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SPATIO-TEMPORAL ANALYSIS OF CUCUMBER MOSAIC VIRUS DISEASE AND ITS SUSTAINABLE MANAGEMENT

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Abstract

Cucumber fields were surveyed in the vicinity of Sargodha to map the spatial and temporal CMV disease distribution. Infected samples were collected for pathogenicity tests, biophysical properties and serological assays. After confirmation of virus; data of disease prevalence, incidence and severity was recorded which showed strong positive linear relationship. There was gradual increase in disease after each temporal assessment. Bhatti Town (BT) showed more than 90% disease prevalence followed by College of Agriculture (COA) and Adaptive Research Farm (ARF). This experiment also demonstrated the significant contribution of NPK solution, Naphthalene acetic acid (NAA), benzothiadiazole (BTH) and elephant ear plant (EEP) extract in improving the cucumber performance against CMV disease. There was positive linear relationship between all disease parameters. All the treatments gave significant contribution for sustainable disease management. NPK was the efficient among rest of the treatments with more than 75% disease inhibition. The second most effective treatment was BTH followed by NAA and EEP with 63%, 55% and 39% disease control, respectively. The study provided useful information for estimation disease dynamics in varied spatio-temporal scales. It could be concluded that NPK and BTH are the suitable tools for sustainable management of CMV disease.

Key words: Naphthalene acetic acid, Micronutrients, Epidemiology, Reducing agents, Inoculums.

Introduction

Cucumber mosaic disease (CMD) is caused by cucumber mosaic virus (CMV) that was first reported in 1916 in New York (Yoon *et al*., 2019). CMV belongs to familyBromoviridae and genusCucumovirus that has ssRNA genome (Nalam *et al*., 2019).The virusinterrupts with somatic and reproductive physiology of plants and cause extensive yield losses (Donnelly *et al*., 2019).The highest percentage of CMD severity may result in 100% yield losses (Groen *et al*., 2017).CMV has wider host range and can attack more than 1000 plant species (Wu *et al*., 2017). It passes through the cell wall ofhost and finds the replicationsites, removes coat protein, becomes a part ofhost and replicates through replicases enzyme (Mauck, 2016). It damages the biosynthetic machinery of host plants as it replicates in that system (Casteel *et al*., 2015).

The salient symptoms of the disease are stunting, dark and light green patterns, malformation and yellow streaking of leaves (Tungadi *et al*., 2017). It is transmitted through sap inoculation (Gao *et al*., 2016), seeds (Arogundade *et al*., 2019), aphids (Tungadi *et al*., 2020) and 10 species of dodder (Ntui *et al*., 2013). Aphids transmit CMV in a stylet borne manner with acquisition feeding period of 10 seconds (Jo *et al*., 2016).

The activation of induced systemic resistance triggers the expression of plant defense genes (Kumar *et al*., 2017). It can be strengthened by using different nutritional amendments (Meena *et al*., 2016) and by the application of salicylic acid, jasmonates and ethylene (Qin *et al*., 2013). Jasmonic acid and ethylene induce the systemic resistance in plants against the signaling mechanisms of pathogens (Aznar *et al*., 2015).

Similarly, spraying of insecticides is also an option available to the farmers for managing CMV through vectors control. However, as CMV is an aphid-transmitted non-persistent virus, only insecticidal spray may not control the disease effectively. Moreover, dependence on a single method is highly vulnerable to failure (Borer *et al*., 2016). However, when integrated with more than one management strategies may repress disease significantly more than any single tactic alone (Anitha, 2016). Therefore, if available an integrated approach is preferred. Many reports are available on the successful application of integrated management tactics for CMV (Al-Zahrani *et al*., 2018).The aqueous extract of *Tanacetumvulgare* was applied for the management of CMV and many other mechanically transmitted plant viruses (Petrov *et al*., 2016).There are essentially two approaches to manage virus diseases. The first approach is to decrease the sources of infection and secondly to minimize the rate of spread by vector control (Rahman *et al*., 2020).

The potential of nutrients and plant extracts for improving the tolerance of cucumber plants against cucumber mosaic virus disease has not been documented so far. Therefore, this study, evaluated the potential of NPK solution, naphthalene acetic acid and elephant plant extract for improving salt tolerance in wheat.

Materials and Methods

Plant material and experimental layout: The experiment was conducted in research area Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan 32.0754° N and 72.41168° E using completely randomized design (CRD). The prevailing environmental conditions during the experiment were; having average

temperature of 29°C with relative humidity 57.43%, 10.4 h sunshine, 15.2 mm rainfall, 14 km/h wind speed and 1.8 mm potential evapo-transpiration. Cucumber seeds of Poinsettee variety were obtained from local market, Sargodha. Clay pots of 40 cm diameter were filled with 25% sand, 75% clay and appropriate amount of farmyard manure. The 30 pots were seeded with cucumber seeds (1 cm deep) at the rate of 10 seeds per pot and kept under natural daylight in glasshouse. The pots were irrigated with tap water regularly till the end of experiment. The fertilizers i.e. potassium sulphate, urea, and diammonium phosphate were applied as sources of potassium, nitrogen and phosphorous respectively.

Sources of viruses and inoculums preparation: Cucumber crop was surveyed at Adaptive Research Farm (ARF), Sargodha (32.1228° N, 72.6786° E) for the collection of diseased samples. The samples were collected in zip lock polythene bags, placed in ice-bucket and stored in the refrigerator at4°C in Plant Pathology laboratory. Virus inoculums were prepared from infected fresh leaves of cucumber having alternate light green patches intermingled with normal green color. Infected cucumber leaves (5 g), were macerated in 5 ml of 0.01 M phosphate buffer in pre-chilled pestle and mortar, filtered through muslin cloth and stored in a falcon tube (Gao *et al*., 2016).

Virus inoculation on cucumber and indicator plants: The primary leaves of cucumber and indicator plants (chili, tomato and chinopodium) were dusted with carborandum powder. A cotton swab was dipped in falcon tube containing inoculums and injured leaf surfaces were rubbed. The inoculated leaves were sprinkled with water to remove extraneous inoculums. The inoculated plants were covered with polythene sheet to avoid from the attack of insects and other pathogens (Crespo *et al*., 2018).

Serological assay (DAS-ELISA) for detection of CMV: Infected samples were collected and double antibody sandwich (DAS-ELISA) was performed in Laboratory of Plant Virology section Ayub Agricultural Research Institute Faisalabad-Pakistan. Two plants from each location were selected for serological assay and sap was extracted from leaf lamina and leaf mid rib. The standard procedure of ELISA as described by Clark & Adams (1977) was followed and after stopping the reaction, micro-titre plate was kept in ELISA reader for recording the absorbance readings.

Spatio-temporal assessments of CMV disease severity: After confirmation of CMV through mechanical inoculation; the cucumber fields at ARF, COA and Bhatti Town were visited weekly for noting disease observations. The D_{50} and D_{95} were calculated for each location by determining the nearest neighbor distance from each CMV block at a specific time. The nearest neighbor distance was calculated by using ArcMap software (Gougherty & Nutter, 2012). The data of disease incidence and severity were recorded four times from three places at weekly intervals by using following formulae.

$$\text{Disease incidence (\%)} = Number\ of\ diseased\ \frac{plants}{Total}\ number\ of\ plants\ \times 100$$

$$\text{Disease severity (\%)} = Number\ of\ diseased\ \frac{leaves}{Total}\ number\ of\ leaves \times 100$$

$$\text{Disease prevalence (\%)} = Number\ of\ symptomatic\ \frac{plots}{Total}\ number\ of\ plots \times 100$$

Area under disease progressive curve (AUDPC) was calculated by the trapezoidal integration $n-1$

$$AUDPC = \sum_{i=1}[(xi + xi + 1)/2](ti + 1 - ti)$$

where n is the number of assessment; x, disease incidence (%); and (t_i+1-t_i), duration between two consecutive assessments (Shaner and Finney, 1977).

$$\text{Disease severity index (\%)} = \sum P \times Q/(M \times N) \times 100$$

where P = severity score, Q = number of infected plants having the same score; M = Total number of plants observed, N = Maximum rating scale number

Disease was categorized according to following disease rating scale as mentioned in table 1 (Bashir *et al*., 2002).

Effect of sap storage on virus infectivity: Sap was prepared with standard method and stored under optimum conditions. Then sap was inoculated with the standard method as described above. Four groups of plants containing 5 pots each were inoculated with varied time interval i.e. same day the inoculums were prepared, 2 days after preparation, 4 days after preparation and 6 days after preparation (Mujtaba *et al*., 2019). The symptoms produced by each set of inoculums were assessed on the inoculated plants.

Table 1. Disease rating scale for CMV

Disease rating	Disease severity (%)	Response
0	All plants free of virus symptoms	HR (Highly Resistant)
1	1-10% infection	R(Resistant)
2	11-20% infection	MR (Moderately Resistant)
3	21-30% infection	MS(Moderately Susceptible)
4	30-50% infection	S (Susceptible)
5	More than 50% infection	HS (Highly Susceptible)

Effect of reducing agents on crude (CMV) sap: To evaluate the efficacy of reducing agents on crude CMV sap, 5ml of 2.5% carbon tetrachloride (CCl_4) and 5ml of 5% chloroform ($CHCl_3$) were added in the crude CMV sap (Ali *et al*., 2020). The sap containing reducing agents was applied on two sets of plants (5 pots each) including one control. After inoculation, plants were covered and symptoms were observed to assess the efficacy of reducing agents on CMV virulence.

Demonstration of dilution end point (DEP): Sap was prepared with the standard method. 1ml of sap was taken and added into 9ml of distilled water in the test tube (10^{-1} dilution). 1ml of sap was taken from 10^{-1} dilution and added into 9ml of distilled water in the test tube (10^{-2} dilution). Similarly, 1ml was taken from10^{-2} dilution and added into 9ml of distilled water in the test tube (10^{-3} dilution). These dilutions were inoculated with the different dates (Sharma *et al*., 2020). All sets of diluted sap were inoculated on three sets of plants including positive and negative control. In positive control, undiluted sap was applied while negative control remained untreated.

Effect of different treatments against CMV disease under field conditions: Elephant ear plant (*Clocasia esculenta* L.) extract was used against CMV disease at three different concentrations. The leaves of test plants were collected from premises of COA, UOS and extract was prepared by grinding the leaves with manual plant extraction machine. Equal v/v of sample and water used to make 100% solution. The relative concentrations of 5%, 7.5% and 10% were made by adding 5 ml, 7.5 ml and 10 ml of extract in 95 ml, 92.5 ml and 90 ml of distilled water, respectively. The extract obtained was considered as standard and was stored at -20^{o}C until use. Three foliar sprays of plant extracts at 5%, 7.5% and 10% concentrations were applied at an interval of 7 days. After each spray disease incidence data was recorded two times at five days' interval.

Ali Akbar product named "Fashion" was taken as NPK and was used at three concentrations i.e. 2.5%, 5% and 7%. This was prepared by adding 25ml, 50ml and 75ml NPK solution into1 liter of water, then apply as a foliar spray. STEDEC product name Phytofixwas taken as a naphthalene acetic acid (NAA). The concentrations (1.5%, 2.5% and 3.5%) were prepared by adding 15ml, 25mland 35ml into1liter of water. Benzothiadiazole (BTH) was applied at three concentrations (0.2%, 0.3% and0.5%) prepared by adding 2g, 3g and 5g in one liter of distilled water.

The data of disease severity was recorded before and after each spray by following above mentioned formula. The efficacy of each treatment was calculated by using the formula given below described by (Helal, 2019).

$$I\% = C\text{-}T/T*100$$

where I% = Inhibition or efficacy; C = Control and T= Treated

Comparison of diseased and healthy leaf to check leafarea: Ten leaves each from diseased and healthy plants were taken to check the leaf area $cm^2$with the leaf area meter (Plate 1).

Dry preservation of inoculums: In order to perform the virus indexing procedure conveniently, diseased samples were collected, cut the veins, chopped into small pieces and put into desiccators for 4-6days.Took the dried inoculums into a bottle having four layers (Silica, muslin cloth, chopped leaves, and muslin cloth) four preservation (Plate 1).

Comparison of chlorophyll contents from diseased and healthy plants: Ten leaves from diseased and healthy plants were taken to check the chlorophyll contents with the chlorophyll content meter (Plate 1).

Statistical analysis

Data were analyzed statistically and means were compared by using Least Significant Difference (LSD) test and standard error was calculated. ANOVA was used to determine the effect of treatments on disease severity (Steel *et al*., 1977).

Results and Discussions

Spatial distribution of CMV disease in different locations: Significant differences were observed for prevalence, incidence and severity of CMV at three locations of ARF, COA and BT in Sargodha district. The parameters of disease estimation showed higher values in all locations of BT and COA, respectively while minimum observed in ARF. Maximum disease prevalence (100%) was recorded in BT at location 1, while location 1 and 2 of ARF gave minimum disease prevalence (60%). Disease prevalence ranged from 60-70%, incidence ranged from 51%-59% and severity from 32-39% in ARF. The overall disease prevalence (60-100%), disease incidence (51-75%), and disease severity (32-55%) was recorded in all locations of three places (Table 2). The locations with more CMV disease prevalence gave more disease incidence and severity while low values of disease incidence and severity were recorded in less prevalent locations respectively. In all three locations, mean disease prevalence depicted strong linear relationship with disease incidence and severity (Figs. 1 & 2). For each per unit increase in disease prevalence there was relative increase in disease incidence (0.21-0.56) and severity (0.34-0.52). The values of D_{95} and D_{50}were calculated for all locations collectively which conformed the disease distribution pattern to real model. The values of (D_{95} and D_{50}) for prevalence are higher than incidence and severity indicating the preciseness of disease distribution. These values indicate the conservative measurement of pathogen spread. Gougherty and Nutter proposed that these values provide a guide for the establishment of a quarantine area for careful future survey. Furthermore, these are also helpful in devising disease eradication programs for an area. Pearson's correlation indicated more than 95% strong relation among disease prevalence and incidence and severity.

Cucumber mosaic virus ranked among the most damaging disease causing agents worldwide. The remarkable yield losses incited by CMV disease may be eliminated by sowing resistant germplasm against the virus. There are very limited studies to explore the natural genetic potential of cucumber cultivars against CMV (Iqbal *et al*., 2012). The present research was undertaken to unveil the hot spots for CMV disease in three different locations of Sargodha that would be helpful in deciding the sustainable management options against quality and yield destruction. All the designated locations were regarded as susceptible and highly susceptible when rated according to a devised scale. The non-availability of resistant sources is a key factor in minimizing the cost-benefit ratio. CMV is the most damaging viral pathogen with wide host range which infects many crops from various families (Ashfaq *et al*., 2014). Due to multiple hosts and high mutation rate, provision of genetically resistant germplasm against CMV is limited. That's why conventional methods including cultural control, physical eradication, chemical and bio-control of vectors are mostly relied upon for CMV management (Hull, 2014)

Table 2. Spatial distribution of CMV disease in various cucumber fields of district Sargodha.

Spatial distribution with GPS location	Locations	DP (%)	DI (%)	DS (%)
ARF 32.1228°N 72.6786°E	1	60 ± 3.16 b	51.57 ± 1.32 c	32.16 ± 1.24 c
	2	60 ± 1.32 b	57.08 ± 1.41 b	35.53 ± 1.73 b
	3	70 ± 2.41 a	59.06 ± 1.73 a	39.09 ± 1.31 a
COA 32.0754°N 72.41168°E	1	80 ± 1.41 a	67.02 ± 1.73 b	44.18 ± 1.59 b
	2	70 ± 2.24 b	62.51 ± 2.24 c	41.97 ± 1.73 c
	3	80 ± 2.83 a	69.15 ± 2.65 a	47.42 ± 1.41 a
BT 32.1087°N, 72.6543°E	1	100 ± 0.0 a	75.17 ± 2.24 a	55.14 ± 1.89 a
	2	90 ± 1.73 b	73.38 ± 1.89 b	52.87 ± 1.63 b
	3	90 ± 2.47 b	72.78 ± 1.63 ab	50.62 ± 1.62 c
	D_{95}	96.01	74.45	54.23
	D_{50}	80.24	67.02	44.18

Means with similar letters in coloumns against each location are not significantly different 5% probability level

To assess the temporal distribution of CMV disease, the cucumber crop was observed. CMV disease was evaluated by recording prevalence, incidence and severity from all locations at fortnight interval for three times. The overall minimum values of disease parameters were found after first assessment in all locations while all parameters increased after second and third assessments. Significant differences were recorded for disease prevalence, incidence and severity in all assessments (Table 3). During first assessment disease prevalence was minimum at all locations which ranged from 63.55%-93.53% that gradually increased after second assessment (66.31-95.67%) and third assessment (69%-97%). It was assessed that disease incidence and severity also increased with each consecutive assessment. The range of temporal increase in disease incidence and disease severity was 55.91%-78.02% and 35.59%-58.19%, respectively.

The evaluation of cucumber crop at all locations indicated that there was no resistance against CMV disease (Table 3). The disease severity index and AUDPC value showed increasing trend at each assessment. The crop surveyed at ARF and COA was regarded as susceptible when compared with disease rating scale while BT gave highly susceptible response. The disease severity index ranged from 0.71-1.39% with disease rating of 4 and 5. The value of AUDPC was lower in temporal assessment at ARF which ranged from 157.98 to 1309.28 which increased in case of COA (1491.08-1572.07) and BT (1660.05-1755.45).

There was highly significant difference in each temporal assessment of CMV disease distribution which showed increasing trend. These results are in line with the findings of a cucumber screening experiment which was conducted for evaluation of tomato germplasm against CMV (Akhtar *et al*., 2010). The spatial and temporal disease dynamics would provide a strong base for eradication plans in the future (Gougherty *et al*., 2015). Spatio-temporal disease distribution maps are very intuitive in plant disease management strategies (Byamukama *et al*., 2010). The spatial analysis for disease measurement was strengthened by devising a new method to differentiate by two consecutive spaces through the calculation of D_{95} and D_{50} values (Gougherty and Nutter, 2012). A more advance method in spatial analysis was to determine the hot spot among locations by applying Ripley's K function which specifies the site needs more attention (Nutter *et al*., 2011). While studying spatial disease distribution, each unit should be sampled systemically due to clustered pattern (Byamukama *et al*., 2011).

Effect of sap storage on virus infectivity (Longivity in vitro-LIV): The studies on LIV done at room temperature which showed 100% transmission and maximum lesions when inoculated with crude sap immediately after extraction from infected leaves. Gradual decrease in transmission was observed when the crude sap was kept at room temperature for varied duration. The results showed that the virus could retain infectivity up to 6days and got inactivated from 6thday when kept at room temperature (Fig. 3).

The effect of aging LIV has been studied by many researchers worldwide. The attenuation of CMV sap infectivity with increased storage was recorded by Chandankar*et al*., (2013). In another study, maximum transmission in main host and indicator plants were observed by inoculation with fresh sap that decreased drastically when inoculation was done 6 days old sap (Mujtaba *et al*., 2019).

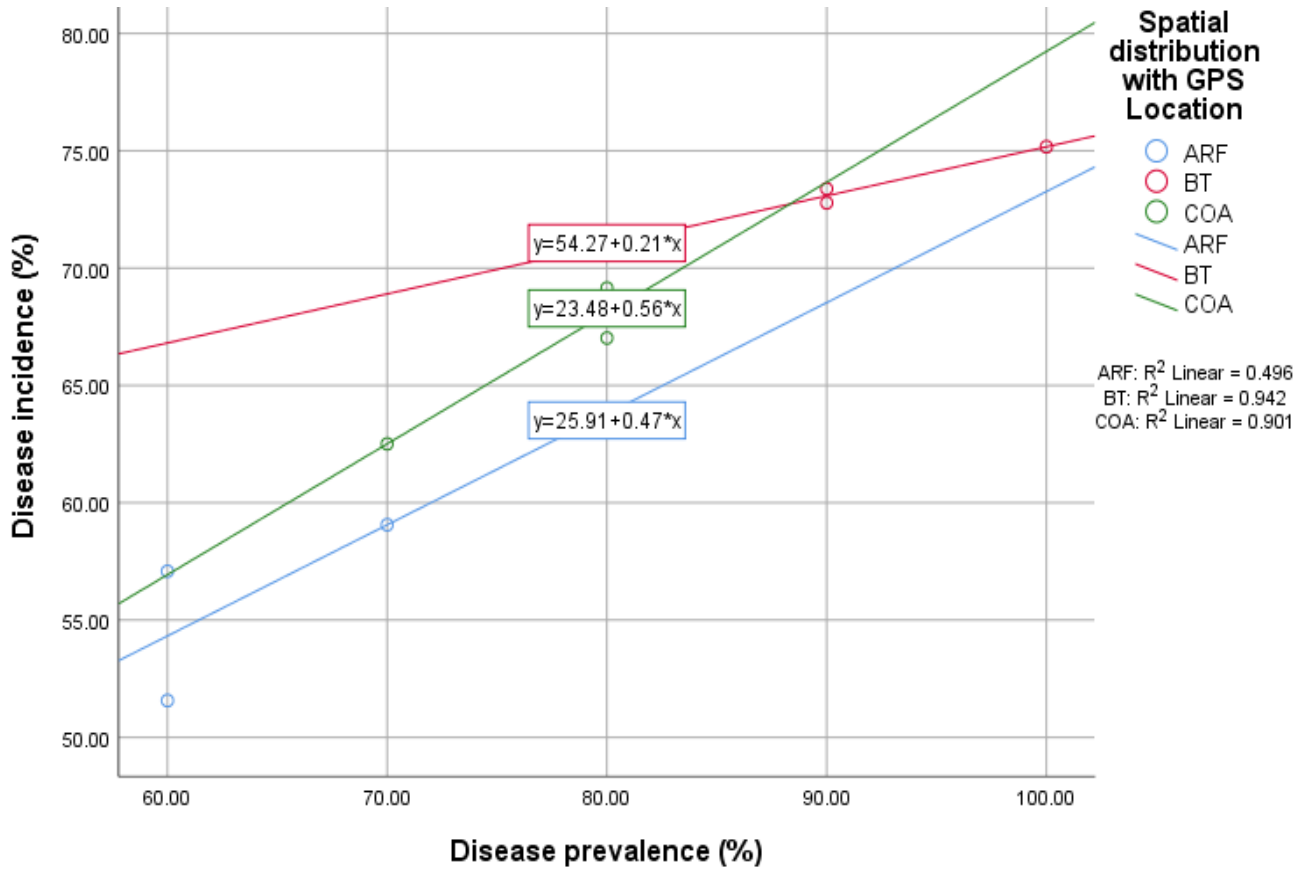

Fig. 1. Relationship between disease prevalence and incidence at three locations.

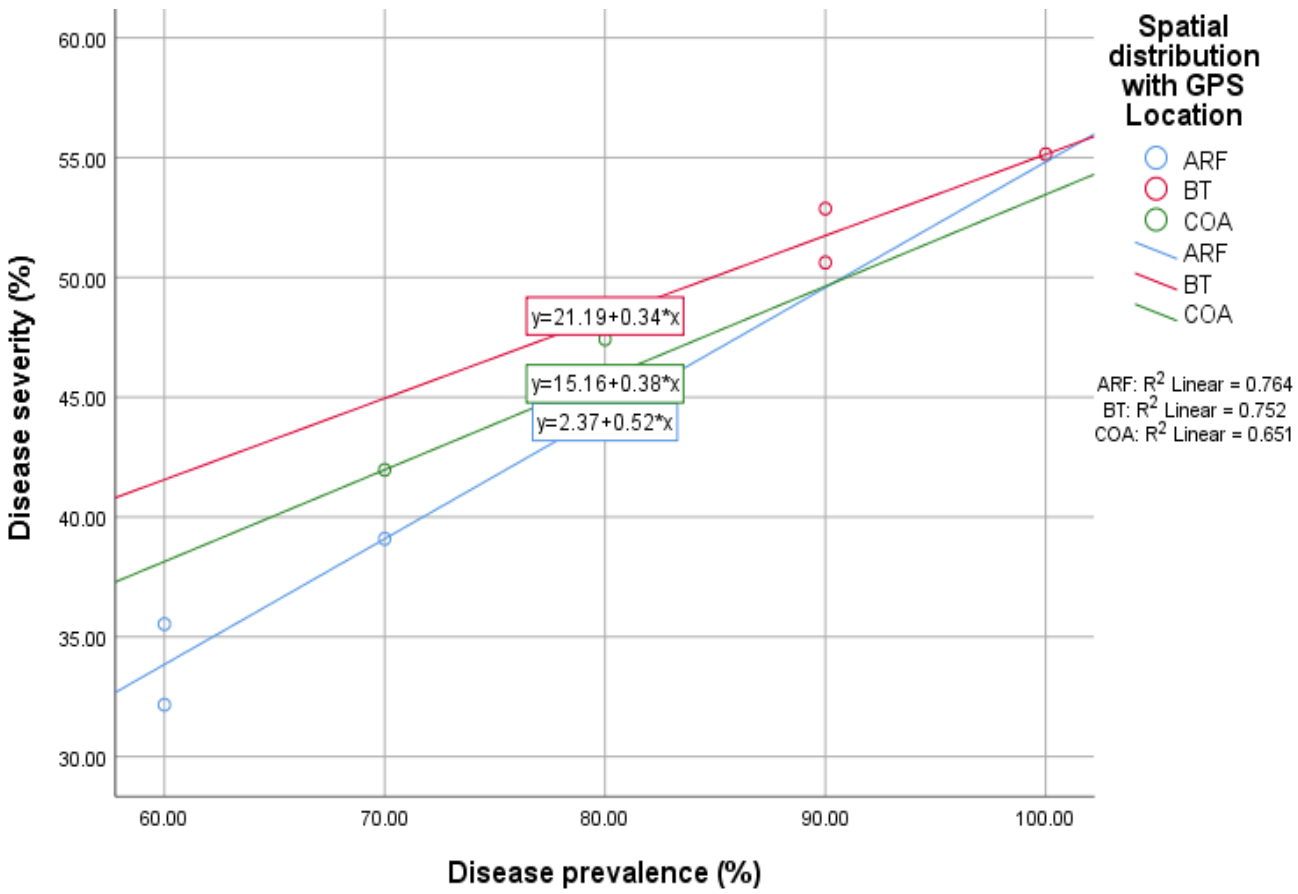

Fig. 2. Relationship between disease prevalence and severity at three locations temporal distribution of CMV disease.

Table 3. Temporal distribution of CMV disease at fortnight intervals.

Locations	Data assessments	DP (%) ^	DI (%)	DS (%)	DSI (%) ^	Disease rating	AUDPC^	Response
ARF	First	63.55 ± 1.44i	55.91 ± 1.41i	35.59 ± 2.42i	0.71	4	1257.98	S^
	Second	66.31 ± 1.23 h	58.03 ± 2.65h	37.13 ± 2.13 h	0.74	4	1305.68	S
	Third	69.53 ± 2.82 g	58.19 ± 1.82 g	38.98 ± 1.93 g	0.77	4	1309.28	S
COA	First	73.53 ± 1.73 f	66.27 ± 1.57 f	42.51 ± 2.01 f	0.85	4	1491.08	S
	Second	75.14 ± 1.37 e	68.05 ± 1.26 e	44.73 ± 1.06 e	0.89	4	1531.13	S
	Third	79.29 ± 1.31 d	69.87 ± 1.21 d	48.22 ± 2.11 d	0.96	4	1572.07	S
BT	First	93.53 ± 1.01 c	73.78 ± 1.18 c	52.88 ± 1.78 c	1.27	5	1660.05	HS
	Second	95.67 ± 1.22 b	75.87 ± 1.34 b	55.06 ± 1.89 b	1.32	5	1707.08	HS
	Third	97.19 ± 1.38 a	78.02 ± 1.63 a	58.19 ± 1.71 a	1.39	5	1755.45	HS

^value given against each assessment in all columns is the mean of three readings

DP= Disease prevalence; DS= Disease severity; DI= Disease incidence

^DSI= Disease severity index; AUDPC= Area under disease progress curve; S= Susceptible; HS= Highly susceptible

Effect of reducing agents on crude (CMV) sap: The virulence of CMV was increased when its sap was mixed with 5% carbon tetrachloride (CCl_4) and 2.5% chloroform ($CHCl_3$). These reducing agents showed significant higher number of lesions and percent transmission of CMV as compared with control. In consecutive inoculations, maximum infection (65%) was recorded in CCL_4 treated sap followed by $CHCl_3$ (53%) as compared to control (Fig. 4).

Plants have constitutive antiviral substances which may interrupt with virus replication, in order to overcome these substances reducing agents were mixed with crude virus sap. Chloroform is a strong reducing agent which gave significant higher transmission as compared to crude virus sap (Sapno *et al*., 2017). Ali *et al*., (2020) also used chloroform and carbon tetra chloride as reducing in cucumber mosaic virus sap.

Demonstration of dilution end point (DEP): The undiluted crude sap produced maximum number of lesions with 100% transmission. However, the sap retained its infectivity up to the dilution of 10^{-3}. From this experiment, it was concluded that the dilution end point of the virus is 10^{-3} (Fig. 5). Assay plants inoculated with the crude sap diluted (10^{-1}-10^{-3}) produced mosaic, leaf distortion and leaf puckering symptoms. However, sap failed to produce symptoms with extract diluted to 10^{-4} and above. These results corroborate with those of Parvin *et al*., (2007) who described decreasing trend of virus transmission with every dilution. The infectivity loss of virus was noted with diluted sap inoculation on indicator plants (Mochizuki & Ohki, 2012).

Evaluation of different treatments against CMV disease: The mean values of disease severity showed that all the treatments were highly significant as compared to control with 71.95% disease severity (Table 4). Among all the treatments, the minimum mean disease severity (17.59%) was recorded in NPK treated plants followed by benzothiadiazole (BTH) 63.68%, NAA (55.06%) and elephant plant extract (39.44%). There was a significant difference in disease severity values after each spray. The comparison disease severity after three sprays showed that the minimum disease severity (22.53%) was recorded after 3rd spray and the maximum disease severity (41.25%) was recorded after 1st spray. The interaction of all the treatments was also significant with three sprays. The efficacy of all the treatments was also calculated which indicated that NPK was the most efficient treatment with 75.55% reduction in CMV disease severity. The second most efficient treatment was BTH followed by NAA and elephant ear plant extract which showed 63.68%, 55.06% and 39.44% efficacy respectively.

TheCMV disease severity was recorded for all the treatments which were applied at different concentrations (Table 5). The analysis showed that there was significant disease severity reduction after each concentration. In C_1, all treatments were applied at lower concentration which gave minimum disease reduction that was gradually increased in case of C_2 and C_3 where higher disease reduction values were noted. Disease severity of CMV under all the treatments was significantly lower than control. The interaction of concentrations with treatments was highly significant which gave maximum disease reduction in C_3 and minimum in C_1. The values of z-score inferred that each treatment was random in its respective concentration and none was clustered at ($p \leq 0.05$). The z-score values indicated that disease severity was random in concentration 1, 2 and 3 in all the treatments.

It's very interesting as none of the above mentioned treatments have insecticidal and viricidal properties. Then how these managed to combat the CMV disease because CMV impairs the plant physiology and these treatments have their independent pathways to repair the damages and triggering the plant defense. The infection caused by CMV disturbs the level of carbon assimilation and metabolic activities in infected plants. Previously in a study, biochemical characterization of infected leaf samples determined reduced carbohydrate concentration and photosynthesis with raised respiration rate (Shalitin and Wolf, 2020). There was significant reduction in biochemical reactions, photosynthetic pigments, protein contents, total phenols and alkaloids of CMV infected plants (Al-Zahrani *et al*., 2018). The functioning of 2b counter defense proteins is impaired due to CMV attack which causes host volatile emissions (Tungadi *et al*., 2017). Effect of CMV infection on host physiology was studied to investigate the alteration of cellular components like chlorophyll, stomatalconductanceand β-carotene (Rahman *et al*., 2019). There was considerable destruction in plant physiology by disruption in signals of hormones, cell deformities and carbohydrate movement (Mauck *et al*., 2019).

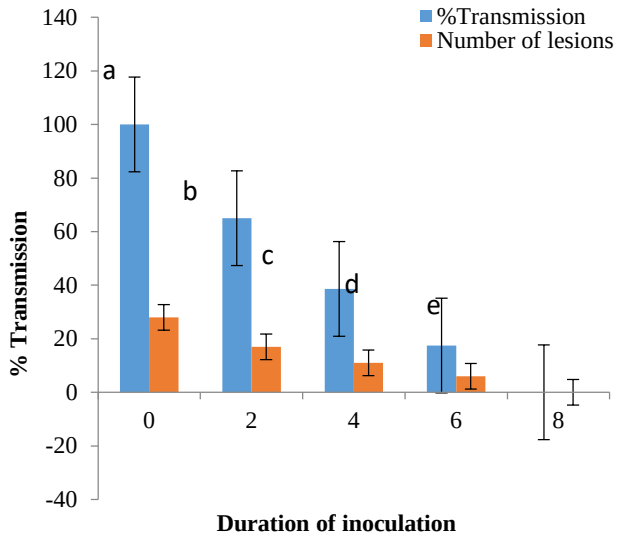

Fig. 3. Effect of storage time on transmission of CMV.

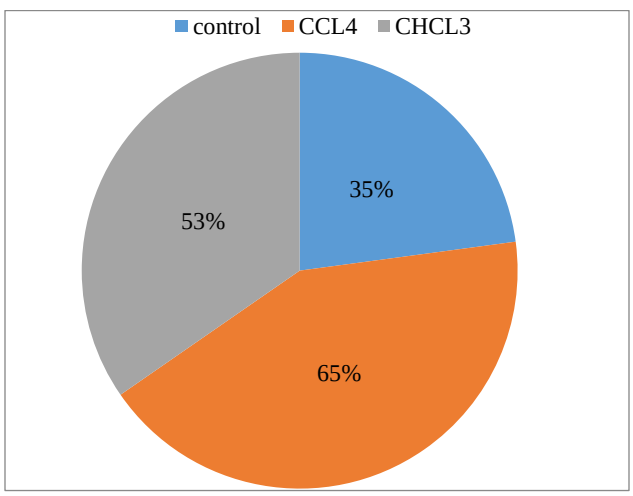

Fig. 4. Graphical representation regarding effect of reducing agents on CMV transmission.

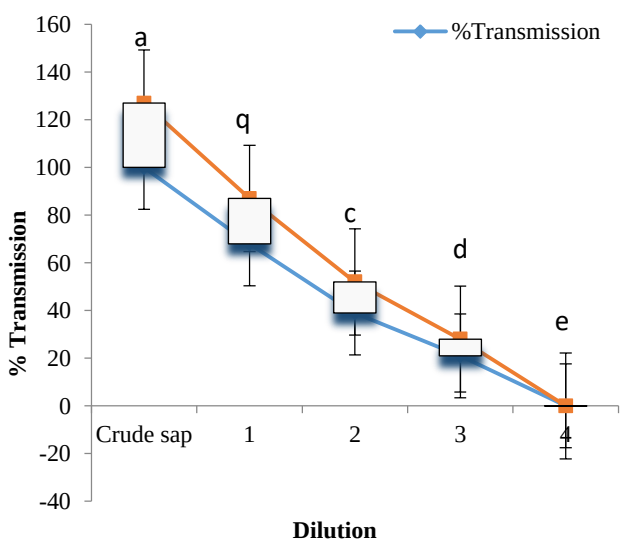

Fig. 5. Effect of series sap dilutions on transmission of CMV (crude sap= no dilution; 1= 10^{-1}; 2= 10^{-2}; 3= 10^{-3}; 4= 10^{-4}). Values with different letters are significantly different from each other at 5% probability level.

The most efficient treatment in managing CMV disease was the foliar application of NPK followed by BTH, NAA and elephant ear plant extract. The plant defense mechanism is activated through the induction of pathogenesis-related (PR) proteins, phytoalexins accumulation and callose depositions (Saldajeno and Hyakumachi, 2011). Nutrients enhance growth, development and initiate defense against pathogenic and physiological stresses (Meddad-Hamza *et al*., 2010). Nitrogen (N) acts as driving force in growth, development and yield of plants by nitrogen assimilation and amino acid transmission (Mur *et al*., 2016). It provides nitric oxide defense signal activation against pathogen invasion (Liu *et al*., 2015). Previous studies focused on plant defense as a function of nitrogen absorption through foliar application (Hout *et al*., 2014). Nitrogen is a constituent of polyamines that follows systemic pathway against biotic stresses through oxidative phosphorylation (Tiburcio *et al*., 2014). Nitric oxide signals stimulated by nitrogen metabolism initiate the salicylic acid dependent gene expression (Yu *et al*., 2014). Nutrient metabolism enhances sugar contents, amino acids and GABA (γ-aminobutyric acid) in the challenged cells (Gupta *et al*., 2013). Nitrogen yields metabolism yields products that take product in detoxification of reactive oxygen species that is linked with disease resistance (Chern *et al*., 2013). NO works as a driving force in generating the hypersensitive response against biotic stresses (Vitor *et al*., 2013). Potassium (K) regulates synthesis of protein, enzyme activation, carbohydrate metabolism, stomatal conductance and photosynthetic processes (Hasanuzzaman *et al*., 2018).K interacts with plant signals which regulates biochemical processes and metabolism that affects plant growth, development and enhances stress tolerance (Shani *et al*., 2017). It has vital role in photosynthesis, carbohydrate translocation and metabolism which ultimately enhance crop yield and quality (Lu *et al*., 2016). Enhanced photosynthetic activity was recorded in phosphorus treated plants (Naeem *et al*., 2010). The biochemical analysis of phosphorus treated plants indicated enhanced growth, development, metabolic reactions and defense signals (Hakeem *et al*., 2012). Similar results regarding increased metabolic activities were also observed in another study (Lambers & Shane, 2007).

The second most significant reduction was recorded in BTH applied plants. BTH is an appropriate treatment due to minute toxic hazards, quick degradation eco-friendly nature (Cao *et al*., 2011). BTH triggers SAR pathway against viruses in various plant species in different plant species (Nischwitz *et al*., 2008).It suppresses viral RNA accumulation in tobacco plants (Mandal *et al*., 2008). BTH interfered with RNA replication resulting in reduced number of local lesion in BTH treated plants (Namitharan *et al*., 2014).The antiviral activity of BTH derived chiral α-amino phosphonate studied by Zhang (Zhang *et al*., 2017). In virus infected plants peroxidases (POD), polyphenol oxidases (PPO) and phenylalanine ammonia lyase (PAL) enzymes were significantly reduced (Helal, 2019). The efficiency of naphthalene acetic acid (NAA) in reducing CMV disease severity could be explained by the fact that increases protein, carbohydrates and sugar contents of the plants (Tůmová*et al*., 2018). The previous research resulted in increased plant growth and development by the application of NAA (Kareem *et al*., 2017). As virus induces

decrease in protein, minerals and vitamin contents of the infected plants; the efficacy of elephant ear plant extract (*Clocasiaesculenta*) was attributed to its abundance of proteins, ascorbic acid, minerals and vitamins in its constituents (Chakrabortyet *al*., 2015). Ascorbic acid provides the basic defense against destructive effects of reactive oxygen species (ROS) (Gallie, 2013).

However, despite the clear importance of virus symptom severity as a driver of both yield losses and virus spread, strategies for mitigating symptoms and enhancing plant tolerance to virus infection are rarely considered as components of integrated disease management. It can be concluded that cultivars selection, proper dose of nutrients and plant extract application could be an effective integrated approach to manage CMV disease. In present study, spatio-temporal disease increment was recorded with each assessment. All the applied treatments induced strong defense responses in plants which resulted in significant disease severity reduction.

Comparison of chlorophyll contents and leaf area in diseased and healthy plants: Chlorophyll content values noted from the chlorophyll meter for each leaf sample and then average was taken which was plotted for five plants. There was significant decrease in the chlorophyll contents of diseased and healthy leaves in all plants. Maximum chlorophyll contents (2.7 mg g^{-1}) were recorded in case of healthy plants which were reduced upto (0.9 mg g^{-1}) in diseased plants (Fig. 6). There was significant reduction in chlorophyll contents of susceptible plants infected with CMV (Patel *et al*., 2013).

There was clear difference in leaf area of diseased and healthy plants with maximum (70cm^2) in healthy plants and minimum (27 cm^2) in diseased plants (Fig. 7). Leaf area reduction was recorded after mechanical inoculations in many cucumber advance lines along with other growth parameters (Azizi & Shams-Bakhsh, 2014).

ELISA for CMV detection: The samples collected from all locations were positive for the presence of CMV both in leaf lamina and midrib (Fig. 8; Table 6). The virus titer was high in leaf midrib than leaf lamina in all the samples. Optical density (OD) values were higher for the samples of BT than COA and ARF respectively and very strong reaction was observed in those samples. There was positive relationship between symptom severity and absorbance values of serological assays in different infected samples (Ashfaq *et al*., 2012). These results in line with previous serological studies where moderately resistant samples gave low absorbance values and high values were observed when ELISA performed on susceptible and highly susceptible samples (Iqbal *et al*., 2012). Arogundade *et al*., (2019) detected CMV from infected samples by using a modified method of ELISA that was antigen coated plate (ACP). Kumar *et al*., (2016) observed high virus titer in leaf mid rib than in lamina which strengthened the results of current experiment. Seed samples of cucumber were used for CMV detection by using anti rabbit polyclonal antibodies (Yoon *et al*., 2019).

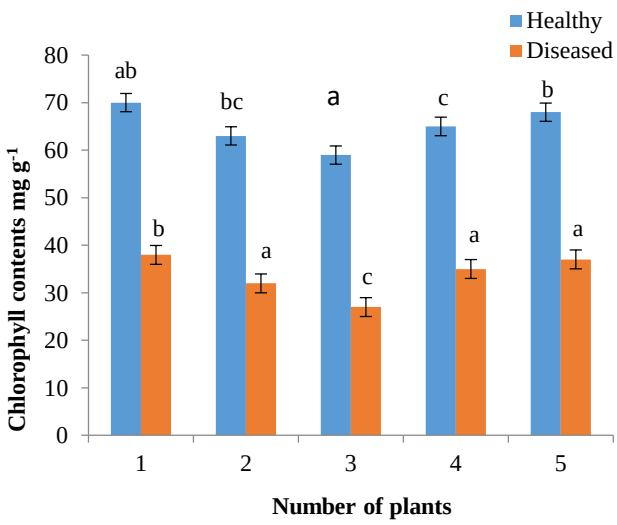

Fig. 6. Comparison of chlorophyll contents in diseased and healthy plants.

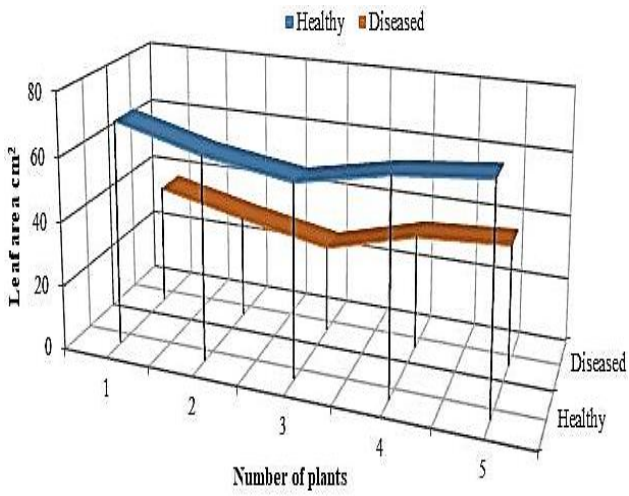

Fig. 7. Comparison of leaf area in healthy CMV infected cucumber plants.

Table 4. Disease severity as influenced by the treatments at various intervals

Treatments	Sprays				
	S_1	S_2	S_3	Mean	Efficacy (%)
NPK solution	25.36±1.12	16.82±1.64	10.59±1.26	17.59 e	75.55 a
NAA*	40.18±1.24	32.75±1.55	24.04±1.34	32.33 c	55.06 c
Elephant plant extract	50.14±1.09	44.32±2.24	36.26±1.26	43.57 b	39.44 d
BTH	33.44±1.26	25.71±2.01	19.24±1.29	26.13 d	63.68 b
Control	70.07±1.22	70.88±1.32	74.91±1.63	71.95 a	----
Mean**	41.25 a	29.9 b	22.53 c		----

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05)
*NAA = Naphthalene acetic acid; **Means don't include control values

Fig. 8. Microtitre plate depicting ELISA results.

Table 5. Disease severity as influenced by the treatments at different concentrations.

Treatments	Concentrations				Pattern
	C_1	C_2	C_3	z-score	
NPK solution	22.08 ± 1.41	12.91±1.73	8.66±1.53	-0.94	Random
NAA	38.34 ± 1.27	29.09±1.36	22.64±1.02	-0.26	Random
Elephant plant extract	48.97 ± 1.13	41.04±1.25	33.58±1.34	0.24	Random
BTH	30.52±1.05	19.13±1.16	14.83±1.82	-0.64	Random
Control	69.05±1.25	71.54±1.56	75.86±1.47	1.61	Random
z-score for conc.	1.07	-0.16	-0.91		
Pattern	Random	Random	Random		

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05)
*NAA = Naphthalene acetic acid

Conclusions

The spatio-temporal disease assessments provide a road map to devise sustainable disease management strategies. The virus disrupts the plant physiology and its biochemical contents. All the treatments used, worked to repair the physiological and biochemical damages incited by CMV. The extraordinaryresults were noted with the plant's ability to defend against viruses with application of NPK. This contribution of NPK is an essentiallandmark for sustainable agriculture. Many environmental concernsconvince the growers to use nutrients like NPK for disease management. It would be helpful to get rid from hazardous synthetic chemicals.

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