results of path analysis indicated that among the soil properties, soil pH had the highest direct effect (59.70%) on TMV concentration (Table 4). Relative saturation (52.57%) and total porosity (34.61%) had the next highest direct effects on TMV concentration after soil pH. While the highest indirect effects of soil pH and relative saturation on ELISA values were obtained through total soil porosity, the highest indirect effects of organic carbon, total porosity and infiltration on ELISA values were mediated by relative saturation. It is known that the adsorption of viruses to clay minerals and organic particulates occurs by physical means, as a result of van der Waals forces and hydrogen bonding (Schaub, Sagik, 1975). Carlson et al. (1968) reported that the adsorption of negatively charged viruses to negatively charged clay surfaces occurs with cation bonds. In this study, the adsorption of viruses to negatively charged organic or inorganic surfaces may have occurred by hydrogen binding due to decreasing soil pH or increasing hydrogen ion concentration in the soil solution. Goyal and Gerba (1979) found that there was a significant negative correlation between soil pH and the percentage of virus adsorption.

Table 4. Direct and indirect effects of some soil properties on the ELISA values

	Direct effect %	Indirect effects %				
		OC	pH	F	RS	I
OC	30.44	–	0.15	26.70	38.22	4.48
pH	59.70	0.20	–	25.76	12.64	1.70
F	34.61	12.34	8.79	–	43.40	0.96
RS	52.57	11.79	2.88	28.92	–	3.82
I	28.15	15.94	4.45	7.41	44.03	–

OC – organic carbon, F – total porosity, RS – relative saturation, I – infiltration rate

As determined with ELISA values, TMV concentration generally increased in all treatments when higher relative saturation and lower infiltration rates occurred in March 2003, compared with the other sampling times (Table 2, Fig. 2). The lowest TMV concentrations were recorded in all treatments when the highest mean monthly precipitation (200 mm) occurred in October 2003. Those results indicate that the virus can be removed from soil by leaching. Despite higher infiltration rates in June 2004, growing green beans in the field increased TMV concentration in all treatments. Yoneyama (1988 a, b) reported that in fields where green beans crops are continuously grown, plants are routinely infected with the virus.

Conclusions

Monitoring of TMV concentration in the experimental clay soil for more than two years showed that virus concentration was influenced by changes in precipitation and some soil properties.

1. Soil pH is one of the most important factors determining virus concentration in that soil. Reducing soil pH caused increases in TMV concentration in the soil.

2. Increases in precipitation and infiltration rate decreased TMV concentration. Results indicated that TMV can be removed from the soil profile by leaching when there is a low water filled pore ratio and high infiltration rate.

3. Conversely, growing green beans or the presence of TMV host plants in the field caused increases in TMV concentration in the clay soil.

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Tabako atliekų įterpimo įtaka tabako mozaikos viruso (TMV) koncentracijai dirvožemyje

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Santrauka

Tabako mozaikos viruso (TMV) koncentracija molio dirvožemyje tirta po tabako atliekų įterpimo praėjus daugiau nei dviem metams. Keturios normos (0, 33, 67 bei 100 t ha^{-1}) tabako atliekų buvo įterptos į dirvožemį 15 cm gylyje trimis pakartojimais. Dirvožemio paviršiniai ėminiai buvo paimti po tabako atliekų įterpimo praėjus 8, 16, 23 ir 30 mėnesių. Siekiant nustatyti TMV koncentraciją dirvožemyje, buvo taikytas DAS-ELISA metodas. TMV ELISA absorbcijos vertes lėmė dirvožemio fizikinės savybės, augalų augimo ir klimato sąlygos. ELISA vertės esmingai koreliavo su santykine dirvožemio prisotinimo drėgme (0.596**), bendruoju poringumu (−0,502**), organinės anglies kiekiu (−0,475**) ir dirvožemio pH (−0,349*). Taikant *Takų* analizę, dirvožemio pH turėjo didžiausią tiesioginę įtaką ELISA vertėms (59,7 %). Didžiausią netiesioginę įtaką dirvožemio pH ir santykinės saturacijos ELISA vertėms turėjo dirvožemio bendrasis poringumas. TMV koncentraciją molio dirvožemyje mažino didėjantis dirvožemio bendrasis poringumas, infiltracijos dydis ir vidutinis mėnesio kritulių kiekis. Auginant salotas ir pupas arba augalus šeimininkus, TMV koncentracija lauke didėjo.

Reikšminiai žodžiai: TMV, tabako atliekos, dirvožemio savybės, laikina stebėsena.