



## The Influence of Soil Nutrients Availability on Banana Bunchy Top Disease Incidence in Banyumas Regency, Central Java Province, Indonesia

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### Abstract

The banana bunchy top is one of the important diseases on bananas, presumably coupled with the influence of soil properties, despite no fixed information regarding their relationship. Therefore, this study aims to map the soil nutrient contents at some banana plantation center and determine the effect of nutrient availability on the incidence of banana bunchy top disease (BBTD). This study set a purposive sampling according to the banana cultivation distribution to gain soil samples and data on BBTD incidence. Soil samples were analyzed for macronutrient content, including N capacity using the Kjeldahl method, Bray method for P analysis, and  $\text{CH}_4\text{OAc}$  methods for K analysis. According to the investigation, the range of total N was 0.2% to 0.75%, showing BBTD incidence from 5.8% to 9.47%, respectively. In line with the total P, BBTD incidence increased from 8.03% to 9.62% in the P content of 15 to 35 ppm. In contrast, in the total K of 0.5 to 1  $\text{cmol}(+) \text{kg}^{-1}$ , the BBTD incidence tended to decline from 9.68% to 9%. It was concluded that BBTD incidence would increase with the higher levels of N and P but decrease with the higher K. In Banyumas Regency, BBTD incidence increased in the altitude range of 100 to 300 m above sea level, then decreased at a higher altitude. BBTD incidence also exhibited an unstable response to pH changes. Bunchy top disease incidence was the highest between pH 5 and 8. This could be a guide to soil management to reduce BBTD incidences.

**Keywords:** banana; disease incidence; nitrogen; potassium; soil nutrient; virus

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### INTRODUCTION

Banana is widely cultivated in the tropics and subtropical countries because it is appreciated by consumers (Ahouangninou et al., 2021). Indonesia's banana production grew 6.85% from 8.18 million tons in 2020 to 8.74 million tons in 2021, while in Central Java Province, banana production increased from 6,215,355 in 2009 to 7,946,267 quid in 2020 (BPS-Statistics Indonesia, 2021). Banana plant requires temperatures between 25 °C and 40 °C, a high level of sunshine, and regular irrigation

(approximately 180 mm of precipitation per month), corresponding to the climate conditions of the wet tropical zones (UNCTAD, 2016). These environmental aspects are found in Banyumas Regency, and therefore, have met the criteria for developing banana commodity. While banana plants grow well on marginal and fertile land, the problems faced in the development of bananas are different soil fertility in each region, banana cultivation techniques, pests and diseases. Huber et al. (2012) stated that nutrients on plant growth and yield are usually explained in terms of the functions of these

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elements in plant metabolism. However, according to Huber et al. (2012) and Olivares et al. (2020), nutrients often have unexpected secondary effects on plant growth and yield by inducing changes in growth patterns, plant morphology and anatomy, or chemical composition, which can increase or decrease plant resistance, tolerance and virulence of pathogens. Nutrients are important for the growth and development of plants and microorganisms, as well as for controlling diseases (Agrios, 2005). Mineral elements directly contribute to plant protection through complete and balanced nutrition (Tripathi et al., 2022). The essential nutrients of plants, such as nitrogen, may affect disease severity, either increase or decrease, depending on the environment (Sun et al., 2020b). In addition, nutrients may simultaneously affect pathogens and plant physiology to influence disease development. This study tried to understand the relationship between soil nutrient availability and the incidence of plant disease. The result will support sustainable agriculture by identifying alternative measures to control plant disease which do not harm the environment while boosting yield and improving quality through plant nutrient management.

Nitrogen affects plant adaptation to abiotic stresses. Low N priming increases the leaf water status and alleviates the inhibition of photosynthesis and increases plant growth (Gao et al., 2019). Similar to Sun et al. (2020b), reducing N application rates increased the yield of maize and wheat. Fertilizer is an important issue in banana production as well (Torres-Bazurto et al., 2019). Nitrogen fertilizer affects plant physiology, such as chlorophyll content, enzyme activities and soluble protein (Sun et al., 2020a). The consequence of the N disorders in the plants is reflected in shorter plants, smaller leaf areas, generalized leaf chlorosis and low yields (Torres-Bazurto et al., 2019). Soil nutrient supplies may change pathogen dynamics indirectly at several stages of the infection cycle by changing host functional traits. Plant nutrient availability could directly limit the production of viral nucleic acids and proteins or limit plant growth, thus indirectly limiting metabolic pathways necessary for viral replication (Lacroix et al., 2014; Whitaker et al., 2015). Nitrogen has been shown to increase the concentration of a plant virus by its impacts on host biomass, rather than thru direct effects on the virus (Whitaker et al., 2015).

Phosphorus is the second most essential nutrition after N (Malhotra et al., 2018; Chan et al., 2021). Low P supplies reduce the severity of plant virus disease (Zafar and Athar, 2013). Phosphorus deficiency is often confused with N since the veins of young leaves appear red under both deficiencies. The P affects plant growth which is attributed to either decrease in photosynthesis or an increase in energy investment. Its limitation negatively impacts crop yield and quality (Malhotra et al., 2018). Phosphorus deficiency increase defense responses. Under P deficiency, the jasmonic acid (JA) signaling pathway is activated, bringing enhanced resistance to insect herbivores (Chan et al., 2021).

Potassium nutritional status is essential for plant resistance to biotic and abiotic stress (Wang et al., 2013; Zörb et al., 2014). Potassium is one of the essential elements for plant growth and physiology. Also, K is a constituent of plant structures and has regulatory functions in several biochemical processes associated with protein synthesis, carbohydrate metabolism and enzyme activation (Hasanuzzaman et al., 2018). Potassium plays a prominent role in enhancing the quality of crops by improving the physical performance, disease resistance, and post-harvest performance of fruits and vegetables for human consumption (Prajapati and Modi, 2012; Wang et al., 2013; Hartati et al., 2018). Potassium is particularly important in photosynthesis, translocation of photosynthates, regulation of stomata movement, phloem transport, yield, quality and stress resistance of crops (Hou et al., 2019).

Banana bunchy top disease (BBTD) is considered a serious threat to bananas. It causes severe economic losses for bananas up to 100% and decreases the cropped areas (Qazi, 2016; Abiola et al., 2020; Mahfouze et al., 2020). BBTD is caused by the banana bunchy top virus (BBTV) transmitted by aphid *Pentalonia nigronervosa* in a circulated non-propagative manner (Watanabe et al., 2013; Rahayuniati et al., 2021). The limiting factor of BBTV distribution and management have been studied before, but little is known about the effects of plant nutrition on viral diseases. Gabriel and Mupenzi (2015) investigated the role of soil and plant nutrients in spreading BBTD in areas surrounding Bugarama Valley and found that the spread of BBTD was limited by some factors including K, Ca, Total N, and P. Despite that, the impact of

soil nutrients on BBTD has not been clear yet. In Banyumas Regency, Indonesia, some center of banana plantation show BBTD symptoms, so this research was conducted to identify the effect of soil nutrients on the incidence of BBTD. This information will be useful for the selection of appropriate banana plantation sites and crop management to suppress the distribution of BBTD disease. It takes better understanding of the effect of N content on soil and plants to help develop appropriate exploration strategies and applications, increase crop yields, and protect the environment.

## MATERIALS AND METHOD

### Study area

This research on spatial distribution of BBTD was conducted from March to September 2022 in Banyumas Regency of Central Java Province, Indonesia. Surveys and sampling activities were conducted to record the incidence of banana bunchy top and collect soil samples to investigate nutrient availability.

The study was conducted especially at the banana plantation center in some districts of Banyumas Regency. Banyumas is located at 7°15'05" to 7°37'10" S and 108°39'17" to 109°27'15" E, with an altitude range from 15 to 420 m above sea level (m asl). The highest rainfall was 707 mm<sup>3</sup> in November and the highest number of rain days registered at 24 days. There were four soil types in Banyumas, namely

Entisols, Inceptisols, Vertisols and Ultisols (BPS-Statistics of Banyumas Regency, 2021).

### Soil sampling

Purposive soil sampling was done in 32 banana plantation centers, including Sumbang, Baturaden, Purwojati, Rawalo, Karanglewas, Somagede, Ajibarang, Gumelar, Wangon and Kemranjen sub-districts (Figure 1). The sample collection included plants with bunchy top symptoms and a total of 129 soil samples. The composite soil samples were taken at a depth of 0 to 40 cm under the infected plant. The samples were subjected to soil analysis for fertility characterization purposes, including soil reaction (pH), electrical conductivity (EC,  $\mu\text{s cm}^{-1}$ ), the content of N (%), available P (ppm) and available K ( $\text{cmol}(+) \text{kg}^{-1}$ ).

### BBTD incidence

The incidence of disease was calculated based on the observation and recording of visual symptoms of the treated plants. The appearance of early symptoms may indicate that the plant is infected (Ngatat et al., 2017; Rahayuniati et al., 2021; Olivares et al., 2022). The disease incidence (DI) was calculated by comparing the percentage of symptomatic plants with the total plants observed, following Equation 1.

$$\% \text{ DI} = \frac{\sum \text{ plant with BBTD symptoms}}{\sum \text{ total plants observed}} \times 100\% \quad (1)$$

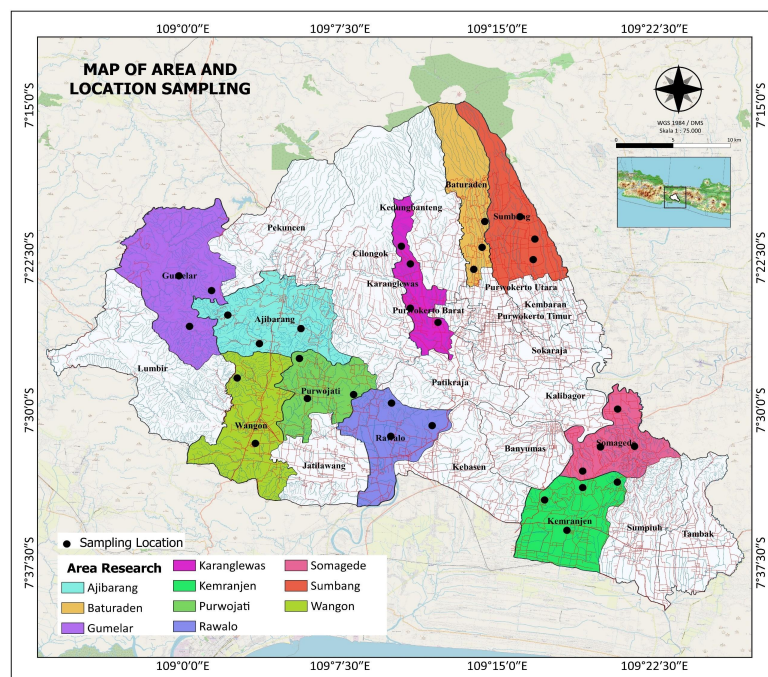


Figure 1. Distribution of NPK and disease incidence at 10 districts in Banyumas, Indonesia

### Soil nutrients analysis

Analysis of nutrients or soil nutrients was carried out by procedures available at the Soil Science Laboratory. The analysis mainly determined pH, EC, N, P, K content of the soil planted with infected bananas. It was expected to reveal the nutritional content in order to determine and carry out the appropriate management.

#### Nitrogen analysis

Soil total N was determined using the Kjeldahl method. This method analyzes N levels in three steps, namely the process of destruction, distillation and titration (Bremner, 1965; Eviati and Sulaeman, 2009). N-total is gained by Equation 2.

$$\begin{aligned} (\%) \text{ N-total} = & \\ & \left( \frac{((A-B) \times N \text{ NaOH} \times 14 \times 100) \text{ mg sample}}{\left( \frac{WC+100}{100} \right)} \right) \times \end{aligned} \quad (2)$$

Where, A = ml NaOH blank, B = ml NaOH sample, N NaOH = 0.05 N, WC = water content of the sample. The WC is obtained through Equation 3.

$$WC = \left( \frac{B-C}{C-A} \right) \times 100 \quad (3)$$

Where, B = weight of the filled vessel, C = weight of vessel after the oven, A = weight of the empty vessel.

#### Phosphorus analysis

According to Sims (2000) with slight modification, P availability was analyzed using Bray method if the pH was < 5.5 and the Olsen method when the pH was > 5.5.

##### a) Bray method

*Reagents Bray and Kurtz P-1 extracting solution (0.025 M HCl in 0.03 M NH<sub>4</sub>F).* Exactly 11.11 g of reagent-grade NH<sub>4</sub>F was dissolved in about 9 l of distilled water. Then, 250 ml of previously standardized 1M HCl was added, and distilled water was incorporated until it reached a volume of 10 l, and mixed thoroughly. The pH of the resulting solution should be pH 2.6±0.05, so pH was adjusted using HCl or NH<sub>4</sub>OH. The final solution was stored in polyethylene carboys until later use.

Exactly 2 g soil sample that passed the 2 mm sieve was put into a flask, then 200 ml Bray extract was added, continued shaken for 30

minutes and filtered. If the solution was not clear, it was re-filtered until transparent. The clear extract was then put into a test tube, added with phosphate dye reagent then shaken until homogeneous, and left for 30 minutes. Colorimetry was observed through the absorbance of the solution using a spectrophotometer at a wavelength of 693 nm.

##### b) Olsen's method

*Preparation for Olsen P extracting solution (0.5M NaHCO<sub>3</sub>, pH 8.5).* Exactly 420 g commercial grade NaHCO<sub>3</sub> was dissolved in distilled water to make the final volume of 10 l. Magnetic stirrer was used to speed up the dissolving process of the NaHCO<sub>3</sub>. Adjust extracting solution pH to 8.5 with 50% sodium hydroxide.

The soil sample was sieved using a 2.00 mm sieve, and the sifted soil samples were put into a flask. A total of 20 ml Olsen solution was added to each flask then shaken for 30 minutes and filtered using Whatman filter number 42. If the extract was not clear, it was re-filter the extract. The clear extract was then put into a test tube, added with phosphate dye reagent, shaken until homogeneous and left for 30 minutes. The absorbance was measured by a spectrophotometer at a wavelength of 693 nm.

#### Potassium analysis

Potassium level was analyzed based on Brown et al. (1973) and Zhang et al. (2017). The collected soils were dried at 40 °C then sieved to a 2-mm mesh screen. Exchangeable K (extracted using NH<sub>4</sub>OAc) was analyzed using 1 mol l<sup>-1</sup> NH<sub>4</sub>OAc at a soil-to-solution ratio of 1:10 for 30 minutes. After this, the suspension was filtered and the K concentration was determined using a flame photometer.

#### Data analysis

The statistical analysis was conducted using IBM SPSS Statistics 24. A polynomial regression analysis was used to determine the relationship between soil nutrient content and the incidence of BBTD.

## RESULTS AND DISCUSSION

There are many environmental factors influencing banana growth, including soil fertility which is closely related to soil enzyme activities. Soil enzyme activities also reveal the biochemical processes in soil and reflect the nutrient capacity supply (Sun et al., 2020b). While soil properties are widely known as predispositions for plant

diseases (Huber et al., 2012), only little attention has been paid to plant disease management.

Chemical soil components, such as N, P, K and soil pH are essential elements that directly impact plant's development. According to Liu and Zhang (2019), N soil addition affects the component of plant and soil systems by weakening the linkages between plant and microbes' communities and the pattern of enzyme activities. They suggested that N excess induced soil acidification and influenced the decomposition activities and nutrient cycle.

There was a variation in macronutrient availability of some banana plantation center in Banyumas. The author categorized the nutrient content into three parts, namely low, moderate and high. The content of N varied from 0.10% to 0.75%, P from 10 to 35 ppm, and K from 0.4 to 1.0 cmol(+) kg<sup>-1</sup>.

#### Nitrogen total

The total N at each sampling location varied from low to medium, and high (Figure 2). Low N was 0.1% to 0.2%, medium was 0.21% to 5.0%, and high was 0.51% to 0.75%. Some areas of Banyumas including Kemranjen, Wangon, Ajibarang, Purwojati, and a few parts of Gumelar, Karanglewas, and Rawalo showed a high N value. While medium N content was found in most areas of Banyumas Regency, much lower N were observed in Sumbang, Purwojati, Gumelar and Somagede. Further, the research found that BBTB incidence significantly increased with N value. The average disease incidence was 5.8% at low N, up to 8.9%

at medium N and up to 9.47% at higher N. Therefore, this result indicated a positive correlation between N and BBTB incidence.

The higher N content in the soil would increase disease incidence. As reported in previous studies, in addition to indirect influence on viral infection, higher N affects plant growth and health (Lacroix et al., 2017), N nutrients also directly affect chlorophyll. More specifically, excessive N harms banana plants (Sun et al., 2020a). This present research indicated that the N nutrient played a role in the incidence of BBTB (Figure 2). Areas with lower N (Sumbang, Purwojati, Gumelar and Somagede) not so many banana plants showed BBTB symptoms. Meanwhile, areas with high N values showed a considerable number of bananas with BBTB symptoms (up to 9.47%). The severity of disease could be supported by high level of N. Lacroix et al. (2014) found that an increase in N content was responsible for the formation of multiple viral infections in plants. Macromolecules like proteins and nucleic acid contained high level of N which could increase the production of virus particles because it was composed of nucleic acid and protein capsid. This biochemical process caused N deficiency in plants. (Tejada and Gara, 2017) stated that lack of N caused all parts of the leaves to turn pale green, leaf midrib became reddish, growth slowed down, and the top of the plant became rosette and bunchy. Although the incidence in the present study was under 10%, it could potentially be the source of diseases in other bananas.

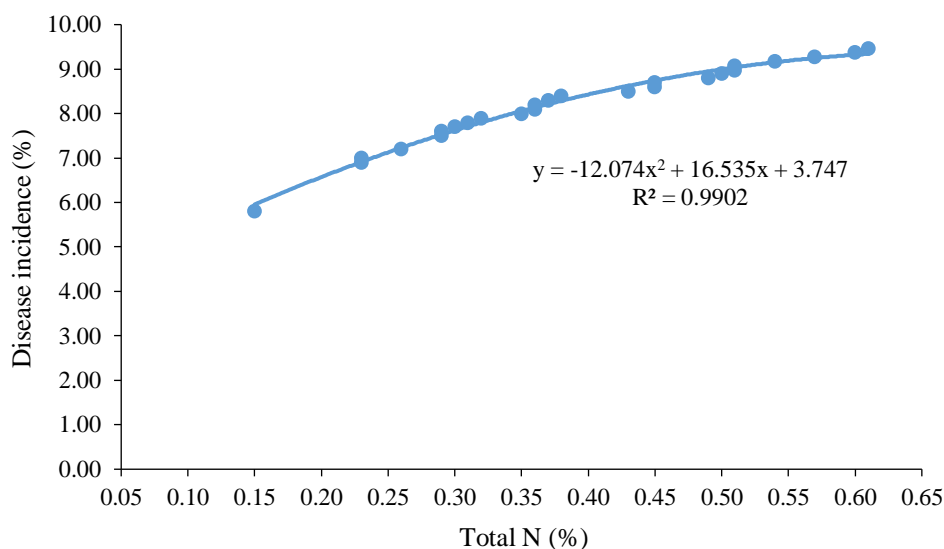


Figure 2. Relationship between total N soil and BBTB incidence



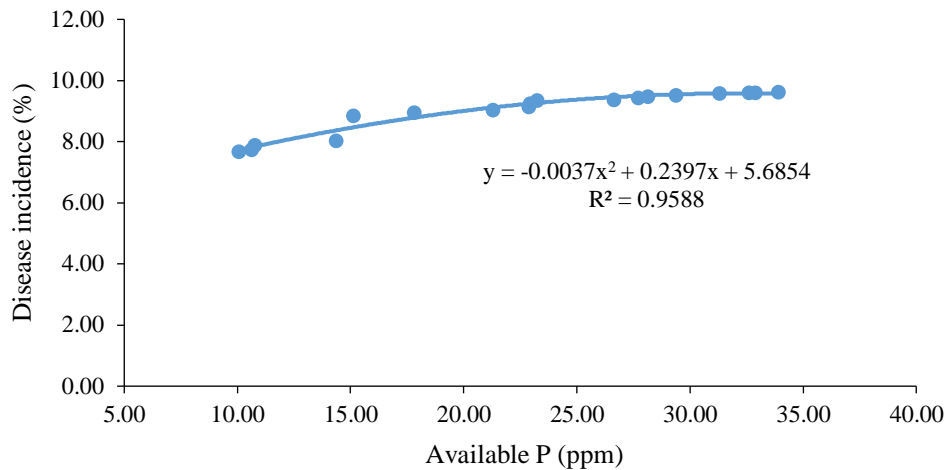


Figure 3. Relationship between available P in soil and BBTD incidence

### Phosphorus availability

In this study, P value was categorized into three groups: low (10 to 15 ppm), medium (16 to 25 ppm) and high (26 to 35 ppm). The research found that P influenced banana response (resistance) to BBTV infection, and disease incidence increased with P concentration (Figure 3). Exactly 15 ppm P caused 8.03% of disease incidence, around 25 ppm P increased disease incidence to 9.35%, and even higher level of P caused 9.62% of disease incidence.

It was evident that in addition to receiving indirect effect of N, BBTD incidence was influenced by P availability. Phosphorus is an essential element for ATP, NADPH, nucleic acids, sugar, phosphates and phospholipids, which are involved in photosynthesis (Carstensen et al., 2018). Despite the fact that P plant resistance to disease will increase with P level, there has been limited information about the effect of P on plant resistance (Huber et al., 2012). In contrast, the results of this study showed that

the higher the P, the higher the incidence of stunting in case P retention from the soil did not occur in ideal manner. Since plants are unable to utilize P in the form of phosphate, microorganisms play a crucial role to help convert P into phosphate for easy absorption.

### Potassium availability

Potassium content (Figure 4) could be considered low at the range of 0.4 to 0.6 cmol(+) kg<sup>-1</sup>, moderate at 0.6 to 0.8 cmol(+) kg<sup>-1</sup>, or high at 0.8 to 1.0 cmol(+) kg<sup>-1</sup>. K nutrient has an important role in plant resistance. The results showed that BBTD incidence was less prevalent in areas with higher K content. Figure 4 shows that 9.69% of disease incidence occurred at 0.5 cmol(+) kg<sup>-1</sup> of K, then decreased to 9.56% at 0.75 cmol(+) kg<sup>-1</sup> of K. The lowest disease incidence (9%) occurred at 1.0 cmol(+) kg<sup>-1</sup> of K. In other words, the decrease of disease incidence could be related to plant stress tolerance.

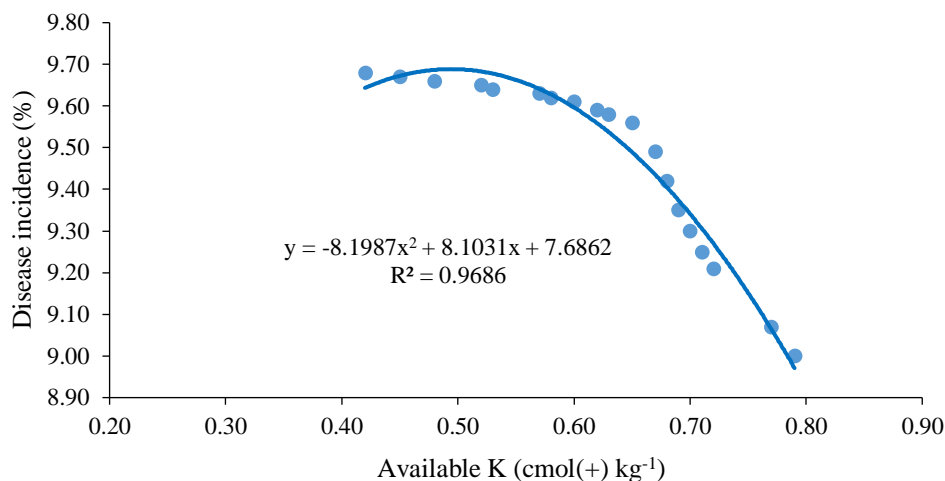


Figure 4. Relationship between available K in the soil and BBTD incidence

K nutrient has different characters from N and P. This study found that the incidence of BBTD was low in soils with high K content, but high in low K content (Figure 4). It was in line with Wang et al. (2013) that lack of K caused plants to be more susceptible, while balanced K conditions would provide banana plants with increased vigor and disease resistance. In general, the application of K fertilizer decreases the incidence of fungal and bacterial disease, as well as indirectly influencing the insects and mites on the plant. While previous study reported a more frequent viral infection in plants with high K (Amtmann et al., 2008), this present study indicated that high K substance in banana plants in Banyumas Regency was correlated with banana resistance to BBTV infection.

#### Altitude correlation

Banyumas Regency is located in the low to the middle area (0 to 500 m asl) despite its close proximity to Mount Slamet (peak at 3,400 m). Figure 5 shows that banana bunchy top disease was distributed at all ranges of altitude in Banyumas. The disease incidence was higher at 200 to 300 m asl (9.6% to 10.6%) than that at 100 m asl (7.5%) and 400 m asl (8.4%). In other words, BBTD incidence increased in areas with 100 to 300 m asl elevation, but decreased at higher altitudes. It was distributed in all Banyumas areas because the climate factors and soil types

were suitable for the spread and contagion of diseases.

The low-to-medium altitude of Banyumas Regency was the reason of non-significantly different temperature or humidity of the areas. However, this research found that the higher altitude showed a slight difference of BBTD incidence (Figure 5) which could be due to its geographical position that were consistently related to environmental and climatic factors, such as temperature and relative humidity, as the primer of vector dynamics. While temperature affected the biology of *P. nigronevosa* (increase or decrease fecundity), biological factors like feeding behavior could determine the success rate of virus transmission (van Munster, 2020). Sucking mouth-type insects, such as aphids, whiteflies, and leafhoppers, have intimate relationships with their hosts, and these relationships can be disrupted by changes in biotic and abiotic factors (Trebicki, 2020). The relationship between altitude, climatic factors and BBTD incidence in Banyumas Regency should be studied further.

#### Effect of pH level

The effect of pH is illustrated in Figure 6. Disease incidence at neutral pH soil (7.5) was higher than at pH 5.5 and 6.5. The pH level could slightly influence the intensity of fusarium wilt disease (Dita et al., 2018). The result of this present study showed that the higher the pH, the higher the disease incidence.

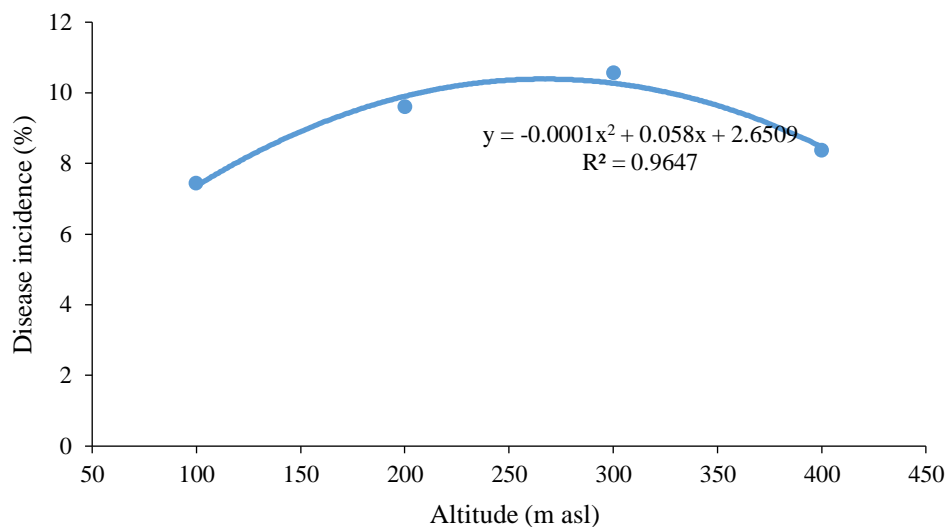


Figure 5. Relationship between altitude and BBTD incidence

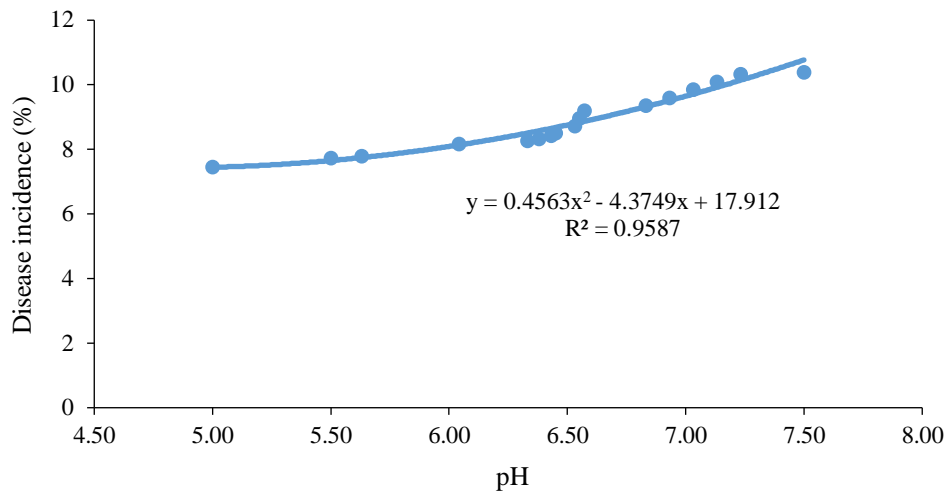


Figure 6. Relationship between pH level and disease incidence

This study also attempted to understand the correlation between soil pH and BBTB incidence. It may have been coincidental because of the instability of the data. The results demonstrated the complexity of responses to pH changes and the importance of the direct impact on virus replication and bunchy top disease. Figure 6 shows that bunchy top disease incidence was found from pH 5 to 8. Previous research reported the role of soil properties in plant disease expression, such as the cases of pH and N in Fusarium Wilt (FW) in bananas, describe land management opportunities to address this disease (Segura-Mena et al., 2021). Soil pH can change due to the input and output of materials, including N and C cycles (Orr and Nelson, 2018). Multiple chemical properties of soil, including pH, organic matter content and availability of some micronutrients are related to the suppressiveness to FW in certain soils. However, this correlation largely depends on the soil type and climate, which render it unlikely to generalize the effect of soil properties on the development of FW diseases (Olivares et al., 2021). Further investigation should be undertaken on the direct role of soil pH and its correlation with other soil properties in the interaction of BBTB and bananas.

A study by Olivares et al. (2022) identified the combination of Zn, Fe, Ca, K, Mn and Clay as the potential new indicators associated with the incidence of banana wilt caused by fungal-bacterial complex in soils of lacustrine originated in Venezuela. Further investigation by Olivares et al. (2022) reported that lacustrine and alluvial soils of Venezuela were characterized by the change of land use from forest to plantation,

morphological properties of the soil such as biological activity, texture, dry consistence, reaction to HCl and type of soil structure. These traits made the areas suitable for high level of productivity and less susceptibility to diseases. Also, the results of several studies in tropical banana zones demonstrated how soil properties influence the production of a complex and variable system. It was further reported that N, P and microbiological (biotic) properties were linked to biometric and productive responses in commercial Cavendish banana plantations and to the susceptibility of bananas to fungal diseases as the key for establishing phytosanitary management of plantations in Venezuela and Panama.

According to studies on tropical banana plantations in Venezuela and Panama using multivariate techniques, under-innovative agricultural strategies and high-cost inputs were insufficient to obtain significant yields (Olivares et al., 2020; Olivares et al., 2022). Additionally, these factors could have linked to the change and accelerated deterioration of certain properties of banana soils and the subsequent appearance of diseases, such as *Xanthomonas* wilt of banana, black Sigatoka (Olivares et al., 2022), FW (Olivares et al., 2021) and banana wilt by bacterial-fungus complex (Olivares et al., 2022). The results showed that banana nutrition was the first to react to physical and chemical changes in the soil. Therefore, it was important to analyze the behavior and interactions of these changes in order to determine the conditions in which diseases could be suppressed in the rhizosphere of the pathosystem of banana cultivation.



## CONCLUSIONS

The increase of N and P in soil caused a higher BBTB incidence, but a higher K content caused BBTB incidence to decrease. Relative to land altitudes, the prevalence of BBTB increased from the lowland to the medium but decreased at higher altitudes. The effect of pH changes on BBTB was also unstable. Future studies can undertake quantitative prediction of the risk of BBTB in banana and new research can be improved through the systematic use of new locations to obtain a robust database of soil and plant nutrition related to plant disease incidence.

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