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Management of Cucumber Mosaic Virus through Organic and Inorganic Extracts in Greenhouse

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ABSTRACT

Cucumber mosaic virus is one of the most devastating and widely distributed pathogen. This was successfully transmitted to indicator plants (chili, tomato and chenopodium) through sap inoculation. After infectivity assay, CMV was inoculated on cucumber plants grown in greenhouse. Management of CMV in cucumber was done with different botanicals (Neem, Eucalyptus) and plant defense activators (Urea, Salicylic acid and milk). These treatments were used 7-10 days after CMV inoculation. Botanicals were used @5m/L, urea @5g/L, salicylic acid @1.5mg/L and milk @1ml/9ml. Among the different treatments neem extract with 15.49% disease incidence was the best in reducing cucumber mosaic virus disease followed by eucalyptus (18.13%), urea (18.65%), salicylic acid (19.46%) and milk (28.12%) in that order. Therefore, neem extract treatment was found to be best for management of CMV.

Key words: Botanicals, CMV, Cucumber, Efficacy, Resistance.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to family Cucurbitaceae. It contains 95% water, folic acid, potassium, carotenoids and vitamin A and C (Akbar *et al*., 2015). In Pakistan, cucumber is cultivated on an area of 23268 hectares and annual production in Pakistan is 52766 tons (GOP, 2017).

Among major devastating factors, cucumber mosaic disease caused by cucumber mosaic virus (CMV) is the most important one (Myti *et al*., 2014). CMV has broader host range as it can infect over 1000 host species, from 85 plant families (Roossinck, 2001). Major symptoms of mosaic disease are stunting, malformation of leaves, alternate pattern of dark and light green colors on the plant (Palukaitis *et al*., 1992). Necrosis, yield reduction and discoloration are also common in infected plants (Petrov, 2015).

CMV is a mechanically transmitted virus but it can also be transmitted through more than 75 species of aphids in a non-persistent manner. The most efficient vector is *Aphis gossypii* Glover (Palukaitis *et al*., 1992). Weed hosts serve as a repository for the virus and a primary source of inoculum for the development of disease epidemics (Grube *et al*., 2000).

CMV is difficult to control because of its extremely broad natural host range and there are no sources of genetic resistance to CMV available in commercial fresh-market tomato cultivars (Sikora *et al*., 1998). Different approaches are currently under way to find suitable control measures for CMV.

Commonly, CMV disease is managed by using different insecticides to control its vector. Extensive use of chemicals creates environmental hazards, insecticide resistance and increase production costs (Ginting, 2006). Keeping in view the above mentioned facts, the present study was hypothesized that plant extracts and defense activators may repair the damages caused by CMV.

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MATERIALS AND METHODS

Sowing of plants for indexing and management

Cucumber (*Cucumis sativus* L.) seeds were sown in pots for indexing. Indicator plants (Chili, Tomato and chenopodium) were plants were also maintained in greenhouse for indexing. The experiment was conducted in University College of Agriculture, University of Sargodha (Pakistan).

For management, experiment was laid out in completely randomized design (CRD) with five replications.

Collection of diseased samples

Cucumber fields were surveyed to collect the young diseased leaves were taken in polythene bags from the field and were transferred to the lab in an ice box.

Preparation of CMV inoculums

CMV inoculum was obtained by grinding infected leaves in chilled pestle and mortar in the presence of extraction buffer.

The ingredients of extraction buffer (0.05 M; pH 7.0) were Potassium dihydrogen phosphate (KH_2PO_4) 2.40 g; dipotassium hydrogen phosphate (K_2HPO_4) 5.40 g; mercaptoethanol 1.56 ml and distilled water 1000 ml. One gram diseased leaves were ground in 10 ml of extraction buffer. The homogenate was sieved by muslin cloth and inoculums was collected in a small vial (Jalender *et al*., 2017) and the filtrate was used as inoculum.

Mechanical inoculation

The obtained sap was then applied on cucumber plants for confirmation and on indicator plants for indexing purpose (Akbar *et al*., 2015). Indicator plants used for this purpose were chenopodium (*Chenopodium album*), tomato (*Lycopersicon esculentum*) and chili (*Capsicum annuum*) plants. The sap was applied on the leaves of indicator plants with the cotton swab and carborendum powder was used to cause the mechanical injury to the leaves. Excess of sap was then removed with the help of distilled water to avoid chemical injury.

After the confirmation of CMV, inoculations were made on cucumber plants for the management of CMV in the pots. Extracts of eucalyptus and neem, urea, milk and salicylic acid was applied for management purposes. All treatments were applied after 7-10 days of inoculation of plants with CMV sap. The details of the treatments are given below in Table 1.

Preparation of plant extracts

Fresh mature leaves of neem and eucalyptus were detached and stored in polythene bags. Leaves were washed with tap water to remove dust particles followed by oven drying at 60°C for 8 hours. Dried leaves were ground to form powder that was stored in small vials an placed in refrigerator until use. For preparation of extract, 5 grams powder (Neem and Eucalyptus) was mixed with 50 ml distilled water in a flask. After mixing, the flask was placed on shaking incubator at 200 rpm for 24 hours. The solution was filtered through muslin cloth. This process was repeated three times after which a clear aqueous extract of the plant was taken (Al-Manhal and Niamah, 2015).

Data recording and Statistical analysis

Disease incidence data recording were started one week after the application of treatments with following formula.

%Disease incidence =

No. of symptomatic leaves/Total no. of leaves*100

Disease incidence was recorded at one-week interval. The data was analyzed by using statistical software (Statistix 8.1). Data was subjected to analysis of variance (ANOVA) and means were compared through least significant difference (LSD) test at P= 0.05 (Steel *et al*., 1997).

RESULTS AND DISCUSSION

All the evaluated treatments reduced the cucumber mosaic virus disease incidence significantly compared to untreated control (Table 2). *Azadirachtaindica* (Neem) extract was found to be the most effective against CMVD followed by *Eucalyptus globules* (Sufaida), urea, salicylic acid and milk, respectively. Milk and salicylic acid were found less effective compared to other treatments for the management of CMVD. These results are in line with (Khan *et al*., 2003) who found significant reduction in virus disease by using neem extract. The reason behind disease reduction was the repellent efficacy of neem extract against the sucking insect pests (Butler *et al*., 1991). The repellent behaviour of plant extracts were also observed in studies of (Butler and Henneberry, 1992) who described that reduced insect infestation resulted in minimum disease incidence. Arif *et al*., (2009) found that plant extracts are less toxic way to control the sucking insect pests. Neemhas many active ingredients likeAzadirachtin, nimbin, nimbidin, nimbolide, limonoids that play role in diseases management. It reduced the CMVD incidence due to antimicrobial effects of these compounds (Gurjar *et al*., 2012; Alzohairy, 2016).

Eucalyptus extract was the second most effective treatment against CMV disease incidence. Eucalyptus extract from the leaves of eucalyptus provide good control of CMV and it is due to its antimicrobial active chemicals or compounds such as 1,8-cineole, eucamalol, limonene citronellal, citronellol, citronellyl acetate, p-cymene, linalool and others. These compounds cause hinderance in microbial action and protect the host from microbes (Bachir and Benali, 2012).

Urea assimilates plant metabolism and increase the growth or vegetative production of plant cells as nitrogen uptake influence the pH of solution and this shift of pH changes the metabolism of the plants so in this studies urea suppressed the disease following the neem and eucalyptus (Kirkby and Mengel, 1967).

Table 1: Treatments applied against Cucumber mosaic disease.

Serial No.	Treatments	Dose
T_1	Neem extract	5ml/L
T_2	Eucalyptus extract	5ml/L
T_3	Urea	5g/L
T_4	Salicylic acid	1.5mg/L
T_5	Milk	1ml/9ml

Table 2: Efficacy of different treatments against cucumber mosaic virus disease.

Serial No.	Treatments	Disease incidence (%)	Efficacy (%)
T_1	Control	62.04 a	
T_2	Milk	28.12 ab	54.67
T_3	Salicylic Acid	19.46 ab	68.63
T_4	Urea	18.65 ab	69.94
T_5	Eucalyptus Extract	18.13 b	70.78
T_6	Neem Extract	15.49 b	75.03

LSD = 0.462 at P = 0.05.

Means with similar letters in a column are not significant.

Salicylic acid help in plant defence by producing hypersensitive response and also salicylic acid is the signal molecule act in the systemic induced resistance, which is regulated by the effect of salicylic acid mediated pathway it is also produced naturally in plants and its external application provide beneficial results in plant defence (Raskin, 1992). In milk,Lysozymes act as bacterial cell wall hydrolase and these are the part of some unspecific innate defence mechanism. While other proteins found in milk also play a important role in antimicrobial reactions (Benkerroum, 2008).

Chemical pesticides are not eco-friendly, toxic to non-target organisms and destroy natural ecosystem. That's why eco-friendly management strategies as plant extracts, nutrients and defence inducers are useful ways to management the disease.

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