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Leaf Mineral Composition of Coffee Infected by a *Hemileia vastatrix* Fungus in Bondowoso, East Java

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Abstract

Leaf rust disease caused by *Hemileia vastatrix* fungus may become an important issue on highland coffee (*Coffea arabica*) especially related to the progressive increase in terms of global temperature. A research aimed at evaluating the mineral composition of some rusted coffee leaves from a single clone S 795 was carried out in Andungsari Experimental Station, Indonesian Coffee and Cocoa Research Institute. A single block experiment was situated at 1451 m asl. The intensity of rust spot in the leaves were identified, and estimated to correspond with the score of 5 to 7 out of 10. The difference in the nutrient status between normal and rusted leaves were statistically evaluated by comparing the mean values using unpaired t-test. The leaf analysis shows an optimal range for potassium (K), calcium (Ca), magnesium (Mg) and silicon (Si). Silicon distribution exhibits a high variability with coefficient of variation (CV) around 30%, while K is slightly lower with CV less than 10%. Principal component analysis shows that Ca, Mg and K may have explained the most variation in the original variables as defined by PC1 (54.76%), PC2 (23.22%), and PC3 (14.01%). The rusted leaves are associated with Ca and Si while normal leaves are associated with K. A considerable high of K may explain for the depression of Ca in normal leaves which is, however, associated with an antagonism between these two minerals. The ratio of $\frac{K}{Ca+Mg}$ has a negative correlation with Ca, and may show a potential as an indicator for leaf mineral evaluation in the context of *Hemileia vastatrix* fungus infected coffee.

Keywords: coffee, leaf mineral composition, *Hemileia vastatrix*, Bondowoso

INTRODUCTION

The high efficiency of fertilization is important to make sure that most of mineral added to soil is absorbed by plant. To some extent, the efficiency of fertilization is frequently low as a result of high losses both occurred in the soil and plant system. Disease attacks-related losses are important due to immobilization and ingestion of minerals by pathogens. Disease attacks affect both mineral nutritional status of plant, and the level of tolerance or resistance to pathogens (Marschner, 1995; Dordas, 2008). They influence the plant physiological and biochemical processes (Huber & Graham, 1999). It is in combination with different factors, i.e. susceptibility of the variety grown, the presence of pathogen races, crop ages, presence or absence of shades, bearing level in relation to photosynthetic capacity and climatic condition (Eskes, 1982; Waller *et al*., 2007).

The discussion related to some important mineral nutrients, i.e. K, Ca, Mg and Si, is presented in this paper as their dynamics in the plant tissues may be important to control plant diseases. However, their complexity in the context of inter-relationship results in a direct assessment of diseases effect to mineral composition may not be easy to determine. Interaction between K and Mg, and Ca uptake has been widely reported due to physiological properties (Fageria, 2009). The dynamics of these minerals may contribute to a corresponding decline or incline in terms of host plant susceptibility to diseases (Marschner, 1995; Dordas, 2008; Huber & Jones, 2013).

The adequacy level of K, Ca and Mg in the leaf is suggested to be a key factor for optimal plant growth (Neilsen & Edwards, 1982). To support coffee growth, they play a specific role individually, i.e. K contributes to development and maturation of coffee fruit, while Ca is required to support the development of terminal buds and flowers, and Mg as a component of chlorophyll is crucial for photosynthetic processes (Wintgents, 2004). Additionally, Si may contribute to the reduced number of *Hemileia vastatrix* lesions up to 66% (Martinati *et al*., 2008). Its indirect effect to plant growth includes to improve leaf erectness, to decrease susceptibility to lodging, and to prevent Mn or Fe toxicity (Marschner, 1995). Generally, the failure to meet the optimum level of plant mineral nutrients will reduce bean quality and unfavourable to coffee yield.

Coffee leaf rust may have become an important issue as a result of constant increase in the context of global temperature. Given that the highlands, the suitable habitat for Arabica coffee, are continuously getting warmer it is potentially broadening the spectrum of *Hemileia vastatrix* fungus infection. The obligate parasite exhibits an important impact to devastate coffee plantation at its peak incidence levels (Suresh *et al*., 2012). It reduces both yield quantity and quality as premature defoliation causes a reduction of photosyntethic capacity and, therefore, hampers the growth of new stem. Consequently, the vegetative growth will be decreasing in the following year. The losses of leaves and berries can be up to 50-70% after rust attacks. However, this may provide an alarming signal to Arabica coffee plantation which occupies a limited mountainous area in the world. Therefore, a deep understanding of coffee leaf mineral composition is urgently required.

This paper will provide a discussion about the preliminary study of leaf mineral composition, i.e. K, Ca, Mg, and Si, related to *Hemileia vastatrix* fungus infection in coffee. To improve the understanding of leaf mineral composition, the mineral status and their interaction were also evaluated using mineral ratios based on diagnosis and recommendation integrated system (DRIS). It uses mineral ratios instead of absolute nutrient concentrations to interpret the result of tissue analysis.

MATERIALS AND METHODS

A study was carried out in Andungsari Experimental Station, Indonesian Coffee and Cocoa Research Insitute (ICCRI), Bondowoso District, East of Java. GPS coordinate and elevation of plot were recorded with data showing S07°55'42.7" and E113°41'30.2" and 1451 m asl.

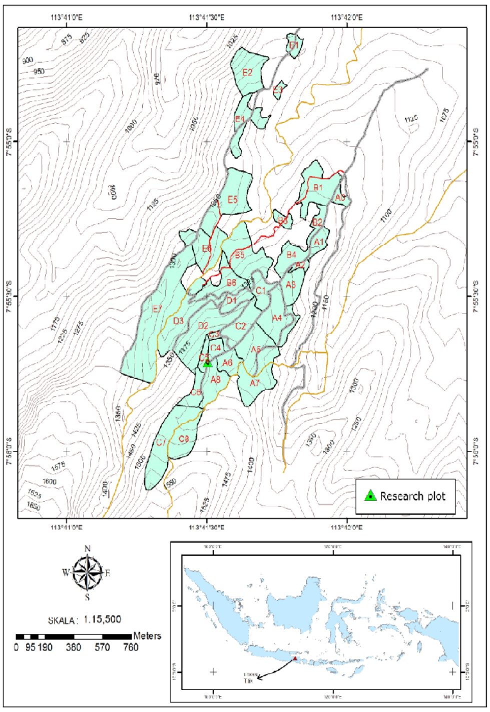

Figure 1. Map of research plot in Andungsari Experimental Station

Data from local climate station shows an average annual temperature ranging from 17 to 26°C and annual precipitation from 2500 to 3500 mm/year. The chemical characteristics of soil as deciphered from previous research (Pujiyanto, 2013), showed a high organic C content ca. 7.29% typical of fresh volcanic soil but with a bit higher C/N ratio ca. 26. The high soil C/N ratio may indicate that the process of soil organic matter decomposition is still in-progress. The availability of P (Bray I) is low ca. 14 ppm indicating a high P fixing capacity. The basic cations are quite dominant in the soil with concentration of Na 0.36 me%, K 3.21 me%, Ca 10.38 me%, and Mg 2.99 me% contributing to base saturation close to 55%. The cation exchange capacity value of 30.96 me% shows a quite high activity of clay minerals.

Leaf Sampling

Leaf samples of both rusted and normal coffee were taken from a homogeneous of 0.90 Ha block area (C5) with Arabica S 795 as a single clone. The investigation was performed in the early of August 2013. The total number of samples were 20 for both 10 pairs of rusted and normal leaves selected randomly. A diagonal sampling was performed on pair of rusted and normal tree with position relatively close each other to minimize field variability. The pair of trees were chosen based on the similarity in the context of performance, i.e. height and width.

The organization of leaf sampling were principally adopted from Pujiyanto and Baon (1995), Wilson (1999) and Wintgents (2004). For full review, reader is referred to these references. In this section a practical way in the context of field operational will be briefly discussed.

The samples were picked from the third pair of mature leaves identified from the tip of an active growing branch. This was performed in the morning to have the high level of leaf turgidity. It requires that tip's leaf posing a minimum 5 cm length, otherwise it is determined from the next section of the same branch. The chosen leaves are subject to a full sunlight exposure from lateral ramification and situated in the halfway of tree height.

The samples were collected from four different direction of lateral branch, i.e. west, east, south and north side of orthotropic stem. An appropriate leaf were picked carefully in every lateral branch to allow four leaf samples per tree, and gives a total number of 40 leaves for both normal and rusted trees.

Defoliation may be occured in the diseased trees, and reduces the leaves to fruit proportion compared to the normal trees (Figure 2.2). In this research, the diseased plants were designated as they have one-third minimum defoliation from full leaf coverage of normal tree. The severity of rust was estimated by a chart to allow a score of rust intensity from 0 to 10. By investigating the number of rust spot observable in leaf surface, an appropriate score of the intensity at the time of observation may be obtained. The relation can be pointed that the greater the intensity of rust spot the higher the score. The observation showed that the estimated score of rusted leaves ranging from 5 to 7, and for normal leaves close to zero. To keep the sample material fresh before the laboratory course the ice box was used to store.

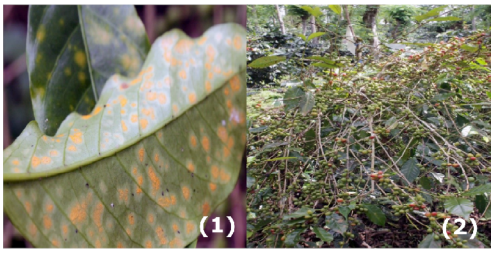

Figure 2. CLR symptoms on underside and upperside of leaves (S 795 clone) (1) and defoliation on rusted leaf (2)

Laboratory Analysis

The preparation and chemical analysis of leaf samples were performed at the Soil and Water Laboratory of ICCRI, Kaliwining, Jember. Prior to chemical treatments, a distilled water was applied to remove dust and any contaminations from both upper- and underside of leaves as soon as they were transported to the laboratory. The contact between leaves and aquades was made as quick as possible to avoid the potential loss of some elements such as K and Cl due to leaching (Jones and Case, 1990).

Two days oven-drying of samples at 60^{O}C were performed to prevent of any potential chemical and biological change during the storage later on. To facilitate the chemical analysis the samples were ground using a mechanical mill. The concentration of K, Ca, Mg and Si were determined by atomic absorption spectroscopy (AAS). The combination of chemicals, i.e. HNO_3 and $HClO_4$, were used to extract the tissue prior to AAS reading. Dry combustion method by applying temperature at 600^{O}C was used to determine Si content.

Statistical Analysis

The extent of leaf nutrient distributions among coffee were investigated by coefficient of variation (CV). To see any differences in terms of nutrient standing between normal and rusted leaves, the average values of data were evaluated. These data were tested statistically by unpaired t-test to investigate any significant effect of *Hemileia vastatrix* fungus infection to K, Ca, Mg and Si concentrations. This test was performed for only specified data that are normally distributed (Dytham, 2011). Therefore, Shapiro-Wilk test was applied to evaluate the distribution of data. Otherwise, the non-parametric Mann-Whitney U test was performed to see any significant differences of data. All data were evaluated at probability $P<0.05$.

Principal component analysis (PCA) was applied to investigate relations between variables and their dependence on rusted leaf samples. The dimensionality of variables is reduced, and the correlation matrix between variables is calculated to produce a new set of independent factors (Buurman *et al*., 2008). These factors are retained to some extent to explain all variation in the individual variables. PCA was calculated by FactoMineR package in R 3.5.1.

RESULTS AND DISCUSSIONS

Leaf Mineral Status

The analysis shows that leaf nutrient distributions vary depending on the type of minerals (Table 1). The high of Si CV up to 30%, for both normal and rusted leaves (Table 1), demonstrates a large range in the context of its concentration in compare to different minerals. The lower variability for K and Mg for normal leaves with CV < 10% indicate a less heterogeneity. With CV ca. 20%, however, Ca shows in the more moderate level in terms of its distribution among leaves. In rusted leaves the CV of Mg is significantly higher almost doubled than in the normal leaves. This large gap, however, may suggest the effect of rust infection to the dynamics of Mg in the leaf tissue.

The leaf mineral standing was evaluated according to some references, i.e. ICCRI manual (unpublished) and Epstein & Bloom (2005). The analysis shows the optimal range of K, Ca, Mg, and Si in both normal and rusted leaves. This may be interpreted as the *H. vastatrix* fungus infection may not affect the leaf mineral status which means that current level of infection may not inhibit the transport of minerals from different tissue to the leaves. However, it could be explained in different way as leaf minerals have already been in the optimal range prior to fungus attack. This is based on the field information that the station applies good agricultural practices, and the fertilization programme is always on schedule.

Based on average values the order of leaf mineral concentrations follow K > Si > Ca > Mg. The dominance of K may suggest the depressing effect to other minerals, i.e. Ca and Mg. The interaction of these three cations in plants tissue may be explained as an antagonism (Jones, 2003; Marschner, 1995; Wintgents, 2004).

Statistical analysis shows a significant difference ($P<0.05$) of K and Ca minerals between in the normal and rusted leaves. The K average content exhibits a 11.39% higher in normal leaves than in the rusted leaves. The higher K content in normal leaves, as expected, confirms its role in modulating disease resistance (Dordas, 2008). In addition, K is favouring the synthesis of high molecular weight compounds such as proteins, starch and cellulose (Römheld & Kirkby, 2010). Consequently, it would depress the concentrations of low molecular weight compounds, namely, soluble sugars, organic acids, amino acids and amides in plant tissues. These compounds are needed for feeding pathogens and therefore, more prevalent in K deficients plants (Marschner, 1995). This may have pointed out the more resistant of K optimal coffee to disease infection.

Conversely, the Ca content was 24.64% lower in normal leaves rather than in rusted leaves. The role of Ca to support plant against pathogens may be important. The high Ca concentration in plant tissue would be reducing the susceptibility of parasite infections (Marschner, 1995), which is unable to observe clearly in this limited scope of study. It may be shown as well the more association of Ca to rusted leaves (Figure 3), which does not confirm the previous study to show the higher level of crop susceptibility, i.e. anthracnose severity in *Cornus florida* L., as the effect of lower inputs of Ca (Holzmueller *et al*., 2007).

Table 1. The range value and coefficient of variation (CV) of leaf mineral compositions in coffee

Nutrient (%)	Normal leaves	CV (%)	Rusted leaves	CV (%)
K	2.31 – 2.79	9.48	2.09 – 2.49	8.72
Ca	1.21 – 1.73	17.67	1.46 – 2.21	20.40
Mg	0.43 – 0.50	7.35	0.38 – 0.51	15.13
Si	1.18 – 2.33	32.71	1.45 – 2.63	29

A considerable high concentration of K in normal leaves, with concomitant low in rusted samples, corresponds to the reduced of Ca, with concomitant high in rusted leaves. The negative dependence between these two minerals has been discussed (Neilsen & Edwards, 1982; Marschner, 1995; Fageria, 2009). The increase in Ca content in rusted leaves may be interpretable in different way following the similar mechanism reported in soybean (*Glycine max L.*) (Smith *et al*., 2001). As Ca is a second messenger involved in elicitor reaction response in plants, the increase of this nutrient is suggested as a defense signaling mechanism for plants undergoing infection of pathogens (Dickinson, 2003). Yet, different finding revealed that the satisfactory level of Ca may contribute to a greater tolerance to disease (Serrano *et al*., 2013). This means that the optimal level of Ca in rusted coffee leaves may drop the *H. vastatrix* fungus infection effect to some extent.

The result of statistical analysis suggested the insignificant difference of Mg and Si concentration between both normal and rusted leaves. The absence of diseases relation to Mg and Si content in crops, i.e. soybean and sorghum, were confirmed (Smith *et al*., 2001; Resende *et al*., 2009). The missing of fungus infection effect to Mg and Si may be suggested as the result of optimal level of coffee leaf minerals. However, the diseases in the crops are mostly associated with deficiency levels (Dordas, 2008).

The effect of optimal level of K, Ca, Mg and Si in coffee leaves to either the susceptibility or resistance to leaf rust infection may not be able to observe clearly in this research. In this case, it is suggested that the sufficiency level of minerals for coffee growth may allow the adjustment an infection to a tolerance level. The term tolerance here means that coffee can survive to grow despite the on-going infections. However, the internal factor for instance the variety of coffee may also be considered as an important point in the context of diseases-minerals regulation (Waller *et al*., 2007). The influence of minerals either to plant resistance or tolerance are related to the characteristics of varieties, namely high, moderate or low resistance to disease (Marschner, 2005).

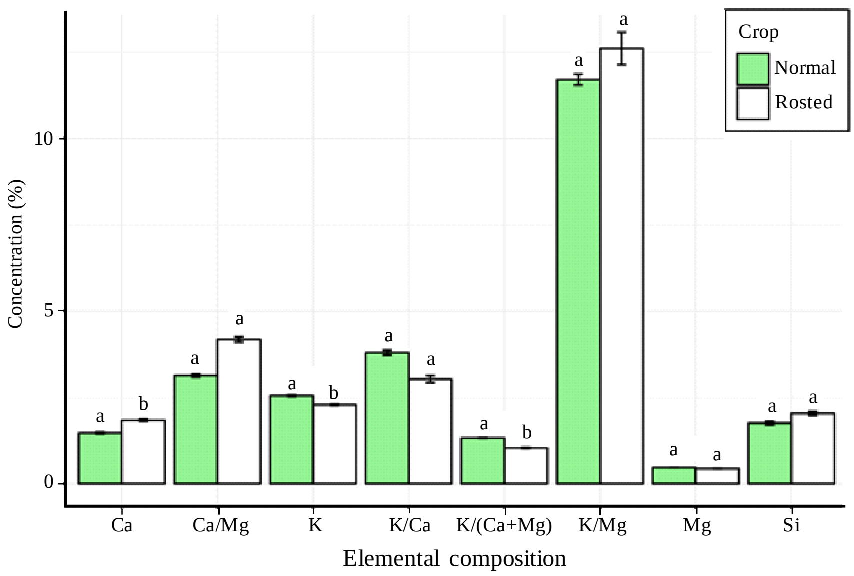

Figure 3. Leaf mineral compositions. Bars followed by different letter means significantly different at P<0.05.

Leaf Mineral Interactions

Leaf mineral composition was evaluated by principal component analysis. Principal components (PC) are retained for those with eigenvalues more than one. The total three components (PC1, PC2, and PC3) account for 91.99% of the total variation in the original variables (Figure 4). The remaining variation of ca. 8.01% is unexplained, and may be considered as random variation due to field variability and various types of error produced during field sampling, laboratory preparation and analysis (Kosaki & Juo, 1988; Buurman *et al*., 2008).

Table 2. Factor pattern for the first three principal components

Variable	PC1	PC2	PC3
K	-0.147	0.073	-0.766
Ca	0.425	-0.173	-0.096
Mg	0.214	0.589	-0.178
Si	0.218	-0.036	-0.518
CM	0.434	-0.086	-0.135
KCM	-0.429	0.085	-0.223
CMR	0.300	-0.508	-0.053
KC	-0.433	-0.136	-0.039
KM	-0.234	-0.570	-0.176

With: CM = (Ca + Mg); KCM = $\left(\frac{K}{Ca+Mg}\right)$; CMR=$\left(\frac{Ca}{Mg}\right)$; KC=$\left(\frac{K}{Ca}\right)$; KM=$\left(\frac{K}{Mg}\right)$

For the first component, as account for most total variation in the original variables,

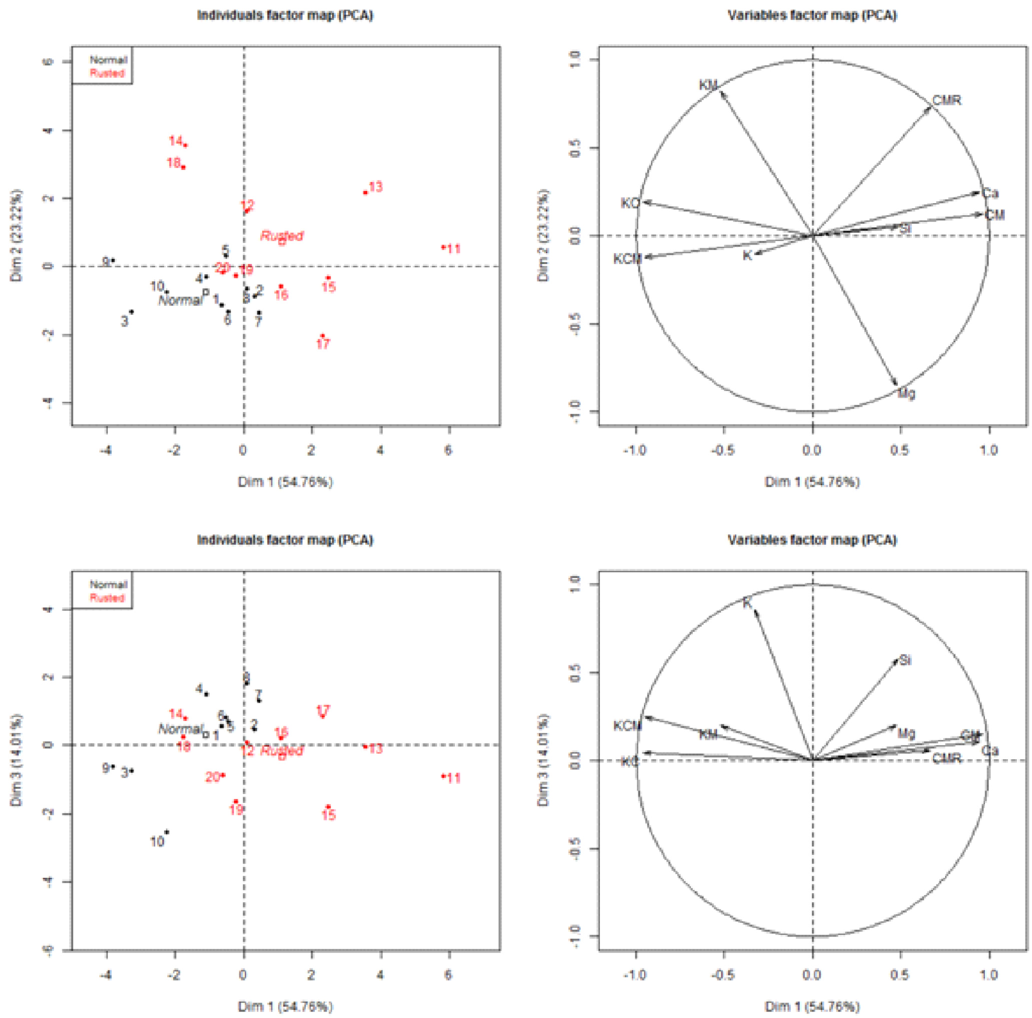

Figure 4. Principal component analysis of mineral composition

the high coefficients both positive and negative were given to Ca, CM, KCM, and KC (Table 2). Calcium defines the total variation both as absolute form and mineral ratios showing the importance of this nutrient in the context of mineral dynamics in the leaf. It plots to the right toward the same direction with Si and Mg implying slightly their positive mutual dependences. In combination with Mg, as represented as CM, they define more the variability of original variables as the coefficient is now increasing to 0.434.

Conversely, K occurs at the space to the left of plot showing the lower score of coefficient (Table 2), which means that this mineral may not importantly explain the total variation of original variables. It plots at the space the other way around of Ca, Si, and Mg implying a negative dependence. Similarly, the ratio of KC and KCM occur at the space toward to the left of plot. With higher coefficient values, they may be important in the context of dynamics of leaf minerals. The opposite position to Ca (Figure 4) may be explained simply as a result of the ratio formulation, i.e. K position in the formula is always as a numerator.

The most of individual samples from rusted leaves cluster to the right of zero line at the space defined by PC1 (Figure 4). This is slightly opposite to normal leaf individual samples to the left. A clear separation of individual samples may indicate a slight negative relationship between normal and rusted leaves. When the variables and individual factor map is arranged to overlap each other, it is now clearly showing a strong association of K to normal leaves, and Ca and Si to rusted leaves.

As already discussed in the previous section, the evaluation of laboratory data showed an optimal status of coffee leaf minerals. In this condition, K may play a role to decrease the susceptibility of host plant to diseases infection (Dordas, 2008). Therefore, the association of K to normal leaves may be attributable to the increasing of resistance level of leaf tissue to *H. vastatrix* fungus infection. The relation of Ca with fungus infected leaves has been hypothetically discussed in the preceding section as a signal emerging due to the ongoing infection.

Magnesium occurs at the space close to zero vertical line reflecting the insignificant difference of its concentration between the normal and rusted leaves (Figure 2). Though the effect of Mg to increase plant diseases in some plants has been reported (Huber & Jones, 2013), yet its interaction with other mineral such as Ca may be crucial to take into account to examine the effect of rust diseases to leaf Mg, which is unable to explain in this research. The slight association of Si with rusted leaves may indicate a low level of disease suppression. In this case, the physical barrier hypothetically created by Si (Dordas, 2008) may not be effective to prevent fungal hyphae penetration, which means that the level of Si in the leaf may be lower than the point of requirement.

The ratio of minerals (Figure 3) are suggested be more crucial than the absolute amount of each element in the context of evaluation for some diseases (Huber & Graham, 1999). The analysis shows that $\frac{K}{Ca+Mg}$ ratio between normal and rusted leaves of coffee were significantly different (Figure 2). The value was higher in normal leaves than those of rusted leaves. This result may show a potentiality of this ratio to be an indicator for rust control in coffee. To some extent, a better mineral composition in the leaf to support coffee growth may be obtained by higher ratio in the leaf.

CONCLUSIONS

The infection of *Hemileia vastatrix* fungus did not make the status of leaf minerals significantly different, as the minerals evaluated, i.e. K, Ca, Mg and Si both in normal and rusted leaves of Arabica coffee clone S 795 in the research plot were in optimal level. The significant difference between K and Ca concentration in coffee leaves may be interpretable in different way as the first is more associated to normal leaves while the latter together with Si is more to rusted leaves. Both minerals show a negative correlation each other. However, Ca shows to have a more mutual dependence with Si and Mg. The use of mineral ratios may be useful to decipher the mineral composition in coffee leaves. The analysis shows that the ratio of has a negative correlation with Ca which is associated more to fungus infected coffee, and it may potentially be an indicator to control leaf rust disease in coffee as the normal leaves showed a higher value.

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