

Accumulation of *Tobacco mosaic virus* (TMV) at different depths clay and loamy sand textural soils due to tobacco waste application

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Abstract The effects of tobacco waste (TW) application to the soil surface on the accumulation of *Tobacco mosaic virus* (TMV) in clay and loamy sand textural soils at various depths were investigated in two different fields. The tobacco waste had been found to be infected with TMV. Eighteen months after TW application to the soil surface, soils were sampled at 20 cm intervals through to 80 cm depth. The DAS-ELISA method was performed to determine infection of soil with TMV. The viruses persisted in clay soil for a long period compared with loamy sand soil. There was no accumulation of TMV at any depth of loamy sand soil in Experimental Field 2. TMV adsorption to soil particles in 0–60 cm depth of clay soil was determined in all TW treatments in Experimental Field 1. The highest ELISA Absorbance (A_{405}) values in all treatments were determined in the 20–40 cm soil depth that had the highest clay content. ELISA A_{405} values of TMV at different depths of clay soil gave significant correlations with clay content ($r=0.793^{**}$), EC values ($r=0.421^{**}$) and soil pH ($r=-0.405^{**}$). Adsorption of

TMV to net negatively charged clay particle surfaces increased with increasing EC values of soil solution. Decreasing soil pH and infiltration rate increased adsorption of TMV to clay particles. Higher infiltration rate and lower clay content in loamy sand soil caused leaching of TMV from the soil profile.

Keywords TMV · Tobacco waste · Soil depth · Soil texture · Adsorption

Introduction

Tobacco mosaic virus (TMV), which belongs to genus *Tobamovirus*, is one of the most significant pathogenic causes of economic loss in many crops worldwide (Agrios 1988). Like other Tobamoviruses, TMV is transmitted through seeds and soil (Komuro and Iwaki 1969, Lamer et al. 1982, Tan et al. 1997). Once a field has been contaminated with the virus, decontamination is extremely difficult. As long as beans are continuously cultivated, the virus persists stably in infected plants and infects newly transplanted seedlings. Methyl bromide is the only chemical known to effectively prevent the spread of the virus. Until now, methyl bromide has been used to fumigate soils (Yoneyama 1988a, b). However, according to the Montreal Protocol, the chemical was banned in developed countries beyond 2005. Consequently, understanding the behavior of viruses in porous media in long term agricultural practices is

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an important issue to have alternative strategies to control TMV in fields.

The application of agricultural wastes with high organic matter content to soil is a current environmental and agricultural practice for maintaining soil organic matter, reclaiming degraded soils and supplying plant nutrients (De Neve and Hofman 2000; Madejon et al. 2001; Tejada and Gonzalez 2004). Broadbent (1976) and Allen (1981) reported that infected plant debris in the soil was considered the main reservoir for initial infections in tomato crops in glasshouses. Moreover, in some studies (Smith et al. 1969; Allen 1981, 1984; Cheo 1980; Cheo and Nikoloff 1980), it was found that a few viruses with stable structure like TMV might also remain infectious in a wide variety of soil types for certain period of time. Many factors affect the mobility and persistence of viruses in soils, including soil pH and moisture content, concentration of ions, cation-exchange capacity, type and amount of clay, organic matter concentration, proteins, salt concentrations in soil, groundwater and hydraulic conditions, infiltration rate, virus type and strain (Kegler et al. 1995; Lipson and Stotzky 1987; Sobsey and Shields 1987; Vaughn and Laundry 1983). Most studies have showed that mineral soils bound more virus than organic soil (Rao and Melnick 1986; Fillhart et al. 1998).

To date, there are some studies of TMV which are transmitted by tobacco powder and stability of TMV in soil in Turkey. The use of tobacco wastes in agriculture as a soil conditioner, after processing by the cigarette industry has been investigated by Özgüven et al. (1999). Tobacco wastes at different rates (0, 7.5, 15 and 30 Mg/ha) were incorporated into soil to 15 cm depth 2 months before seeding to provide time for partial decomposition of the tobacco wastes. Subsequently, the transmission of tobacco mosaic virus from tobacco wastes to wheat, rape, sesame and corn plants was investigated. Although tobacco wastes were contaminated with tobacco viruses, test plants were not infected (Özgüven et al. 1999). Erkan (1987) indicated that slight changes in temperature, pH and moisture caused the gradual reductions in the rates of TMV recovery from soils. Fewer virus particles were recovered when soil pH values were adjusted from 10 to 3.40. Also, TMV degraded quicker in moist soil than in drier soil, but when the soil was saturated, TMV appeared to be stable.

The determination of viral concentration in soil is important for the prevention of soil-borne viral diseases such as TMV. If numerous viral particles in the soil, they may present a threat of infection to the next cultivation. However, there is little information about the long term effects of surface application of tobacco waste on accumulation of TMV at different soil depths. Therefore, the objective of this study was to investigate the effects of surface application of tobacco waste on the accumulation of TMV at different depths of clay and loamy sand textural soils due to changes in soil properties under long term field conditions.

Materials and methods

Field experiments

Field experiments were conducted at Experimental Field 1, (Vertic Haplustolls) of the Agricultural Faculty of Ondokuz Mayıs University (41.3° N, 36.11° E) and Experimental Field 2, a farmer's field (Aquic Udipsamments) in Engiz district (41.3 N, 36.63 E) of Samsun–Turkey. After plowing to 15 cm depth and rototilling of soil, 12 plots (1.20×1 m²) were designed in a randomized block design in each field. There was 0.40 m buffer between plots and between rows. A plastic sheet was buried 0.80 m below the soil surface between plots and rows to avoid leaching of materials among the plots. Tobacco waste (TW) was incorporated from 0 to 15 cm depth at four different rates (0, 33, 67, and 100 Mg ha⁻¹) and three replications. After 18 months, soils were sampled from 0–20, 20–40, 40–60 to 60–80 cm depth of each plot.

Biological tests

For foliar rub-inoculation, after tobacco powder was ground in 100 mM phosphate buffer (pH: 7), 1 ml extract was centrifuged at 10.000×g for 2 min. The supernatant was applied onto carborundum-dusted leaves of *Nicotiana tabacum* cv. *xanthi* nc plants. Inoculated plants were kept in a growth room at 25°C.

Extraction of virus from soils

One gram of air-dried clay or sandy TMV-infected or uninfected soil samples were suspended in 1 ml extraction buffer in Eppendorf tubes. The buffer for