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Usage of Non-chemical Methods to Counter the Damages Caused by Tomato Mosaic Virus

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ABSTRACT

Background: Tomato mosaic virus (ToMV) is the most common and important pathogen of tomato crop. The infected plants show mosaic, mottle, wrinkling, reduced leaf size and uneven ripening of fruits. ToMV is transmitted mechanically, by cultural operations, farm implements and also through seed.

Methods: The present study was planned to evaluate environment friendly strategies for the management of ToMV disease. Different non-chemical means *viz*., Elephant Ear plant extract, Eucalyptus extract, Zinc and Boron solution, NPK solution, Milk and Urea were used on four tomato varieties (Tanja, Chico, Manik and Sahil). All the treatments were used at three different concentrations (1%, 2% and 3%) and replicated three times.

Result: The maximum disease reduction was recorded in plants treated with NPK solution (16.35%) and minimum in plants treated with Elephant Ear plant extract (29.65%). The maximum mean disease severity was recorded at 1 percent concentration of all the treatments (32.46%) which was statistically significant from that of 2 and 3% (24.74% and 19.23%), respectively. Among the varieties, minimum disease severity was recorded in Manik (24.64%) variety and maximum in Chico (31.27%) variety. All the treatments contributed towards reduction in disease severity of which NPK solution being most efficient.

Key words: Biotic stress, Botanicals, Disease severity, Management, Nutrient, ToMV.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) as a vegetable has high nutritive value and is a predominant vegetable as well as cash crop after potato and is being cultivated worldwide (Regassa *et al*., 2016). It is consumed by humans in different forms; used in almost all cooking foods and is an excellent source of vitamins (Tewodros and Asfaw, 2013). Pakistan produces 5.5 lac tons of tomato annually (GOP, 2018).

The production of tomato is low in Pakistan as compared to other countries due to several biotic and abiotic factors (Akhtar *et al*., 2019). Plant diseases contribute to 15% of yield losses and among these diseases 30% losses are considered to be caused by plant viruses (Cerda *et al*., 2017). Tomato mosaic virus (ToMV) belongs to genus Tobamovirus and family Virgaviridae and is the most common and important disease causing pathogen of tomato plants (Arinaitwe *et al*., 2018). Tobamoviruses destroy the crop plants worldwide owing to their wider host range and are transmitted easily (Spence *et al*., 2001). ToMV is widely distributed across Pakistan (Imran *et al*., 2012). The genome of ToMV consists of ss RNA that infects plants (Ullah *et al*., 2017). Mosaic, curling and lead distortion along with internal browning and malformed fruits are some symptoms of ToMV shown by susceptible plants (Kumar *et al*., 2011). The leaves of diseased plants show alternate patches of yellow and green colour, wrinkles, reduced size and bending of leaves and uneven ripening of fruits (Sui *et al*., 2017). Virus is mostly transmitted by infected seeds, crop debris and dodder. Transmissions through insect vectors have also been reported (Ullah *et al*., 2019). The disease can also be easily disseminated through contact, cultural tools and infected seeds (Aseel *et al*., 2019). Tomato mosaic virus disease (ToMD) is prevalent wherever tomato crop is being cultivated both under glasshouse and in open field (Kubota *et al*., 2003). ToMV cause 34.30% losses in in number of fruits while 59.77% losses in weight of tomato fruit when remain uncontrolled (Giri and Mishra, 1990). ToMV is the most persistent virus which survives for longer time in plant tissues (Letschert *et al*., 2002). ToMV disease has been managed by using healthy seeds and resistant varieties (Pfitzner, 2006). Among twenty genotypes screened against ToMV disease under field conditions, only a single advance line was resistant according to disease rating scale (Imran *et al*., 2012). Thermotherapy was also been used for the management of ToMV in tomato seeds before sowing (Wang *et al*., 2018). Cross protection has also been used as

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management strategy but with less success on commercial scale (Hansen *et al*., 2010). Use of chemicals is considered harmful approach for the disease management and is also harmful for humans (Zheng *et al*., 2016). There are almost negligible studies on the conventional management of tomato mosaic virus disease. Considering the above mentioned facts, the experiment was planned to evaluate different non-chemical treatments for management of ToMV disease. It can be postulated that the plant extracts, nutrients solutions, milk and urea used in the present investigations may help in disease management by stimulating plant defence or by repairing the damages caused by ToMV.

MATERIALS AND METHODS

Site of study and experimental material

The experiment was conducted at research area, College of Agriculture, University of Sargodha, Pakistan (Latitude 32.13186° Longitude 72.68791°) in 2017-18. Seedlings of four tomato varieties (Tanja, Chico, Manik and Sahil) were obtained from local market and transplanted in pots under greenhouse. Pots with dimension of 9" height and 28 cm diameter were arranged in completely randomized design (CRD). Pots were filled with clayey soil, sand and farm yard manure (FYM) before transplantation of seedlings. The purpose of greenhouse experiment was to avoid the plants from insect attack such as whitefly so that the successful infectivity assay of ToMV may be ensured. Plant contact was minimized by transplanting a single seedling per pot and each pot was placed at a distance of 45 cm from the other. Recommended irrigation with hand shower and hand weeding was done whenever needed to keep the plants in good condition.

Survey for collection of infected samples

Tomato fields were surveyed at In-service Agricultural Training Institute (IATI), Sargodha (Pakistan) and symptomatic leaves were collected. The samples were placed on ice-bucket after storing in zip lock bags till further use in laboratory.

Preparation of ToMV inoculums

The infected samples were removed from the ice bucket and placed at room temperature for few minutes. Leaves were washed with water to remove dust particles followed by disinfection with bleach solution and then dipped in three changing of distilled water to remove extra surface disinfectant. Washed leaves were dried in paper towel and then crushed in a pre-chilled pestle and mortar. Sample crushing was performed in 0.01 M phosphate buffer. After preparation sap was passed through two folds of the muslin cloth and stored in a falcon tube till further use.

Infectivity assay

In order to confirm the presence of ToMV in sap, mechanical inoculation was performed in separately grown tomato and other indicator plants (Chili, Chenopodium and Soybean). Leaves of the target plants were washed with water to remove dust particles and then carborandum powder was sprinkled to create injury followed by spray of the inoculums. Leaves were washed again after inoculation with water to remove extra inoculums. The inoculated plants were covered with polythene sheet to avoid from insects and other injuries. The relevant symptoms were observed after 4-7 days for the confirmation of ToMV.

Inoculation of test plants to monitor the role of non-chemical treatments against ToMV

After virus inoculation, plants were sprayed with plant extracts (Elephant ear plant, Eucalyptus) nutrient solutions (Zn and B, NPK), milk and urea. Three sprays of each treatment were applied at weekly intervals. All treatments were used at three different concentrations, the detail of which is given below.

Data recording

Disease severity data was recorded from the inoculated plants by using following formula:

$$\text{Disease severity (\%)} = \frac{\text{No. of diseased leaves}}{\text{Total no. of leaves}} \times 100$$

Statistical analysis

The disease severity data recorded from treated and untreated tomato plants were subjected to ANOVA to determine the differences between the means. The means of treatments were also tested by least significant difference (LSD) test at 0.05 probability level (Steel *et al*., 1997).

RESULTS AND DISCUSSION

All the treatments applied on tomato reduced tomato mosaic virus infection significantly than control (Table 2). In current study, NPK solution gave maximum reduction in disease severity (16.35%) over control. It has been recorded that nitrogen concentration plays significant role in inducing resistance in plants against several pathogens (Snoeijers *et al*., 2000). This probably is due to the fact that NPK solution fulfilled the nutritional necessities of plant which helped them to grow and perform their functions properly. Nitrogen is also widely involved in plant stress responses and can influence the deployment factors. Nitrogen also has

Table 1: Detail of treatments used against ToMV disease.

Common name	Scientific name/Trade name	Concentrations	Dose
Elephant ear plant	*Clocasia esculenta*	1%, 2%, 3%	5ml/L
Eucalyptus extract	*Eucalyptus globules*	1%, 2%, 3%	5ml/L
Zn and B	Classic	1%, 2%, 3%	5ml/L
NPK	Fashion	1%, 2%, 3%	5ml/L

Table 2: ToMV disease severity dynamics in treated tomato plants after various sprays.

Treatments	Disease severity (%)			
	1st Spray	2nd Spray	3rd Spray	Mean
Elephant ear plant (*Clocasia esculenta*)	36.68 a	28.84 b	23.42 c	29.65 c
Eucalyptus extract (*Eucalyptus globules*)	33.59 a	25.41 b	18.79 c	25.93 d
Zinc and boron solution	29.54 a	16.91 b	12.74 c	19.73 f
NPK solution	27.72 a	13.78 b	07.56 c	16.35 g
Milk	43.48 a	36.85 b	28.71 c	36.34 b
Urea (0.5%)	30.81 a	16.45 b	11.52 c	19.59 e
Control	60.78 a	64.29 b	66.75 c	63.94 a
LSD	0.92			

Mean values with similar letters in same column and row are non-significant with each other at 5% probability level.

negative effect on physical defence's *i.e.* phytoalexins production but has positive effects on defence-related enzymes and proteins to affect local defence as well as systemic resistance (Sun *et al*., 2020). Phosphorus has vital role in cell division, energy transfer and sugar transport regulation. Phosphorus can affect pathogen by inhibition of metabolic activity of pathogens and can have a direct toxic effect (Datnoff *et al*., 2007). Potassium (K) is involved in essentially all cellular functions that influence disease severity. K regulates the nitrogen ratio and effect disease resistance and susceptibility in plants. Amino acids, phytoalexins, phenols and auxins which help in disease control, their accumulation also depend on the availability of potassium levels (Huber and Arny, 1985).

Second best treatment was Zinc and Boron solution (19.73%) followed by Urea (19.59%), Eucalyptus extract (25.93%), Elephant ear plant (29.65%) and Milk (36.34%) as compared to control (63.94%) (Table 2).

Boron helps in plant disease management by strengthening the structure of the cell wall by formation of carbohydrate-borate complex. Boron also regulate the permeability, stability and function of the cell membrane and also mediate the metabolism of phenols and lignin in plants (Brown *et al*., 2002). Zinc has role in plant defence against pathogens (Dordas, 2008). Zn has a direct effect on pathogen metabolism (Li *et al*., 2016) and mediates the production of antibodies in the plants (Datnoff *et al*., 2007).

In this study urea was very effective against tomato mosaic virus disease management. Urea assimilates the plant metabolism and has a role to play in the vegetative growth of the plant. Urea uptake influences the plant pH values and changes the metabolism of the plants by changing pH levels (Kirkby and Mengel, 1967). The application of urea caused denaturing of coat protein of the TMV and interrupted with virus replication (Bawden and Pirie, 1940). Under *in-vitro* studies extensive physiological changes were observed in TMV particles after treating with urea (Santos *et al*., 2008). Urea caused the liberation of nucleic acid from the virion of TMV (Bonafe *et al*., 1998).

Eucalyptus extract reduced the viral diseases incidence. Eucalyptus leaves help in disease control due to its antimicrobial active chemicals or compounds (1, 8-cineole, eucamalol, limonene citronellal, citronellol, citronellyl acetate, p-cymene, linalool and others). These compounds cause hindrance in microbial activity and protect the host from the disease (Bachir and Benali, 2012). Eucalyptus extract activates salicylic acid and jasmonic acid pathway against biotic stresses (Naidoo *et al*., 2013). After absorption into plant, the active ingredients present in Eucalyptus extract, enhances the production of secondary metabolites due to which defence is boosted up (Henery *et al*., 2008). It also stimulates indole acetic acid and ethylene mediated signals in the plants (Ditengou and Lapeyrie, 2000). The aqueous extract of Eucalyptus initiates pathogenesis related proteins (PR-proteins) in the plants which help in defence against pathogens (Rosa *et al*., 2010).

In *Clocasia esculenta* different antimicrobial chemical compounds are present such as thiamine, riboflavin, oxalic acid, sapotoxin and also has antioxidant activities (Chakraborty *et al*., 2015). Thiamine regulate pathogenesis-related (PR) genes and also potentiates stronger and the up-regulateprotein kinase C activity (Ahn *et al*., 2005). Riboflavin act as coenzymes in physiological reactions, take part in peroxidation and antioxidation and hypersensitive response (Saikia *et al*., 2006). Oxalic acid participates in programmed cell death (PCD) in plants tissue required for disease development (Kim *et al*., 2008).

Different lysozymes are present in milk which affect and act as cell wall hydrolase enzymes for bacteria and also the part of some unspecific innate defence mechanism. Milk proteins also have antimicrobial role in disease management (Benkerrorum, 2008). Many studies concluded that whey proteins present in milk have effective antiviral properties (Marshal, 2004). Milk reduces the plant's susceptibility against viruses (Waarts *et al*., 2005).

Maximum disease severity was recorded after first spray which gradually reduced after second and three sprays in all the treatments as compared to control (Table 2). The disease severity in Elephant ear plant was 36.68 percent after first spray which reduced to 23.42% after third spray. Eucalyptus extract application resulted in 33.59% and 18.79% disease severity after first and third spray,

Table 3: ToMV disease severity dynamics in treated tomato plants at different concentrations.

Treatments	Disease severity (%)		
	Conc. 1 (1%)	Conc. 2 (2%)	Conc. 3 (3%)
Control	62.46 a	66.46 b	63.54 c
Elephant ear plant (*Clocasia esculenta*)	34.43 a	31.24 b	26.12 c
Eucalyptus extract (*Eucalyptus globules*)	31.47 a	28.07 b	21.49 c
Zinc and boron solution	27.23 a	18.09 b	15.24 c
NPK solution	25.13 a	14.44 b	09.32 c
Milk	45.42 a	37.29 b	29.08 c
Urea (0.5%)	31.09 a	19.28 b	14.13 c
Mean	32.46 a	24.74 b	19.23 c
LSD	0.53		

Mean values with similar letters in same row are non-significant with each other at 5% probability level.

Table 4: Effect of non-chemical treatments on ToMV disease severity in different tomato cultivars.

Treatments	Disease severity (%)			
	Tanja	Chico	Manik	Sahil
Elephant ear plant (*Clocasia esculenta*)	32.56	33.84	30.79	31.67
Eucalyptus extract (*Eucalyptus globules*)	30.98	31.69	24.76	27.91
Zinc and boron solution	24.72	26.41	16.21	19.47
NPK solution	17.58	23.84	11.67	13.33
Milk	35.61	37.11	33.59	35.27
Urea (0.5%)	32.91	34.74	30.86	31.95
Mean	29.06 b	31.27 a	**24.64 c**	**26.61 d**
Control	65.53	67.33	66.25	65.61
LSD	0.75			

Mean values with similar letters in same column are non-significant with each other at 5% probability level.

respectively. In Zinc and Boron solution treated plants, 29.54% disease severity was recorded after first spray that was reduced to 16.91% and 12.74% after second and third spray, respectively. First and third sprays of Milk gave 43.48% and 28.71% disease severity, respectively. There was 30.81% disease severity in urea treated plants after first spray which reduced 11.52% after third spray.

All the treatments were used at three different concentrations *i.e.* 1, 2 and 3%, to find out the most effective dose. The disease severity significantly reduced after each concentration in all the treatments (Table 3). Maximum disease severity was recorded at 1% application which was reduced gradually after 2% and 3%, concentration.

The overall effectiveness of the treatments was same *i.e* . NPK>Zinc and Boron>Eucalyptus>Elephant Ear Plant> Milk in all varieties (Table 4). Maximum mean disease severity was recorded in Chico (31.27%), followed by Tanja (29.06%) while minimum mean disease severity was found in Sahil (26.61) followed by manic (24.74%).

The use of plant extracts, nutrients and milk as the natural remedies for the plants that has no harmful effects and provide broad spectrum aspects for research in eco-friendly cure.

CONCLUSION

The present study was conducted to evaluate the comparative efficacy of botanicals, nutrients and milk against tomato mosaic virus disease. It can be concluded that NPK at 3% concentration is the most effective in to minimizing tomato mosaic virus disease severity under field conditions. The remaining treatments (Zn and B solution, botanicals and milk) could also be used as eco-friendly management strategies against tomato mosaic virus disease.

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