ISSN:2523-0522
Volume 6: issue 2, April 2023
ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

ORIGINAL ARTICLE

Incorporating the Plant Disease Triangle Framework for Analyzing the Effect of FWB Incidence on Soil Attributes

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Received:26/10/2022 ***Accepted:13/01/2023*** ***Published:01/04/2023***

OPEN ACCESS

Doi:
https://doi.org/10.52865/vwbd9414

Competing Interests: The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

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ABSTRACT:

FWB (FWB), also known as Panama disease and caused by *Fusarium oxysporum f. sp. cubense* (Foc), is the most destructive disease in the banana industry. Foc is the most virulent pathogen and is capable of surviving in agricultural soil for an indefinite period. The complex interaction between Foc, bananas, and the environment has restricted efforts to develop FWB prevention and control. Thus, it is essential to investigate all dimensions of the interactions relating to the occurrence of FWB in plantations. The purpose of this preliminary study was to assess the reversed interaction of soil attributes and FWB. The relationships between Fusarium wilt disease incidence and soil characteristics were analyzed using a forward linear regression analysis with dummy variables. Age, as well as shrubs and weeds, were incorporated as dummy variables. The first model of forward linear regression with soil moisture as the dependent variable significantly revealed that R^2 is larger and more strongly correlated when plant conditions and banana tree age variables are included in the model (p = 0.0). Still, a comprehensive experimental approach is required for better understanding since an increasing human population in the future may pose a threat to food security.

KEYWORDS: *Backward interactions; Dummy variables; Linear regression; Musa spp; Panama disease.*

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INTRODUCTION:

Banana, scientifically known as *Musa* spp., is one of the most important fruits globally that contributes as a source of food for human consumption and commercial uses (Le Thi et al., 2022; Zhang et al., 2022), notably for developing countries (Scott, 2021). According to the Department of Agriculture (DOA), the banana is the second most extensively farmed crop in Malaysia, after durian, with a planted area of approximately 26,210 hectares and a production of 313,811 metric tons in 2020 (DOA, 2021). Banana in Malaysian food systems, particularly production, has changed over the years due to disease and pests, which are among the key restraints in banana farming (Köberl et al., 2017), in addition to other limiting factors such as massive urbanization and climates (Alho et al., 2021). The first pandemic of FWB was documented in 1890 (Wong et al., 2019). Nearly every publication regarding this disease stated that FWB is the greatest threat to banana growth worldwide (Le Thi et al., 2022). FWB, or Panama disease, is caused by a soilborne fungal pathogen with extreme resistance in soil, called *Fusarium oxysporum f. sp. cubense* (Foc) (Dita et al., 2018). Furthermore, Foc is a virulent pathogen of significant global importance (Maymon et al., 2020).

Despite the expanding number of FWB publications, there is little concern over the indirect interaction of FWB and soil characteristics. Previously, researchers hypothesized that soil characteristics influenced the occurrence of FWB. Thus, such data have constrained our understanding of the complicated interplay between the occurrence of FWB and soil conditions. Thus, there is a need to identify any possibility of reversed direction for FWB and soil attributes by utilizing statistical tools. As advised by Jones (2014) and Madden et al. (2007), plant disease epidemiology research must incorporate agronomical, biological, ecological, and statistical perspectives. A comprehensive and diverse understanding of various subjects could greatly assist in detecting and controlling plant diseases. Thus, the purpose of this preliminary study was to provide relevant materials and further reading for those interested in FWB investigation and modeling. A novel strategy and more statistical analysis may be recommended to better understand the FWB's interaction with soil qualities and the reversed direction of their interactions.

Materials and Methods

Study Area

The sampling location for this study was a small-scale banana farm in Jeli, Kelantan. The banana farm is situated on sloping and uphill terrain, with healthy banana trees in the upper half and suspected diseased banana trees in the lower section. It is about 12km from the Universiti Malaysia Kelantan, Jeli Campus.

Sample Preparation

Randomly, three infested and three non-infested banana trees from the sampling area were collected for this study. Four distinct sampling locations were selected to collect the soils at each banana tree. The external symptoms of infested banana trees served as an indicator of their infestation. For each condition, soil samples were taken below 50 cm of depth. Then, 24 soil samples were placed in plastic bags and transported to the laboratory for the air-drying process. In the laboratory of the Faculty of Agro-Based Industry and the Faculty of Earth Science at Universiti Malaysia Kelantan, all soil samples were prepared, identified, and analyzed for this study.

Soil Preparation, Soil Analysis, and Fungal Identification

The soil samples were air-dried at room temperature (27ºC ± 1) for seven days. The dried soil samples were ground using a mortar and pestle before sieving with a 2 mm sieve for physicochemical analysis and Foc isolation. The soil moisture, pH, and electrical conductivity (EC) were analyzed to determine the critical values associated with the banana tree conditions (healthy or infested). All analyses followed the soil analysis manual published by Rokupr Agricultural Research Centre (RARC) and Japan International Cooperation Agency (JICA) in 2014 with slight modifications.

The gravimetric method was used to determine the soil moisture content in the soil samples. A glass petri dish was weighed before 10 g of fresh soil samples were placed. The weight of 10 g of fresh soil sample in a petri dish was weighed again and dried at 105 °C for 24 hours until its weight remained consistent. The samples were then taken from the oven and chilled before the dried soil samples were weighed. Using the following formula, the percentage of soil moisture was calculated.

$$soil\ moisture\ content\ (\%) = \frac{(wt\ of\ fresh\ soil + petri\ dish) - (wt\ dried\ soil + petri\ dish)}{(wt\ of\ dried\ soil + petri\ dish) - wt\ of\ petri\ dish}\ x\ 100$$

The pH of the soil was determined by adding 100 ml of water (H2O) to a 10 g sample of dried soil that was weighed in a conical flask. After adding 25 mL of distilled water and capping the bottle, it was occasionally shaken for one hour. The soil suspension was shaken again before being deposited into the portable pH meter (model: compact pH Meter LAQUAtwin). The reading is recorded when the pH value becomes stable after five replications.

Ten grams of dried soil sample were placed in a 100 mL conical flask for EC analysis. Then, 50 mL of distilled water was added before the soil sample was shaken for one hour using a reciprocal shaker. The soil suspension was shaken once more before being placed into the portable EC meter (model: Milwaukee Martini EC59). Before the EC measurement, the portable EC meter was calibrated first using 20 mL of 1288 µs/cm and 1413 µs/cm buffer solution. The EC value was measured and recorded in five replicates when the value became stable.

All the successful isolates from soil samples were prepared for identification, as described by Lesli and Summerell (2008). The fungus was cultured, isolated, and identified to confirm the presence of *Fusarium oxysporum* spp. according to the manual provided by Pérez-Vicente et al. (2014).

Statistical Analysis

In this study, a t-test was used to test for significant differences between the soil of infested and healthy banana trees. Then, linear regression analysis with dummy variables was used to reveal the relationships between Fusarium wilt disease incidence and soil attributes. The banana tree age, as well as bushes and weeds were included as dummy variables. All statistical analyses were performed using SPSS Statistics version 25.0 software (IBM, New York, USA).

Results and Discussion

Table 1 displays the descriptive results of soil moisture, pH, and EC for both infested and healthy soils. According to the findings, the soil pH and percentage of soil moisture in the soil samples of healthy bananas are somewhat lower than in the soil samples of infested bananas. Like other variables, soil moisture is also crucial for the occurrence of plant diseases, including FWB (Peng et al., 1999;

Wahyuni & Nasution, 2019). The soil moisture could be employed as an essential soil parameter for early FWB diagnosis (David & Guico, 2018) and is highly related to the other soil parameters in banana plantations, including pH and temperature (Guo et al., 2013). The association between pH and FWB disease has been complex in prior research. In certain investigations, researchers discovered a correlation between a low pH value and the severity of FWB (Blaya et al., 2015; Deltour et al., 2017; Griffin, 2012; Segura et al., 2018). Another study indicated that high pH leads to the occurrence of FWB (Cao et al., 2016; Peng et al., 1999). However, soil pH plays a crucial role in FWB expression (da Silva Junior et al., 2000; Segura et al., 2021).

Table 1. Summary of descriptive statistics for 24 soil sample points

Banana Tree	Soil sampling point	pH	Soil moisture (%)	EC (µs/cm)
Healthy 1	1	4.66 ± 0.05	17.79 ± 5.80	24.60 ± 1.82
	2	4.60 ± 0.00	21.45 ± 0.46	24.40 ± 0.55
	3	4.44 ± 0.05	22.36 ± 0.47	23.00 ± 0.00
	4	4.52 ± 0.04	24.82 ± 0.38	24.60 ± 0.55
Healthy 2	1	4.54 ± 0.05	22.14 ± 0.43	18.20 ± 0.45
	2	4.46 ± 0.05	20.59 ± 0.41	24.20 ± 0.45
	3	4.38 ± 0.04	21.88 ± 1.24	24.60 ± 0.55
	4	4.46 ± 0.05	23.16 ± 0.98	31.80 ± 0.84
Healthy 3	1	4.32 ± 0.04	23.00 ± 0.70	24.80 ± 0.45
	2	4.50 ± 0.00	21.58 ± 0.26	21.40 ± 0.55
	3	4.94 ± 0.05	25.16 ± 0.22	27.40 ± 0.55
	4	4.58 ± 0.04	23.17 ± 0.37	37.80 ± 0.84
Mean healthy		4.53 ± 0.16*	22.26 ± 2.46*	25.57 ± 4.89
Infested 1	1	4.30 ± 0.00	26.81 ± 1.39	34.60 ± 0.55
	2	4.72 ± 0.08	37.53 ± 0.99	23.20 ± 1.10
	3	4.84 ± 0.05	29.83 ± 0.97	17.40 ± 0.55
	4	4.58 ± 0.04	32.14 ± 1.65	21.60 ± 0.55
Infested 2	1	4.70 ± 0.00	30.96 ± 0.67	23.20 ± 0.45
	2	6.26 ± 0.05	31.75 ± 1.03	26.00 ± 0.71
	3	4.64 ± 0.05	29.55 ± 1.44	28.80 ± 0.45
	4	4.80 ± 0.00	32.65 ± 0.92	26.60 ± 0.55
Infested 3	1	4.82 ± 0.04	27.49 ± 0.95	20.20 ± 0.45
	2	6.58 ± 0.11	27.51 ± 0.46	34.20 ± 0.84
	3	5.24 ± 0.05	27.53 ± 0.67	19.40 ± 0.55
	4	5.08 ± 0.05	30.17 ± 0.70	15.80 ± 0.45
Mean Infested		5.03 ± 0.65*	30.32 ± 3.06*	24.25 ± 5.87

Note: The values in the table represent mean ± standard deviation (SD), *p-value < 0.05 and significant difference in the T-test.

However, there was no significant difference in EC levels between infested and healthy banana trees ($p > 0.05$). Regarding EC, fewer studies have been conducted to determine the association between EC and the occurrence of Panama disease. Despite a lack of understanding of the relationship between EC

ISSN:2523-0522
Volume 6: issue 2, April 2023
ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

and the occurrence of Fusarium wilt, Domınguez et al. (2001) highlighted that EC is a factor in the development of Fusarium wilt disease in banana cultivars. In a greenhouse study conducted by Deltour et al. (2017), the range of EC values linked with the high disease severity of Fusarium wilt in banana cv. Mac is 0.06 to 0.14 dSm^{-1}.

Due to the complicated connections between banana trees as plant hosts, the Foc pathogen, and soil factors, it is difficult to anticipate the occurrence of FWB. Theoretically, by combining the three components of the plant disease triangle, it is possible to estimate disease outbreaks and choose practical and successful control or management strategies. The development of effective management based on the level of plant disease necessitated a comprehensive understanding of the interaction between the elements that contribute to plant disease occurrence. Such information could aid in the modeling of plant disease progression. Clearly, the more complex the relationships, the more challenging or changeable the forecasts will be. For instance, if Foc could remain in the soil for an extended period with or without favorable soil conditions, there may be a need to investigate other relationships between FWB disease incidence (banana trees and Foc) and soil characteristics. Is there any possibility of changes in soil properties during the occurrence of FWB? But the fourth element, such as farm practices, might play a role in the FWB outbreaks (Dita et al., 2018). Best farm practice allows the farmer to delay the time before a virulent pathogen spreads and causes a plant disease outbreak. This concept is depicted in Figure 1 by the directions of a modified FWB disease triangle.

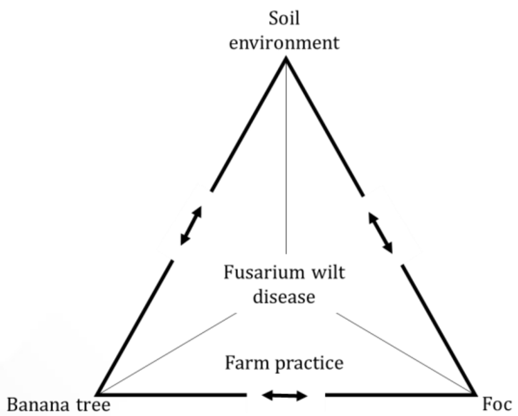

Figure 1. Fusarium wilt disease triangle illustrated the complex interplay between environments, banana trees, and Foc pathogen, which could be influenced by the farm practice

Researchers commonly hypothesize that soil characteristics influenced the occurrence of Fusarium wilt. However, the possibility of reversed interaction between FWB and soil attributes was analyzed in this present study. To answer the research question, this study used a statistical approach to examine the interaction between FWB and soil attributes by including the present value of Foc together with dummy variables (banana age; weeds and bushes) as predictors and soil attributes . The link between FWB occurrence and soil characteristics was examined by utilizing forward linear regression. In this present study, there are three predictors, and therefore, the linear regression model can be denoted as , where is a dependent variable, while x1, x2, and x3 are the independent variables. This study has three dependent variables: soil moisture, pH, and EC. Therefore, the regression analysis was repeated for three datasets that included three selected dummy variables. In data analysis, dummy variables allow researchers to analyze categorical variables using regression (Aguinis et al., 2005). A dummy variable is designed to connect numerical data with categorical factors. Each dummy variable is coded to correspond to a single category of the explanatory variable; banana tree age (0 if less than one year, 1 if greater than one year), plant condition (0 if healthy, 1 if affected), and bushes and weeds (0 if no or less have been covered by weeds and bushes, 1 if the banana tree area was overgrown weeds and bushes). Using dummy variables, researchers can improve their examination of the relationship between components and capabilities to predict disease occurrence (Aguinis et al., 2005).

Since this was a reversed interaction study, the researchers used the same soil characterization data in connection to the occurrence of FWB. The summary analytical result is reported in Table 2. R^2 and *p*-value might indicate how accurately the model represents the data (Alexopoulos, 2010). R^2 can assist researchers in determining whether a model is valid. If R^2 is bigger, the model is superior (Tjur, 2009).

As the *p*-value is less than 0.05, these results indicate that the model is significant. This is a nice representation of the relationship between FWB, banana tree age, and weeds and shrubs in relation to soil characteristics. As this study included dummy variables as predictors, forward linear regression was utilized to simplify the models and reduce the predictors.

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Table 1. Summary of forward linear regression analysis

Model	Dependent variable	Predictors	F change	R	R^2	p
1(a)*	Soil moisture	Plant conditions	253.092	0.826	0.682	0.00
1(b)**	Soil moisture	Plant conditions + banana tree age	21.066	0.855	0.731	0.00
2(a)*	pH	Plant conditions	34.177	0.474	0.225	0.00
2(b)**	pH	Plant conditions + banana tree age	24.684	0.600	0.360	0.00
3***	EC	Weeds and bushes	5.534	0.212	0.045	0.02

Note: EC: Soil electrical conductivity, R: correlation coefficient, R2: R-squared, P: significant value ($p < 0.05$).
*The model excluded banana tree age and weeds and bushes
**The model excluded weeds and bushes
***The model excluded plant conditions and weeds and bushes

The first model of forward linear regression, 1(b) with soil moisture as the dependent variable showed that R^2 is bigger and more strongly correlated when plant conditions and banana tree age variables are included in the model ($p = 0.0$). A second forward linear regression 2(b) with soil pH as the dependent variable found that R^2 is bigger when plant conditions and banana tree age variables are included in the model ($p = 0.0$). In contrast, a third forward linear regression model with the soil EC as the dependent variable revealed that only weeds and bushes are included in the model ($p = 0.02$). According to Table 2, the most important criterion is in model 1(b), where it can be concluded as the best model in the present study. Usually, the larger the R^2, the better the regression model fits the observations (Tjur, 2009). Thus, it encourages researchers to seek an additional prognotic explanation.

Regarding FWB, Foc spores enter banana roots and clog the xylem, inhibiting water and nutrient transfer (Kannan et al., 2022). This resulted in premature yellowing or wilting of older leaves and splitting the pseudostem at the base (Zhang et al., 2022). The dead leaves and shattered pseudostems on the ground can improve microclimates, particularly the soil moisture content. Consequently, dead plants and residues may increase the number of microorganisms in the topsoil. Although dead plants provide nutrients for plant growth, Köhl et al. (2019) highlighted that most plant pathogens utilize nutrient sources in a far less specialized manner by decomposing dead organic plant materials. This is consistent with observations made in the field, where the farmer rarely removed dead leaves and split pseudostem, resulting in increased soil moisture content and disease development. To prevent the spread of FWB, farmers must cut and burn infected banana trees, create isolated areas, and refrain from sharing planting materials. Additional research is required to comprehend the relationship between good agricultural practices and FWB disease incidence. Foc has spread because of ineffective farm management techniques and the uncontrolled transportation of infected banana tree components from one region to another (Dita et al., 2018).

In 1995, Brake et al. stated that further research into the effect of plant age on disease development is necessary. Similarly, Jie et al. (2009) reported that plant age is one of the influencing elements that led to a variable inoculum composition during the development phase of biological control for FWB. In contrast to foliar diseases such as Wheat Stripe Rust, younger wheat plants were much more susceptible to disease than older wheat plants (Farber & Mundt, 2017). In terms of taxonomy and morphology of banana tree, the numerous giant leaves in older leaves provide more shading effect, which might change the microclimate around that particular banana tree. Thus, further systematic experimental design is required to understand the effect of banana tree ages on soil microclimate and FWB.

Conclusion

This study aims to identify any potential interactions between FWB and soil characteristics. Without proper farm management, the data analysis and field observation suggested that the occurrence of FWB may alter the microclimates in certain regions. Inappropriate agricultural practices and pathogen survival strategies result in devastating plant disease epidemics that cause enormous economic losses. However, continuous monitoring, regular evaluation, and a systematic experimental design should be implemented and expanded to various locations of various farm management practices.

As a future path for FWB disease, a disease forecasting system is necessary to determine plant health conditions and growth development. Such a strategy is crucial for comprehending the relationship between soil conditions, banana trees, and the Foc pathogen. In addition to predicting, a mathematical approach in the form of modeling could be computed to provide further decision-making and prediction that provides additional insights into the study of plant disease epidemiology.

Acknowledgments

Thank you to the Jeli District Agriculture Officers for suggesting the banana farm for this study. In addition, we would like to thank the Faculty of Earth Science and the Faculty of Agro-Based Industry for providing facilities for analyzing samples.

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