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SIMULATION OF A MODEL FOR DIAGNOSIS AND PRESCRIPTION FOR CUCUMBER MOSAIC DISEASE

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ABSTRACT

The Experiments were carried out during the period of 2009 to 2013 on the epidemiological status and prescriptions used for the management of cucumber mosaic disease with a view to simulate a diagnosis and prescription model. The survey area covered five upazillas *viz.* Mymensingh sadar, Kushtia sadar, Kumarkhali, Chandina and Sher-e-Bangla Nagar under four districts *viz.* Mymensingh, Kushtia, Comilla and Dhaka where altogether 250 farmers field were considered for observation. The highest disease incidence of mosaic of cucumber (37.1%) was recorded at Kumarkhali, while the lowest were (33.9%) in Boira Keyotkhali. Gemini virus was detected as causal pathogen for cucumber mosaic disease. The model prescriptions issued by Plant Disease Diagnostic Clinic (PDDC) found effective in the farmer's field against the Cucumber Mosaic disease in selected locations were subjected to re-evaluation under different experimentations at Plant Pathological field laboratory at BAU farm. Economically affordable management practices were re-tested in five locations of Bangladesh. Based on the re-investigation results, the model prescriptions were finally selected for the vegetable growers of the country. Model diagnosis and prescription have been proposed. These were Sumi-Alpha @ 1 ml/l sprayed 4 times at 15 days intervals starting from first sign of the disease at 60 days after sowing for cucumber mosaic disease. The management practices were reduced of disease incidence (73.5%), disease severity (73.8%), yield increased (74.4%) over control and benefit cost ratio (BCR) was 3.40, respectively.

Keywords: simulation, model, diagnosis, CMV, prescription

INTRODUCTION

A model can be presented as a simple rule, an equation, a graph, or a table. The output of a model can be a numerical index of disease risk, predicted incidence or severity, and/or predicted inoculums development. Eventual goal is plant disease identification and issuing model prescription. Plant disease models typically are developed in specific climates and regions around the world (Janet, 2004). Recent developments in molecular diagnostic techniques overcome of these barriers to good diagnosis and open the way to improve decision support systems, and to apply fungicides or insecticides more effectively (Meah, 2006). Cucumber belongs to the family *cucurbitaceae* and is one of the important and popular condiment vegetables of Bangladesh. The crop is of Asian origin, the progenitor may be closely related to the wild Cucumis sativus *viz.* hardwickii, which was first found in the Himalayan foothills of Nepal. Cucumber cultivation goes back to last 3000 years in India and in China (Rabinson and Decker, 1997). Today cucumber is grown throughout emergence in the field (Meah and Khan, 1987). Different phytopathogenic seed, soils, as well as vector borne pathogens are responsible for disease development, which attacks the cucurbits during seedling stage to maturity and with both quantitative and qualitative yield reduction (Alimova *et al.* 2002). It suffers from more than 24 different diseases out of which 14 are seed borne. Among them, cucumber mosaic disease has been recorded as vector borne virus pathogen of cucumber in Bangladesh (Anonymous, 2003). Cucumber is important popular vegetables of Bangladesh. It was grown in 7480.31 hectares of land and the

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production was 50,000 tons in 2011-2012, the average yield was 6.68 t/ha (BBS, 2012). These diseases pose threat to cultivation of those vegetables often causing 20-30% yield loss, in severe condition may cause total crop failure. Apposite care and management practices are therefore, necessary. Cultural, biological and chemical controls are some options. Proper diagnosis is the prime basis of the application of the appropriate management practices. Quickest and economic are the two important criteria for the efficient diagnostic process. Field diagnosis of the disease is the first step of an effective management. Integrated Pest Management (IPM) approach is the basis and should be the basis of all options for plant disease control in Bangladesh. PDDC has been doing it since its inception in 2006. It is time to monitor the extent of its application in the field. The objectives of the present studies were to simulate model for diagnosis of cucumber mosaic diseases and to formulate model prescription for cucumber mosaic diseases based on proper diagnosis and management options.

MATERIALS AND METHODS

***In-vitro* Experiments:** The *in-vitro* experiments were conducted at IPM-laboratory, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh and Plant Pathological Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh.

Field Experiments: Field experiments were conducted at Plant Pathology Field Laboratory, Department of Plant Pathology, Bangladesh Agricultural University.

Experimental period: The experiments were conducted during the period of 2009-2013.

Set of Experiments: The following five sets of experiments were carried out in order to simulation of a model diagnosis and prescription for management of cucumber mosaic.

Experiment I: Epidemiological survey on incidence and severity of cucumber mosaic in selected growing areas of Bangladesh during Rabi season of 2009-2010.

Detail research methodology of the experiments: Five upazillas under four districts of major cucumber growing areas of Bangladesh were selected. Ten (10) vegetable farmers/growers/roof gardeners/nursery owners in one village, five (5) villages in one upazilla were randomly selected based on the size of vegetable growing areas. Data on the incidence and severity of cucumber mosaic disease and their control measures adopted by the farmers in the five specific locations were collected. Data on % fruit infection, soil pH, soil temperature, soil moisture, air temperature, air moisture (RH %) and rainfall were also recorded.

Disease incidence: Collecting the information on diseased and healthy plants, the disease incidence was calculated using the following formula (Islam *et al.*, 2001).

$$\text{\% Disease incidence} = \frac{\text{Number of infected plant}}{\text{Number of total plant inspected}} \times 100$$

Disease severity: For foliar diseases, % LAD (leaf area diseased) and for fruit diseases, % FAD (fruit area diseased) was measured by eye estimation. Area of a single leaf/fruit was considered as 100%. Percent disease severity (DS) was measured by using the following formula (Islam *et al.*, 2001):

$$\text{\% Disease severity (leaf)} - \frac{\text{\% infected leaf area}}{\text{Total leaf area inspected}} \times 100$$

$$\text{\% Disease severity (fruit)} = \frac{\text{\% infected fruit area}}{\text{Total fruit area inspected}} \times 100$$

Experiment II: Collection of comprehensive information about the problems on diseased samples brought to plant disease diagnostic clinic (PDDC), BAU for disease diagnosis and issuing prescription

Information about the problem of selected diseases on the different aspects was collected from the client who had submitted/sent the disease sample to PDDC (Plant Disease Diagnostic Clinic). Samples of selected diseases when brought by farmers were studied in the PDDC and prescriptions were issued.

Immunostrip test for virus detection: Immunostrip (Catalog Number: TSWV-01; Manufacturer Information: AGDIA) was used to quickly identify cucumber mosaic virus (CMV) from plant specimen with or without disease symptoms. The sample extraction bag was cut open along the top of the label, filled up with buffer to its one-third volume (Zein *et al.*, 2007). The sample was inserted between the mesh linings near the bottom of the sample extraction bag. It substitute was extracted by rubbing it gently between the mesh linings with a blunt object such as a pen or permanent marker. For observation of the reaction, the immunostrip were inserted into the channel portion of the buffer-filled bag or vial no more than ¼"or to the white line on the immunostrip label.

Table 1. List of isolates of cucumber mosaic viruses with cultivars, plant parts and method used for identification

Isolates number	Cultivars	Plant parts	Method
Cucumber-1	Mollika	Leaf	Immunostrip
Cucumber-2	Deshi	Leaf	Immunostrip
Cucumber-3	Mollika	fruit	Symptom study
Cucumber-4	Baramashi	fruit	Immunostrip
Cucumber-5	Shohag	Leaf	Immunostrip
Cucumber-6	Mollika	fruit	Symptom study
Cucumber-7	Deshi	Leaf	Immunostrip
Cucumber-8	Mollika	Leaf	Immunostrip
Cucumber-9	Mollika	fruit	Symptom study
Cucumber-10	Mollika	Leaf	Immunostrip
Cucumber-11	Baramashi	fruit	Immunostrip
Cucumber-12	Shohag	Leaf	Immunostrip
Cucumber-13	Mollika	fruit	Symptom study

Experiment III: Collection of comprehensive information on selected disease problems and their management practices adopted by the farmers

Data on the disease incidence and severity of cucumber vegetable crops encountered and the control measures adopted by the farmers in the five specific locations were collected. The weather data of the specific area were collected.

Experiment IV: Investigation into validity of the management practices prescribed by PDDC and adopted by the farmers of CMV disease during Rabi season of 2011-2012

Information gathered from both the field and PDDC on the selected diseases were interpreted, combined and conclusion drawn on the common practices for the management of those diseases. Based on the compiled data field experiments were conducted.

Preparation of pits and sterilization of soil: Pit (height × width × depth= $1 \times 1 \times 1$ m^3) substratum was prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2: 1: 1 and sterilized with 5 ml formalin (40%) diluted with 20 ml water for 4 kg soil (Dashgupta, 1988).

Design and layout: The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. Block to block and pit to pit distances as 2.0 m and 3.0 m, respectively.

Seeds sowing: 4 seeds were sown per pit on November 24, 2011. Regular watering was done to maintain soil moisture.

Treatment and treatment combinations: The experiments consisted of six treatments combinations. All treatments were applied four times @ 15 days intervals starting from 60 days after sowing (DAS). They were as follows:

i. T_1 = Control
ii. T_2 = Admire–DF @ 0.5ml/l
iii. T_3 = Admire–DF @ 1ml/l
iv. T_4 = Sumi-Alpha @ 0.5ml/l
v. T_5 = Sumi-Alpha @ 1ml/l
vi. T_6 = Admire–DF @ 0.5ml/l+Sumi-Alpha @ 0.5ml/l

Application of the treatments: Solution insecticide was prepared by taking required amount of insecticides in the tap water and mixed thoroughly to disperse the active ingredient and was applied as foliar spray by Knapsack hand sprayer. Admire–DF and Sumi-Alpha were applied @ 0.05 and 0.1% each conc.

Experiment V: Simulation of a model for diagnosis and prescription for mosaic of cucumber for the grower

Results of the field experiments were analyzed and interpreted. Three treatments, which yielded significantly better performance, were selected further study.

Re-testing of the primarily constructed model: For re-testing, the following factors were considered:

Host: Variety (most susceptible) -based on BAU field experiment.

Disease: Incidence/Severity (%) estimated at BAU field trial.

Management practices: Three management practices those yielded better performance in BAU field trials were retested.

Re-testing of the primary model: Re-testing experiments were conducted at the Plant Pathological field laboratory, Bangladesh Agricultural University, Mymensingh.

Data Collection: i. Disease incidence/Severity (%) ii. Yield/ Yield loss and iii. BCR

Model preparations: Results obtained through field trials were computed, analyzed and interpreted. The best two results were selected and used for the construction of specific disease diagnostic model. Construction of the model was based on:

I. Disease management practices: IPM approach, pesticide(s), stage of the crop for intervention, frequency of the application of crop protection measures.

II. Yield and yield loss relationship.

III. Benefit Cost Ratios (BCR): Costing of application of integrated approaches for management of mosaic of cumber was done based on the current market price of input, rate of hiring labor and agricultural machineries. Price of the field produce was determined on the basis of current market value. Estimation of Benefit Cost Ratio (BCR) was done according to Gittinger (1982) using the following formula:

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Gross return (Tk./ha.)}}{\text{Total cost of production (Tk./ha.)}}$$

Data analysis: Compilation and analysis of the research survey and experimental data were done by the computer MSTAT(C) software program following the statistical procedures (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Epidemiological survey on incidence and severity of cucumber mosaic in selected growing areas of Bangladesh during Rabi season of 2009-10

Disease incidence (% plant infection) of cucumber varied with different locations, soil texture and environmental factors *viz.* soil pH, soil moister, soil temperature (^{0}C), air temperature (^{0}C), Rainfall (cm) and RH% (% relative humidity).The highest disease incidence (37.1%) and disease severity (16.7%) of cucumber mosaic were observed in Kumarkhali, Kushtia Sadar and Marichar Char. Significantly, the lowest disease incidence (33.9%) and severity (14.2%) were observed in Chandina that were statistically identical with that of Boira Keyotkhali as shown in Table 2.

Table 2. Incidence of cucumber in selected locations of Bangladesh (pooled for the years 2009-2010)

Locations	Mosaic of cucumber	
	Disease Incidence	Disease Severity
Marichar Char	36.4ab	16.7ab
Boira Keyotkhali	33.9b	14.3ab
Kushtia Sadar	37.0a	16.6a
Kumarkhali	37.1a	16.4ab
Chandina	34.1b	14.2b
LSD (P<0.01)	2.834	2.299
(CV %)	2.90	5.39

Table 3. Compilation of prescriptions issued by plant disease diagnostic clinic (PDDC) on CMV disease samples brought by farmers during 2009-2010

Disease (Cause)	Samples brought by the farmers	No. of samples	Prescription issued by PDDC
Mosaic of Cucumber (CUMV)	BAU campus Mymensingh. Kushtia Sadar Kumarkhali Chandina, Comilla.	68	i. Remove mosaic affected leaves, burn or bury in the soil. ii. Spray Admire@ 0.1% four times @ 10 days interval in all plants of the field and nearby fields.

Table 4. Summary of the effect of management practices recommended by PDDC adopted by the farmers against cucumber mosaic disease

Crop and Disease	Management practice (s)	% DI under prescribed treatment		Mean of two years	% DI under control		Mean of two years	% DI decreased over control	
		2009-2010	2010-2011		2009-2010	2010-2011		2009-2010	2010-2011
Cucumber, Cucumber Mosaic	i.Sumi-Alfa	33.9	33.2	33.5	37.1	51.2	44.1	8.6	35.1
	ii. Admire	34.1	35.5	34.8	37.1	51.2	44.1	8.0	30.6
	iii. Malathion	36.4	38.7	37.5	37.0	51.2	44.1	1.6	24.4

i Sumi-Alpha @ 1ml/l sprayed 4 times at 12 days interval
ii. Admire @ 1ml/l 3 times sprayed at 12 days interval.
iii. Malathion 57 EC @ 2ml/l sprayed 3 times at 12 days interval.

Typical symptoms and experimental pit of cucumber crops

Symptoms of cucumber mosaic as recorded in different treatments in the experimental field. Aphids were observed on the affected cucumber leaves. Immunostrip test was employed for detection of the virus in the infected leaves of cucumber.

Effect of different treatments on incidence of mosaic of cucumber

Treatment effects on incidence of mosaic disease of cucumber were recorded with 15 days interval starting from 60 DAS (Days after sowing) to 105 DAS and the results were presented in Table 5. At 60 DAS, the effects of the treatments were non-significant but with the advancement of time the disease condition of the treated plot started to change. At 105 DAS, the lowest disease incidence (17.0%) was recorded in case of T_3 (Admire @ 1ml/l) 17.0% that was statistically similar with T_2 (Admire @ 1ml/l) 28.0%, T_5 (Sumi-Alpha @ 1ml/l) 22.5% and T_6 [(Admire @ 0.5ml + Sumi-Alpha @ 0.5ml) /l] 27.5%) treatments but differed significantly with T_4 (Sumi-Alpha @ 0.5ml/l) 30.0%. As per the performance of reducing the disease incidence, T_3 (Admire @ 1ml/l) was promising where 80.0% reduction of the disease incidence was observed followed by T_5 (73.5%), T_6 (67.6%), T_2 (67.0%) and T_4 (64.7%) over control.

Table 5. Incidence of mosaic of cucumber (cv. Mollika) in response to different treatments at different days after sowing (DAS)

Treatments	% Disease incidence (DI %)				% Reduction of DI over control at 105 DAS
	60 DAS	75 DAS	90 DAS	105 DAS	
T_1 - Control	15.2	38.5a	64.0a	85.0a	---
T_2 = Admire @ 0.5ml/l	14.5	19.2b	22.5b	28.0bc	67.0
T_3 = Admire @ 1ml/l	14.2	15.0b	15.7b	17.0c	80.0
T_4 = Sumi-Alpha @ 0.5ml/l	15.5	19.5b	23.7b	30.0b	64.7
T_5 = Sumi-Alpha @ 1ml/l	14.5	17.0b	21.2b	22.5bc	73.5
T_6 = (Admire 0.5ml + Sumi-Alpha 0.5ml)/l	15.2	19.5b	23.2b	27.5bc	67.6
LSD (0.01)	NS	5.6	9.8	11.6	---
CV %	13.0	12.5	16.5	15.9	---

NS = Not significant, Means followed by the same letter(s) in columns are not significantly different at $P < 0.01$ by Duncan's Multiple Range Test.

Effect of different treatments on severity of mosaic disease of cucumber

Treatment effects on the disease severity of mosaic of cucumber were recorded with 15 days interval starting from 60 DAS (days after planting) to 105 DAS and the results were presented in Table 6. At 60 DAS, no significant difference were found on the treatments effects but with the progress of time the disease condition of the treated plot started to change. At 75 DAS; the treatment effects were statistically significant among themselves but different with control. At 90 DAS; performances of the treatments were more or less similar to that of 75 DAS. At 105 DAS, the lowest disease severity 18.7% was recorded in case of T_3 (Admire @ 1ml/l) that was statistically similar with T_5 (Sumi-Alpha @ 1ml/l). Moderate effects were observed in case of the rest of the treatments, while the highest disease severity 81.2% was recorded in control plots. With respect to disease severity, T_3 (Admire @ 1ml/l) scored 76.9% reduction followed by T_5 (Sumi-Alpha @ 1ml/l) 73.8%, T_4 (Sumi-Alpha @ 0.5ml/l) 49.2%, T_2 (Admire @ 0.5ml/l) 48.3% and T_6 (Admire @ 0.5ml/l + Sumi-Alpha @ 0.5ml/l) 44.6% over control at 105 DAS.

Table 6. Disease severity of mosaic disease of cucumber (cv. Mollika) in response to different treatments at different days after planting (DAS)

Treatments	% Leaf infection (disease severity %)				% Reduction of DS over control at 105 DAS
	60 DAS	75 DAS	90 DAS	105 DAS	
T_1 - Control	17.2	45.5a	70.0a	81.2a	---
T_2 = Admire @ 0.5ml/l	15.5	26.2bc	32.5b	42.0b	48.3
T_3 = Admire @ 1ml/l	14.2	17.0c	18.7c	18.7c	76.9
T_4 = Sumi-Alpha @ 0.5ml/l	15.5	26.7bc	36.7b	41.2b	49.2
T_5 Sumi-Alpha @ 1ml/l	15.5	18.2c	20.7c	21.2c	73.8
T_6 - (Admire 0.5ml + Sumi-Alpha 0.5ml)/l	15.0	28.7b	37.5b	45.0b	44.6
LSD (0.01)	NS	9.8	9.6	9.0	---
CV %	19.1	17.4	12.9	10.4	---

NS = Not significant, Means followed by the same letter(s) in columns are not significantly different at $P < 0.01$ by Duncan's Multiple Range Test.

Calculation of costing of management practices and determination of Benefit Cost Ratio (BCR)

For the management of Cucumber mosaic virus, spraying Sumi-Alpha @ 1ml/l (T_5) proved most effective which gave the highest yield and highest disease reduction. The second best was spraying Admire-DF @ 1ml/l (T_3) followed by the combined spray of Admire-DF @ 0.5 ml/l + Sumi-Alpha @ 0.5ml/l (T_6). These three practices were considered in Table 7.

Table 7. Benefit Cost Ratio (BCR) of different management practices for cucumber mosaic

Treatments	Yield T/ha, a	Increase in yield over control (%)	Gross return (TK/ha) b	Total cost of treatment (TK/ha) c	Gross margin d= (b-c) (TK/ha)	BCR
T_1 = Control	3.7	--	47125	48300	-1175	0.97
T_2 = Admire @ 0.5ml/l	8.2	54.3	103125	60380	42745	1.70
T_3 = Admire @ 1ml/l	15.2	75.2	190625	69020	121605	2.76
T_4 = Sumi-Alpha @ 0.5ml/l	10.5	64.0	131250	52860	78390	2.48
T_5 = Sumi-Alpha @ 1ml/l	14.7	74.4	184375	54140	130235	3.40
T_6 = (Admire 0.5ml + Sumi-Alpha 0.5ml)/L	13.3	71.6	166250	62940	103310	2.64

Cucumber market price of 12,500 TK/Ton. Calculated on basis of market price of 2013.

Selection of effective management practices

Based on the reduction of disease severity, increase in crop yield and the cost incurred in managing the disease, two best practices were finally selected as economically promising. The selection leaves a scope of option of one or the other management practices depending on the availability of the product in the market and resources of the farmers were recommended at Admire-DF @ 1ml/l 4 times sprayed at 15 days interval and Sumi-Alpha @ 1ml/l 4 times sprayed at 15 days interval presented in Table 8.

Construction of the diagnostic model and prescriptions

Six management practices selected for each of cucumber mosaic was further explored in the field laboratory of the Department of Plant Pathology at Bangladesh Agricultural University during 2011 to 2012. As per the performances of the treatments, in respected to disease incidence, severity and yield. Treatment T1CMV and T2CMV were finally selected for of Cucumber mosaic diseases as shown in Table 8.

Table 8. Summary of the effective management practices for construction of the diagnostic model and prescription

Problems	Effective Management Practices	DI%	DS%	Yield (t/ha)	(%) Increased in yield over control
Cucumber mosaic	i. T1CMV: Admire-DF @ 1ml/l 4 times sprayed at 15 days interval.	17.0	18.7	15.2	74.4
	ii. T2CMV: Sumi-Alpha @ 1ml/l 4 times sprayed at 15 days interval	22.5	21.2	14.7	75.2

Proposition of a diagnostic Model and prescription for the selected vegetable diseases

Two best treatments were selected based on percentage disease severity reduction, percentage yield increased over control and benefit-cost ratio (BCR) in the field in different locations of Bangladesh during 2012 to 2013. Based on the diagnosis techniques used in plant disease diagnostic clinic (PDDC) and the results of the management practices retested in the field, a diagnostic model and economically affordable prescription for cucumber mosaic disease has been proposed as shown in Table 9.

Table 9. Proposed diagnostic model and prescription for cucumber mosaic disease

Diagnosis in the field/lab	Economic /affordable prescription	BCR
Field Test i. Green and yellow mottling on curled leaves ii. Presence of aphids	i. Sumi-Alpha @ 1ml/l 4 times sprayed at 15 days interval	3.40
Lab Test: i. Symptoms study ii. Immunostrip test	ii. Admire-DF @ 1ml/l 4 times sprayed at 15 days interval.	2.76

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