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DIAGNOSIS AND PRESCRIPTION FOR MANAGEMENT OF BACTERIAL WILT OF POTATO

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ABSTRACT

The experiments were carried out during 2009 to 2013 on diagnosis and prescriptions for management of bacterial wilt of potato. The studies were undertaken in 250 growers from five locations *viz.* Mymensingh Sadar, Kushtia Sadar, Kumarkhali, Chandina and Sher-e-Bangla Nagar. The highest and lowest incidence (20.8%) and (12.6%) of bacterial wilt of potato was recorded at Marichar Char and Kushtia Sadar. *Ralstonia solanacearum* produced bacterial wilt of potato disease. The prescription given by Plant Disease Diagnostic Clinic (PDDC) was effective in the farmer's field against the disease. From this study it may be prescripted that the Cupravit-50 WP [Copper-oxchloride ($CuOCl_2$)] @ 7 g/l sprayed 4 times around root zone at 15 days interval with first time application of 50 gm mixed fertilizer (Ash: KCl=20:1) per plant for the management of bacterial wilt of potato. It was also revealed that the practices reduced the disease incidence by 80.79% over control. The yield increased by 84.57% and benefit cost ratio (BCR) was 4.62.

Keywords: Diagnosis and Prescription, Bacterial wilt, Potato.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the popular tuber food crop of the world belongs to the family of Solanaceae. Currently 2.06 million hectares of land is under potato cultivation in Bangladesh where 45.56 million metric tons yield is produced per annum (BBS, 2016). It is very low in comparison to that of other leading potato growing countries in the world, *viz.*, USA (43.49 t/ha), Denmark (39.41 t/ha) and UK (41.38 t/ha) (FAO, 2005), 43.3 t/ha in the Netherlands (Chadha, 1995 and Swaminathan, 2000). Millions of people in Bangladesh are suffering from malnutrition. Potato can play an important role for food security in the country. It is an important food crop of Bangladesh (Hashem, 1990). Beneath its covering are liberal stores of energy and a high quality protein. There are valuable minerals such as iron, magnesium, potassium, phosphorus and essential vitamins such as vitamin-C and several of the vitamin-B (McCay and McCay, 1967). Diseases are one of the most important factors for the low yield of potato in Bangladesh. In Bangladesh, in total 39 diseases (both biotic and abiotic) of potato have been recorded (Ali and Khan, 1990). The major soil and tuber-borne diseases are bacterial wilt, common scab, stem canker and black scurf. Among them bacterial wilt caused by *Ralstonia solanacearum* is the most common and widespread disease throughout the country (Ali and Dey, 1994). In Bangladesh, the crop loss due to bacterial wilt of potato may rise from 10 to above 80% if the cropping season is humid along with low temperature (Zahid *et. al.* 1993).

Plant disease models are typically developed in specific climates and regions around the world. The model is tested for one or more seasons under local conditions to verify that it will work in this location. Plant Disease Diagnosis Model which simulation for evaluation of a plant disease during the season give important information to assess the seriousness of the situation. The activity precedes the choice of an appropriate action to be implemented for reducing the economic damage. Having effective models is a critical issue in modern agriculture especially with low environmental impact. Pathology models, population and epidemiological models have been developed for several diseases (Janet, 2004). Eventual goal is plant disease identification and issuing model prescription. Identifying diseases

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and levels of infection through visual assessments are not always easy, even for experienced plant pathologists. Recent developments in molecular diagnostic techniques overcome some of these barriers to good diagnosis and open the way to improve decision support systems, and to apply fungicides more effectively (Meah, 2006). Plant disease diagnosis and issuing prescription will educate extension specialists, students and farmers on how to identify common plant pathogens and pests, and incorporate of this model or techniques. Reducing unnecessary applications of agricultural chemicals provide quality control for certifying pathogen-free plant material for export and import, and identify important and/or emerging disease problems. This helps increase yield of crops and ensures financial security of farmers detailing effective, economical, environmentally and eco-friendly agriculture responsible of disease management (Meah, 2006). To ensure better quality, prime importance need to be given for control of the disease to drive maximum yield. For the strategy of developing control measures against bacterial wilt of potato, a disease management model is essential. The objectives of the present studies were to simulate model for diagnosis of bacterial wilt of potato disease and to formulate model prescription for the disease based on proper diagnosis and management options.

MATERIALS AND METHODS

Experiment was conducted at the IPM-laboratory and Experimental Field of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh and Plant Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka, Bangladesh during 2009-2013. Survey on the incidence of bacterial wilt of potato was done during Rabi 2009-2010. CV. Diamond was selected in survey and field experiment of the bacterial wilt of potato, Four Districts, Dhaka, Mymensingh, Kushtia and Comilla, were the survey areas. Five villages from each of five Upazillas were selected from four districts under five upazillas of major potato growing areas of Bangladesh. Ten (10) farmers were selected from each village. Data on reveal of plants infected and tuber counted were recorded. Incidence of the disease was calculated. Control measures adopted by the farmers were also recorded. Four visits were made during November-March. Soil pH, temperature, soil moisture, air temperature, air moisture (RH %) and rainfall were noted. Disease was measured by eye estimation. Percent disease incidence (DI) was measured by using the following formula (Islam, 2009).

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

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

Typical symptoms diagnostic process of bacterial caused by wilt (*Ralstonia solanacearum*): Case-history information and several biochemical/physiological tests were followed. Oozing test in water and streaming test under microscope was the hand-on techniques for identification of the bacteria. Subsequently gram strain test, UV-florescence, colony, colony color and biochemical tests were performed. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. The experiments consisted of six treatment and treatment combinations. They were as follows:

T_1 = Control, T_2 = Ash mixed potash (Ash : KCl = 20:1) @ 50g/plant

T_3 = Cupravit-50 WP @ 5g/L, T_4 = Cupravit-50WP @ 7g/L

T_5 = Ash mixed potash (Ash : KCl = 20:1) @ 50g/plant + cupravit-50 WP @ 5g/L

T_6 = Ash mixed potash (Ash : KCl = 20:1) @ 50g/plant + cupravit-50 WP @ 7g/L

Ash mixed potash and cupravit-50 WP were used as treatments in different combinations. It's were mixed @ ash: potash=20:1. The mixer was applied @ 50g/plant. Cupravit-50 WP was applied @ 0.5 and 0.7% as soil drenching at root zone. All treatments were applied four times @ 15 days intervals. Typical bacterial wilt symptoms were observed in untreated plots (Figure 1- 2). The bacteria was that caused wilt of potato was isolated and cultured in Nutrient Agar medium (Figure 3-4). The culture was studied under microscope and subject to diagnostic test such as gram strain test and identified as *Ralstonia solanacearum* (Figure 5-6).

Fig. 1. Experimental field of potato

Fig. 2. Symptoms of bacterial wilted field of potato at 45 DAP

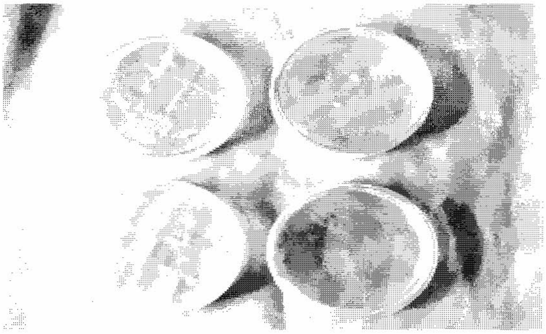

Fig. 3. Two day's single colonies appeared on NA culture media

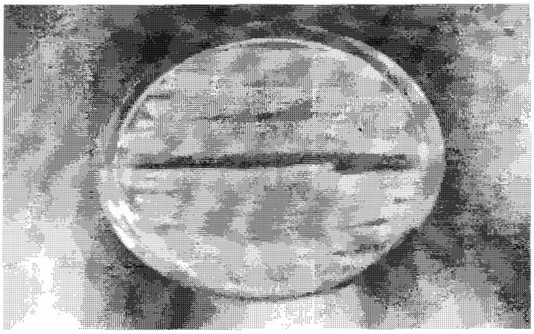

Fig. 4. Four day's colony in sub-culture

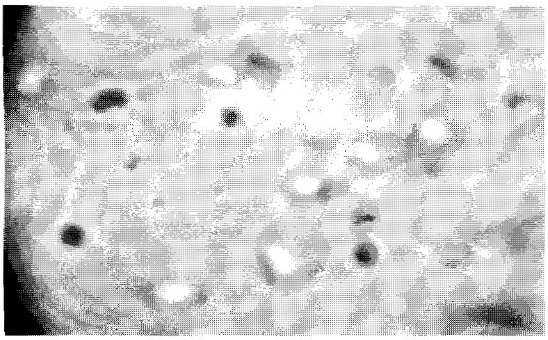

Fig. 5. Cells of *R. solanacearum* under compound microscope.

Fig. 6. KOH (Potassium hydroxide) Test

Oozing test: Observation was on sign and symptoms. A piece of the diseased stem 15 - 20 cm long from the base was cut and suspended the cut stem in distilled water in a glass container held in a vertical position. Observations were made for streaming of any ooze. Within a few minutes, if the smoke like milky threads discharged from the cut stem then were assumed it is bacterial wilt disease organism (Meah and Wick, 2006).

Culturing of bacteria: Tissues rinsed in distilled water were blotted on sterile paper towel with sterile forceps. Then tissue pieces were macerated and placed in tube of sterile water and made serial dilution by transferring 1 ml. of bacterial suspensions from one tube to another and so on. After then 0.5 ml. of each dilution was placed into separate petridish. Then added melted cool agar, stirred gently and let solidified, a two-day's single colonies appeared at one or more of the plates. Single colonies were sub-cultured and bacteria were identified (Fahy and Persley, 1983).

Identification of *R. solanacearum* by morphological characteristics: *Ralstonia solanacearum* is gram-negative rod shaped bacteria and usually produces off-white or light cream color in NA (nutrient agar) medium. Morphological characteristics (shape, size, surface texture, edge, elevation, color) of *R. solanacearum* developed after 24-48 hrs of incubation in TTC medium were studied (Fahy and Persley, 1983).

Table 1. List of isolates of Bacterial wilt of potato with cultivars, plant parts, place of isolation and media used for isolation and identification

Isolates number	Cultivars	Plant parts	Place of isolation	Media/ Method
Potato-1	Diamant	Stem	IPM-lab	NA
Potato-2	Cardinal	Stem	IPM-lab	NA
Potato-3	Diamant	Stem	Field	Oozing
Potato-4	Cardinal	Stem	SPL-lab	Gram-strain
Potato-5	Diamant	Stem	IPM-lab	TTC
Potato-6	Diamant	Stem	Field	Oozing
Potato-7	Diamant	Stem	SPL-lab	Gram-strain
Potato-8	Diamant	Stem	IPM-lab	NA
Potato-9	Diamant	Stem	SAU-lab	TTC
Potato-10	Cardinal	Stem	IPM-lab	Oozing

Triphynyl Tetrazolium Chloride (TTC) medium test: This medium was used to test pathogenic strains of the vascular bacterial pathogen, *Ralstonia solanacearum*. The medium was streaked with bacterial smear, incubated for 48 hours, and then observed for colony shape, consistency and color for identification. 5 ml. of 1% TTC solution was added to one litre of melted basal medium to give a final concentration of 0.005% TTC in the medium. The petridishes containing TTC and NA media were streaked with the pure culture of each group of isolates of *R. solanacearum* with the help of sterilized platinum wire dipping in rectified sprit and flaming over a sprit lamp. Then the isolates of *R. solanacearum* representing each location were classified based on the colony morphology on TTC medium. The colonies of the virulent isolates were to be pink or light red color or characteristics red center and whitish margin on TTC medium (Meah and Wick, 2006).

Benefit cost ratios (BCR): Cost of application of integrated approaches for management of *Cercospora* leaf spot of chilli was done based on the current market price of input, rate of hiring labor and agricultural machineries. Price of the field product was determined on the basis of current market value. Estimation of Cost Benefit Ratio (BCR) was done according to Gittinger (1982) and Islam (2009) using the following formula:

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

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

RESULTS AND DISCUSSION

Disease incidence of bacterial wilt of potato varied in different locations, Soil texture and environmental factors like soil pH, moister, soil & air temperature (^{0}C), Rainfall (cm) and % RH. The highest disease incidence (20.8%) of bacterial wilt of potato was observed in Marichar Char followed by Boira Keyotkhali, while the lowest disease incidence was (12.6%) observed in Kushtia Sadar as shown in Figure 7.

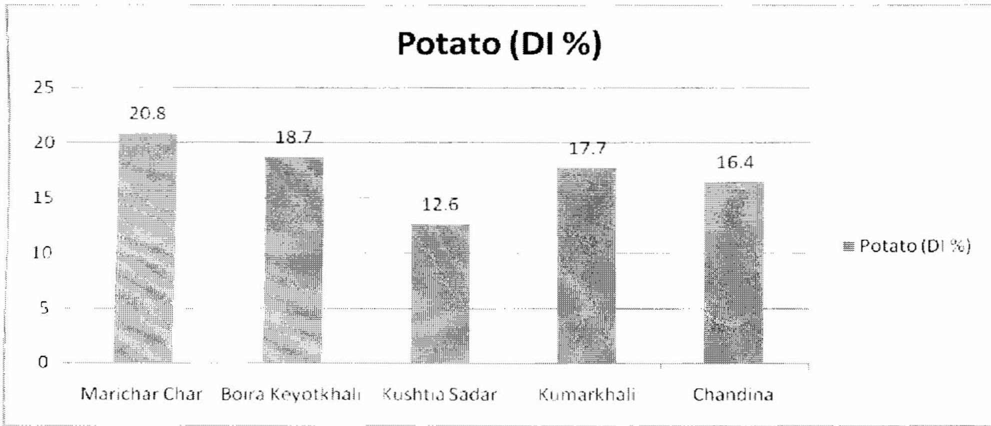

Fig. 7. Effect of soil on disease incidence of bacterial wilt of potato

Effect of different treatments on incidence of bacterial wilt of potato (cv. Diamant)

Treatment effects on the disease incidence of bacterial wilt of potato were recorded with 15 days interval starting from 1st disease symptom at 40 DAP (days after planting) to 85 DAP and the results are presented in Table 2. At 70 DAP, performance of the treatments displayed more or less similar trend as of 55 DAP but different with control. At 85 DAP, the lowest disease incidence (13.25%) was recorded in case of T_6 [(Ash: KCl =20:1) @ 50g/P + Cupravit-50 WP @ 7g/L] that was statistically similar with T_3, T_4 and T_5 treatments but significantly different from T_2 treatment. Moderate effects were observed in case of the rest of the treatments, while the highest disease incidence (69.0%) was recorded in case of control. As per the performance, disease incidence was reduced by 80.79% in case of T_6 followed by T_5 (72.82%), T_4 (71.01%), T_3 (64.85%) and T_2 (42.02%) over control at 85 DAP.

Table 2. Incidence of bacterial wilt of potato (cv. Diamant) in response to different treatments at different days after planting

Treatments	% Plant infection (disease incidence)				% Reduction of DI over control at 85 DAP
	40 DAP	55 DAP	70 DAP	85 DAP	
T_1 = Control	13.25	35.75a	55.00a	69.00a	--
T_2 = Ash mixed potash (Ash :KCl 20:1) @ 50g/plant	13.75	25.00b	30.50b	40.00b	42.02
T_3 = Cupravit-50 WP @ 5g/L,	13.25	19.00bc	22.50bc	24.25c	64.85
T_4 =Cupravit-50WP @ 7g/L	13.25	17.50c	19.25bc	20.00c	71.01
T_5 = Ash mixed potash (Ash :KCl = 20:1) @ 50g/Plant + Cupravit-50WP @ 5g/L	13.50	15.00cd	16.75c	18.75c	72.82
T_6 = Ash mixed potash (Ash :KCl = 20:1) @ 50g/Plant + Cupravit-50 WP @ 7g/L	13.75	10.50d	12.75c	13.25c	80.79
LSD(0.01)	NS	6.69	12.16	10.91	--
CV %	10.67	15.69	22.33	16.96	--

Means followed by the same letter (s) in columns are not significantly different at P <0.01 by Duncan's Multiple Range Test (DMRT).

The treatment effect was recorded on yield and yield contributing characters varied significantly in comparison to control (Table 3). Treatment T_6 (Ash mixed potash @ 50g/plant + cupravit-50 WP @ 7g/L) gave the promising effect on increasing number of tuber, average tuber weight, yield per plant and yield per hectare where yield increased by 84.57% over control. Treatment T_5 (Ash mixed potash @ 50g/plant + cupravit-50 WP @ 5g/L) also gave the remarkable effect on increasing number of tuber. average tuber weight and increased of yield next to treatment T_6 where yield increased by 57.42% over control. The performance of T_4 (only Cupravit-50 WP @ 7g/L) regarding yield contributing character was ranked third among the treatments where 56.89% yield was increased over control.

Table 3. Effect of different treatments on yield and yield contributing characters of potato as affected by the bacterial wilt

Treatments	Yield and yield contributing characters				% Yield increased over control
	Number of tuber/plant	Weight/ tuber (g)	Yield/ plant (kg)	Yield/ha (t)	
T_1 = Control	4.0b	29.25d	0.189c	24.38d	--
T_2 = Ash mixed potash (Ash :KCl = 20:1) @ 50g/plant	5.0ab	38.38c	0.207c	30.25c	19.40
T_3 = Cupravit-50 WP @ 5g/L,	6.0ab	42.17c	0.208c	31.00c	21.35
T_4 =Cupravit-50WP @ 7g/L	6.0ab	52.31b	0.259b	38.25b	56.89
T_5 = Ash mixed potash (Ash :KCl = 20:1) @ 50g/Plant + Cupravit-50WP @ 5g/L	6.0ab	52.38b	0.260b	38.38b	57.42
T_6 = Ash mixed potash (Ash :KCl = 20:1) @ 50g/Plant + Cupravit-50 WP @ 7g/L	7.0a	59.50a	0.300a	45.ooa	84.57
LSD (0.01)	2.12	4.32	23.31	4.49	--
CV %	18.70	4.54	4.72	6.24	---

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

Calculation of costing of management practices and determination of BCR

Based on the cost incurred and the gross return, the management practices T_6, T_5 and T_4 gave higher BCR. Management practice comprising the combined application of Ash mixed potash (Ash: KCl= 20:1) @ 50g/plant + Cuptavit-50 WP @ 7g/L resulted height BCR followed by T_5 with lower dose of Cupravit-50 WP (Table 4).

Table 4. Benefit Cost Ratio (BCR) of different management practices for bacterial wilt of potato

Treatments Combination	Yield /ha.(t)	Gross return (TK/ha)	Total cost of cultivation (T/ha.)	Gross margin (T/h.)	BCR
T_1 = Control	24.38	243800	82400	161400	2.95
T_2 = Ash mixed potash (Ash : KCl = 20:1) @ 50g/plant	30.25	302500	84400	218100	3.58
T_3 = Cupravit-50 WP @ 5g/L	31.00	310000	92150	217850	3.36
T_4 =Cupravit-50WP @ 7g/L	38.25	382500	95250	287250	4.01
T_5 = Ash mixed potash (Ash : KCl = 20:1) @ 50g/Plant + Cupravit- 50WP @ 5g/L	38.38	383800	93350	290450	4.11
T_6 = Ash mixed potash (Ash :KCl = 20:1) @ 50g/Plant + Cupravit- 50 WP @ 7g/L	45.00	450000	97250	352750	4.62

Potato 10,000 Tk/ton was calculated as per average market price

Construction of the diagnostic model and prescriptions

Six management practices selected to control bacterial wilt of potato were 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 respect to disease incidence, severity and yield, treatment T_6 and T_5 were finally selected to control bacterial wilt of potato (Table 5).

Table 5. Summary of the effective management practices for construction of the diagnostic model and prescription

Crop and Problems	Effective Management Practices	DI%	Yield (t/ha)	(%) Increased in yield over control
Potato, Bacterial wilt of potato	1. T_6 Cupravit-50 WP @ 7g/L sprayed 3 times in root zone at 15 days interval along with 50g mixed fertilizer (Ash:KCl= 20:1).	13.25	45.00	84.57
	2. T_5 Cupravit-50 WP @ 5g/L sprayed 3 times in root zone at 15 days interval along with 50g mixed fertilizer (Ash:KCl=20:1).	18.75	38.38	57.42

Proposition of a diagnostic model and prescription for the bacterial wilt of potato

Two best treatments were selected based on percentage disease incidence reduction, percentage yield increased over control and benefit-cost ratio (BCR) in the. 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 bacterial wilt of potato disease has been proposed in Table 6.

Table 6. Proposed diagnostic model and prescription for the bacterial wilt of potato

Diagnosis in the field/lab	Economic /affordable prescription
Field + Lab Test: Oozing Test in a glass of water: Streaming of milky ooze. Lab Test: i. Streaming of bacterial cells under microscope ii. Gram strain test and TTC Test	i. Cupravit-50 WP @ 7g/L sprayed 4 times in root zone at 15 days interval along with 50g/plant mixed fertilizer (Ash : KCl = 20:1).

CONCLUSION

An epidemiological survey was carried out in selected regions of Bangladesh in order to estimate the incidence of bacterial wilt of potato. Various strategies for controlling this disease have been introduced over the years including soil disinfection, cultural practices and chemical treatments but losses still occur. The effectiveness of these approaches is variable and short-lived. Moreover, bacteriocides of broad spectrum produce undesirable consequences on non-target organisms, environment and community health. The present study reveals that many farmers have adopted the management practices recommended by PDDC. Farmers have successfully applied PPDC's prescriptions and have gained control of diseases resulting in a significant increased in yield. Ash mixed potash (Ash : KCl = 20:1) @ 50g/plant + cupravit-50 WP @ 7g/L sprayed 4 times at 15 days interval for controlling the disease.

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