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DEVELOPMENT OF WEATHER-BASED EMPIRICAL FORECASTING MODELS OF TOMATO LEAF CURL DISEASE IN NORTHERN MINDANAO, PHILIPPINES

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ABSTRACT

Tomato leaf curl is a major virus disease in the Philippines. This study was conducted to develop and validate tomato leaf curl empirical forecasting models in Northern Mindanao. Tomato leaf curl disease incidence (%) and index (%) were assessed and number of whiteflies was counted per plant every two weeks after transplanting in the 2018 dry and wet seasons in field experiments in two sites: Malaybalay, Bukidnon and Claveria, Misamis Oriental. The relationships of disease incidence and index with whitefly number and biweekly-averaged weather variables were analyzed using linear correlation analysis. Disease incidence and index were positively correlated with minimum, maximum and average temperatures, but were positively and negatively correlated with relative humidity (RH), wind speed and rainfall in different sites and seasons. Whitefly number was negatively correlated with disease incidence and index in specific sites and seasons. Using stepwise multiple linear regression analyses, empirical models were developed that included temperature, RH, rainfall, wind speed and whitefly number that explained 90-94% of the variation in disease incidence. Multiple linear regression models specific to site and season were evaluated as good predictive models based on low average prediction error (MDIFF), and narrow length of prediction error (LPE).

Key words: correlation analysis, multiple linear regression, whitefly

INTRODUCTION

Tomato leaf curl is one of the most destructive diseases of tomato affecting many tropical and subtropical regions in the world (Navas-Castillo et al. 2011, Zerbini et al. 2017). It is caused by several viral species of the genus *Begomovirus*, family *Geminiviridae* that has a circular, single-stranded DNA genome with two incomplete icosahedral geminate particles (Zerbini et al. 2017). In the Philippines the disease is caused by *Tomato leaf curl Philippines virus* (ToLCPV), *Tomato leaf curl Cebu virus* (ToLCCeV) and *Tomato leaf curl Mindanao Virus* (ToLCMinV) (Kon et al. 2002; Tsai et al. 2011; Brown et al. 2015; Zerbini et al. 2017). The virus is transmitted by an insect vector, whitefly (*Bemisia tabaci* Genn.), classified in the family *Aleyrodidae*, in a circulative persistent manner but not transmitted via seed or mechanically (Uchibori et al. 2013). The tomato leaf curl virus affects yield by greatly reducing the number of fruit produced. Severe yield losses which, depending on the age of the plant at the time of infection can reach up to 100% (Levy and Lapidot, 2008). In the Philippines, particularly in Northern Mindanao, most of the farmers cease to plant tomatoes due to leaf curl occurrence in every tomato season that affects the productivity of tomatoes resulting in 40-60% reduction of crop yield and even higher with very severe infections (Lapoot, personal communication, 2018).

Management of geminiviral diseases including tomato leaf curl is a worldwide challenge. The integrated pest management (IPM) approach involving measures before, in, and after the growing season would be most effective. It starts with the use of resistant cultivars and virus- and vector-free transplants, and then roguing of infected plants and insect vector management in planting. Combined with the practice of host-free period of two to three months before the planting season, the above measures would be more effective (Rojas et al. 2018). Weather has been recognized as a key determinant in the dynamics of tomato leaf curl disease. Modeling approaches will allow the investigation of the complex interactions of weather factors with the biology of the insect vector and spread of the disease which eventually leads to the development of weather-based prediction tools (De Wolf and Isard 2007). No predictive model has been developed that can be used for the management of tomato leaf curl virus disease in the Philippines. The relationships of weather variables with the disease would provide a baseline for developing empirical disease prediction models to help farmers take up plant protection measures more precisely and economically.

This study sought to determine the effects of weather factors and whitefly vector population count on tomato leaf curl disease development, and to develop weather-based empirical forecasting models of tomato leaf curl disease in Northern Mindanao, Philippines. The tomato leaf curl forecasting models based on multiple linear regression were developed and validated in Malaybalay, Bukidnon and Claveria, Misamis Oriental.

MATERIALS AND METHODS

Experimental design and data collection. Two simultaneous field experiments were carried out from April to July 2018 (wet season) and from August to November 2018 (dry season) in the Research Center for Hilly Land Development (RCHD), Claveria, Misamis Oriental (8º37'6.90" N 124º56'49.90" E), while two simultaneous field experiments were conducted from May-August (wet season) and August-November 2018 (dry season) in Northern Mindanao Agricultural Research Crops and Livestock Research Complex (NMACLRC), Malaybalay, Bukidnon (8º12'52.05" N 125º2'9.99" E) (Fig. 1). Malaybalay, Bukidnon and Claveria, Misamis Oriental are located in high elevation areas. Malaybalay is 878 meters above sea level (masl) with Adtuyon soil type and Claveria is 971 masl with Jasa-an clay soil type. Results from the first experimental field trial were used for empirical model development while data from the second experimental field trial were utilized for the validation of the empirical models in both locations in the dry and wet seasons.

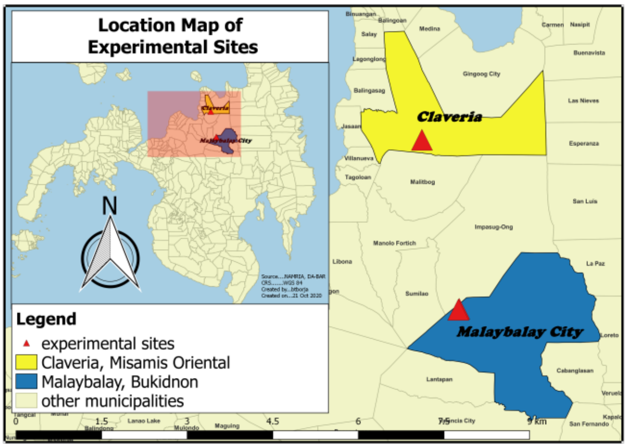

Fig. 1. Map showing the geographical locations of experimental sites in Claveria, Misamis Oriental and Malaybalay, Bukidnon in Northern Mindanao, Philippines used for tomato leaf curl disease forecasting model development and validation experiments from April to November 2018.

Seedlings of susceptible tomato cv. Victory were transplanted in the experimental fields 21 days after sowing. The experiment was carried out in a randomized complete block design with naturally-infected (diseased) and insecticide-applied treatments in four replications with a plot size of 5 m x 6 m with 2 m distance between rows and 1 m distance between replicates. Plants were spaced at the interval of 1 m x 0.50 m, with a total of 72 plants per plot. Standard cultural practices and maintenance of test plants were done in the growing season.

Tomato leaf curl disease incidence. The incidence of tomato leaf curl disease was counted every two weeks from transplanting until 10 weeks after transplanting (WAT) by visual observation of disease symptoms such as curling, yellowing, downward cupping and reduction in size of the leaves in the tomato plants (Zhang et al. 2009). Leaf curl disease incidence was computed as the proportion of plants displaying symptoms over the total number of plants (Allen 1983):

$$Disease\ incidence\ (\%) = \frac{number\ of\ infected\ plants}{total\ number\ of\ plants} \times 100$$

Tomato leaf curl disease index. The disease index (DI) which accounts for both disease incidence and symptom severity was computed using the formula:

$$Disease\ index = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{5N} \times 100$$

where: N is the total number of plant samples; 5 represents the highest scale; $1n_1 + \ldots + 5n_5$ – refers to the number of plants examined showing a rating of 1, 2, 3, 4 and 5, respectively. It was computed using the disease incidence and symptom severity rating taken every two weeks from transplanting.

Symptom severity scoring was conducted from 20 randomly selected plants per plot following the rating scale of Ong and Sta. Cruz (2016). The observation was restricted to the 10 middle rows and 10 columns, leaving two outer rows and columns as border rows.

Whitefly population count. The number of adult whiteflies was determined by gently turning over the leaves of the plant early morning (7-9 am) then counting the total number of whiteflies on the underside of 5 randomly selected plants in the plot since adults congregate and lay eggs predominantly on the undersides of leaves (Subba et al. 2017). The number of whiteflies on 5 young leaves from different branches was counted from 20 sample plants. The linear associations of disease incidence and index with number of whiteflies were analyzed by Pearson and Spearman correlation analyses ($P \leq 0.05$), respectively.

Meteorological data. Weather data were obtained from the nearest meteorological station (distance ≤ 20 km) from each experimental area. Daily values of mean, maximum and minimum temperature (°C), rainfall (mm), relative humidity (RH) (%), wind speed (km/h) and wind gust (km/h) were collected from the Automated Weather Stations (AWS) managed by the University of Science and Technology of Southern Philippines (USTP) Claveria Campus, Claveria, Misamis Oriental for the experimental set-up at RCHD. For the set-up at NMACLRC, Malaybalay, Bukidnon, meteorological data were collected from the AWS installed at Bureau of Soil and Water Management, Dalwangan, Malaybalay, Bukidnon. All weather variables were averaged from 1 to 14 days before the day of whitefly count and disease assessment except for total rainfall that was summed. The linear associations of disease incidence and index with weather variables were analyzed by Pearson and Spearman correlation analyses ($P \leq 0.05$), respectively.

Development of empirical models. An empirical approach was used to determine the collective influence of weather factors (rainfall, temperature, RH and wind speed that were significantly correlated with disease incidence) and whitefly count on disease incidence by multiple linear regression analysis in Malaybalay and Claveria in the dry and wet seasons using the procedure REG in SAS using stepwise selection (SAS Institute Inc., 1988). Whitefly count was included as an independent variable in the regression analysis because whitefly is the vector of the tomato leaf curl virus (Uchibori et al. 2013) and whitefly number was correlated with tomato leaf curl disease incidence in various studies (Mehta et al. 1994; Rahman et al. 2006; Ssekyewa et al. 2010; Prasannath et al. 2014). Weather factors at a duration or window of two weeks before the day of disease assessment were used as predictor variables in the regression analysis to develop the significant models (Butt and Royle 1974). This window would allow the prediction of leaf curl disease before its occurrence in order to apply prompt disease management strategies.

Evaluation of empirical models. Models developed for disease incidence in Malaybalay, and Claveria in the wet and dry seasons were evaluated based on the following regression diagnostics in SAS: normal studentized residual with Shapiro and Wilks $p > W$ near unity, coefficient of variation (CV) less than 25%, low predicted residual error sum of squares (PRESS) statistic value, low Root Mean Square Error (RMSE) (Snee 1977), high adjusted R^2 (Myers 1990), and low variance inflation factor ($VIF < 5$) where $VIF \geq 5$ indicates multicollinearity (Esker et al. 2008) with $VIF > 10$ is unacceptable (Ngo 2012). Models satisfying the above-mentioned conditions were considered good models. RMSE measures how the model predicts the response accurately, and it is the most important criterion for fit if the main purpose of the model is prediction (Snee 1977) and PRESS statistic is a form of cross-validation used in regression analysis to provide a summary measure of the fit of a model to a sample of observations that were not used to estimate the model (Myers 1990).

Validation of empirical models. The empirical models derived from the significant relationships of weather factors and tomato leaf curl incidence were validated as forecasting models by conducting field validation experiments using natural infection at both sites and seasons. Accuracy of model prediction was determined by computing the average prediction error (MDIFF) (prediction error is the difference between predicted and actual disease values), and estimating the length of prediction error (LPE) calculated as the difference of minimum prediction error from maximum prediction error. Models with MDIFF near zero, and narrow LPE for observations in the validation set were judged as good predictive models of tomato leaf curl disease incidence in both sites and seasons.

RESULTS AND DISCUSSION

Correlation of tomato leaf curl disease incidence with weather variables. Pearson correlation analyses of weather variables and whitefly count with disease incidence in the wet and dry seasons in Malaybalay and Claveria are shown in Table 1. In Malaybalay, all temperature variables (minimum, maximum and average) were positively correlated with disease incidence in the dry season while minimum and maximum temperatures had positive and negative correlations with disease incidence, respectively, in the wet season. On the other hand, in Claveria, there were positive correlations of minimum, maximum, and average temperatures with disease incidence in the dry season while minimum temperature was the only positively correlated variable with disease incidence in the wet season. Positive correlations between disease incidence and temperature corroborate with various studies indicating that tomato leaf curl disease incidence was correlated with minimum and maximum temperatures (Zeshan et al. 2016; Haider et al. 2017; Jahanzaib et al. 2017).

Contrasting relationships between relative humidity (RH) and disease incidence were found in both sites and seasons (Table 1). In the wet season, RH was positively correlated with disease incidence while it was the opposite in the dry season. Mehmood et al. (2018) showed positive effects of RH on tomato leaf curl disease incidence, while the negative correlation is similar to studies which have reported an increase in tomato leaf curl disease incidence as RH decreased (Zeshan et al. 2016;

Jahanzaib et al. 2017; Yasin et al. 2017). More research is needed to elucidate the positive and negative effects of RH on tomato leaf curl disease incidence.

Contrasting effects of wind speed on disease incidence were found with positive correlations between wind speed and disease incidence in Claveria wet season and Malaybalay dry season and between wind gust and disease incidence in both sites in the dry season (Table 1). On the other hand, wind speed and wind gust were negatively correlated with disease incidence in the dry season in Claveria. Wind speed helps in the spread of the vector whitefly (Jahanzaib et al. 2017) and influences long distance and trivial migrations of whitefly (Lefeuvre et al. 2010). Tomato leaf curl incidence was positively correlated with wind speed in 2 out of 6 varieties (Jahanzaib et al. 2017) but the oppposite was observed in 1 of 6 varieties (Haider et al. 2017) while a study showed no significant effect of wind speed on tomato leaf curl incidence (Zeshan et al. 2016).

Rainfall correlation with disease incidence was inconsistent in both sites and seasons (Table 1). A positive correlation was observed between average rainfall and disease incidence in Malaybalay wet season while negative correlations of disease incidence with average and total rainfall were observed in the dry season in Claveria. Positive correlations between tomato leaf curl disease incidence and rainfall were observed in 2 out of 6 varieties (Haider et al. 2017) but the opposite was observed in 2 out of 6 varieties (Haider et al. 2017) and in 4 out of 6 varieties (Jahanzaib et al. 2017). However, one study indicated no significant correlation between rainfall and tomato leaf curl incidence (Zeshan et al. 2016).

Correlation of tomato leaf curl disease index with weather variables. Several weather variables were found by Spearman correlation analysis to be correlated with disease index in both sites and seasons (Table 2). In the dry season, all temperature variables (minimum, maximum and average) were positively correlated with disease index in both sites. However, in the wet season, minimum and maximum temperatures were correlated positively with disease index in Malaybalay. Leaf curling started from the shoots, then to the first and second leaf petioles and continued to be observed on the whole plant as disease index increased showing severe symptoms such as stunting, and severe yellowing and leaf curling. These results corroborate with the study of Mehmood et al. (2018) indicating positive effects of minimum and maximum temperatures on tomato leaf curl severity.

Correlations between RH and disease index were inconsistent (Table 2). There were positive correlations between RH and disease index in Claveria and Malaybalay in the wet season.. However, in the dry season, negative correlations between disease index and RH were observed in Malaybalay and Claveria. Naveed et al. (2015) reported a negative correlation between RH and tomato leaf curl disease severity but the opposite was found by Mehmood et al. (2018). More experiments are needed to elucidate the relationship of RH and tomato leaf curl.

The correlation results of disease index with average wind speed and wind gust are similar to the correlation results of disease incidence, and also for average and total rainfall except for no significant correlation in the wet season (Table 2).

Correlation of tomato leaf curl disease incidence and index with whitefly population. Whitefly count was significantly and negatively correlated with disease incidence only in the wet season in Claveria (Table 1) and with disease index in the dry and wet seasons in Claveria (Table 2). Disease incidence and index increased over time while there was a decrease in whitefly population starting at 6 WAT. This result is in contrast to several studies (Mehta et al. 1994; Rahman et al. 2006; Prasannath et al. 2014) which reported significant positive correlations between whitefly number and disease incidence. However, this result was also observed by Ssekyewa et al. (2010) that showed an inverse relationship between whitefly number and tomato leaf curl incidence wherein whitefly number dropped at 10 WAT but symptoms became severe 5 weeks after.

Table 1. Pearson's correlation coefficients between tomato leaf curl disease incidence (%) and biweekly weather variables and whitefly count in the 2018 wet and dry seasons in Malaybalay, Bukidnon and Claveria, Misamis Oriental, Philippines.

Location	Temperature (ºC)			Wind speed (km/h)	Wind gust (km/h)	Relative Humidity (%)	Average Rainfall (mm)	Total Rainfall (mm)	Whitefly count
	Minimum	Maximum	Average						
Wet season									
Malaybalay	0.575**	-0.665**	-0.316^{ns}	0.047^{ns}	0.640**	0.683***	0.607**	0.147^{ns}	-0.377^{ns}
Claveria	0.696**	-0.331^{ns}	-0.356^{ns}	0.704**	0.461*	0.957***	0.110^{ns}	0.110^{ns}	-0.467*
Dry season									
Malaybalay	0.717***	0.796***	0.744***	0.916***	-0.322^{ns}	-0.551*	0.072^{ns}	0.071^{ns}	-0.187^{ns}
Claveria	0.623**	0.671**	0.651**	-0.887***	-0.900***	-0.894***	-0.502*	-0.502*	-0.301^{ns}

^{ns} - not significant;
* - significant at $P \leq 0.05$;
** - highly significant at $P \leq 0.01$;
*** - very highly significant $P \leq 0.001$

Table 2. Spearman's correlation coefficients between tomato leaf curl disease index (%) and biweekly weather variables and whitefly count in the 2018 wet and dry seasons in Malaybalay, Bukidnon and Claveria, Misamis Oriental, Philippines.

Location	Temperature (ºC)			Wind speed (km/h)	Wind gust (km/h)	Relative Humidity (%)	Average Rainfall (mm)	Total Rainfall (mm)	Whitefly count
	Minimum	Maximum	Average						
Wet season									
Malaybalay	0.493*	0.591**	-0.296ns	0.220ns	0.700**	0.556*	0.296ns	0.099ns	0.297ns
Claveria	0.394ns	-0.344ns	-0.344ns	0.640**	0.406ns	0.910***	0.332ns	0.332ns	-0.615**
Dry season									
Malaybalay	0.657**	0.689**	0.657**	0.887***	-0.197ns	-0.591**	-0.197ns	-0.197ns	-0.251 ns
Claveria	0.638**	0.555**	0.516**	-0.834***	-0.877***	-0.948***	-0.592**	-0.592**	-0.456*

ns - not significant;
* - significant at $P \leq 0.05$;
** - highly significant at $P \leq 0.01$;
*** - very highly significant $P \leq 0.001$

Relationship between tomato leaf curl disease incidence and index. Linear relationships were found between disease incidence and index in both sites and seasons except for a nonlinear relationship in the dry season in Claveria (Fig. 2). It is necessary to describe the relationships between disease incidence and disease index that would provide an indirect way of estimating disease index from incidence. Disease index is tedious and time consuming to assess in the field while disease incidence is easier to evaluate, which is advantageous for disease monitoring in the validation of the empirical models of disease incidence. The directly proportional relationships between disease incidence and index imply that by simply counting the number of tomato plants with leaf curl disease, it is possible to estimate the disease index for a susceptible variety, e.g. Victory.

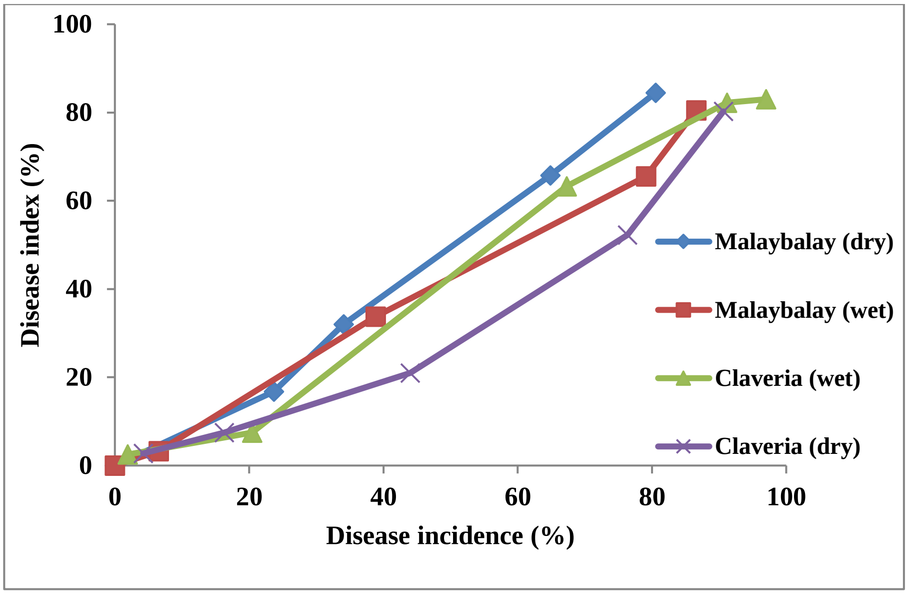

Fig. 2. Relationship between tomato leaf curl disease incidence and index in Claveria, Misamis Oriental and Malaybalay, Bukidnon in the Philippines in the 2018 wet and dry seasons .

Development and evaluation of tomato leaf curl disease empirical models. Overall, temperature (minimum (17.96-21.32 °C) and average (22.38-25.13 °C)), wind speed (0.31-2.48 km/h), RH (72.43-89.57%) and average rainfall (0.71-18.15 mm) were the significant variables included in the multiple linear regression models of disease incidence (Table 3). Minimum temperature was included in 2 of the 4 models while average temperature was included in the Malaybalay dry season model. RH and wind speed were included in 3 out of the 4 models. Average rainfall was included in the Malaybalay wet season model. These results corroborate with the multiple linear regression models of tomato leaf curl index of Maity et al. (2019) that included average temperature, rainfall and RH.

In general, the multiple linear regression models showed that all weather variables included in the models explained 90 to 94% (adjusted-R^2 value) of the total variation in disease incidence in both sites and seasons. Furthermore, the regression models are good predictive models based on low RMSE values (6.47-10.73), low coefficients of variation < 25 % (18.53-24.42%), normalized studentized residuals with Shapiro-Wilks near unity (0.11- 0.79), low variance inflation factors (1.04-5.68) and low PRESS statistic values (671.83-1842.69) (Table 3).

Table 3. Multiple linear regression equations of tomato leaf curl disease incidence with weather variables and whitefly count in the 2018 wet and dry seasons in Malaybalay, Bukidnon and Claveria, Misamis Oriental, Philippines.

Location	Regression equation	Statistics					
		R^{2a}	RMSE[b]	CV[c]	Shapiro-Wilk[d]	PRESS[e]	VIF[f]
Wet season							
Malaybalay	Y= -884.2350±79.80[g] + 43.3172±4.28 MinTemp + 0.6841±0.11 RH+ 6.3998±0.96 Rain	0.93**	6.47	24.42	0.11	671.83	1.03-1.37
Claveria	Y= -663.0943± 54.81+16.0228±5.81 Wind + 8.5955±0.075 RH	0.94**	10.23	18.53	0.14	1777.63	1.57
Dry season							
Malaybalay	Y= 802.3705±280.86 - 20.1987±7.83 AveTemp + 206.2628±22.65 Wind - 7.0691±1.64 RH	0.91**	8.79	19.89	0.79	1237.69	2.66-5.58
Claveria	Y= 412.9375±110.78 – 13.1556±5.13 MinTemp – 112.7340±10.98 Wind -5.0803±0.98 Whitefly	0.90**	10.73	23.18	0.20	1842.69	1.39-2.65

Y = tomato leaf curl disease incidence (%); MinTemp = minimum temperature (ºC); AveTemp = average temperature (ºC); Wind = wind speed (km/hr); RH = Relative humidity (%); Rain = average rainfall (mm); Whitefly = whitefly count; [a]Adjusted coefficient of determination; [b]Root Mean Square Error; [c]Coefficient of variation; [d]Shapiro and Wilk's probability less than W to test normality of studentized residuals; [e]Allen's predicted error sum of squares (PRESS); [f]Variance inflation factor; [g]Standard error after plus-minus symbol; ** - Significant at P≤0.01

Empirical model validation. Validation of the empirical regression models was done by forecasting disease incidence in the following location and season prediction scenarios: i) prediction for the same site in the same season, ii) prediction using a different site for the same season, and iii) prediction using the same site for a different season, .and determine the accuracy of the models for prediction (Table 4). Low MDIFF value and narrow LPE were obtained by the regression models used to predict disease incidence for the same site in the same season with MDIFF values of 1.53-22.39 and LPE values of 14.46-29.76 . However, the regression models used to predict disease incidence for different sites within the same season and for the same site in different seasons have high MDIFF values and wide LPE showing lesser accuracy of prediction..

Table 4. Statistical results of the validation of empirical models developed for tomato leaf curl disease incidence in the 2018 wet and dry seasons in Malaybalay, Bukidnon and Claveria, Misamis Oriental, Philippines.

Location and season	Statistics	
	MDIFF[a]	**LPE**[b]
i) Same site for the same season[c]		
Claveria (dry) for Claveria (dry)	1.53	14.46
Claveria (wet) for Claveria (wet)	3.75	14.40
Malaybalay (dry) for Malaybalay (dry)	3.60	11.04
Malaybalay (wet) for Malaybalay (wet)	13.49	29.76
ii) Same site for different season[c]		
Claveria (wet) for Claveria (dry)	22.07	185.11
Claveria (dry) for Claveria (wet)	140.81	252.28
Malaybalay (wet) for Malaybalay (dry)	48.14	150.10
Malaybalay (dry) for Malaybalay (wet)	25.21	275.17
iii) Different site for the same season[c]		
Malaybalay (dry) for Claveria (dry)	133.5	262.21
Malaybalay (wet) for Claveria (wet)	48.14	105.21
Claveria (dry) for Malaybalay (dry)	56.13	102.75
Claveria (wet) for Malaybalay (wet)	30.02	312.64

[a]Average prediction error (MDIFF); [b]Length of prediction error (LPE); [c]Prediction scenarios

Overall, the empirical regression models validated on the same site and same (dry or wet) season were good predictive models based on low MDIFF values and narrow LPE. These results imply that empirical regression models developed for Claveria and Malaybalay in the dry and wet seasons were site and season-specific but these could still be tested as a prediction tool in forecasting tomato leaf curl disease incidence in other locations over seasons.

CONCLUSION

Empirical forecasting models of tomato leaf curl disease incidence were developed for Northern Mindanao using multiple linear regression analyses that included weather variables found significantly correlated with disease incidence (temperature, relative humidity, rainfall, and wind speed) and whitefly number. These regression models explained 90-94% of the variation in disease incidence. Multiple linear regression models specific to site and season were evaluated as good predictive models based on low average prediction error (MDIFF), and narrow length of prediction error (LPE).

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