



## **Critical Level of Manganese in Soil and Leaves: It's Relationship to Fruit Quality of Mangosteen (*Garcinia mangostana* L.)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Manganese (Mn) is one of the essential micronutrients that plays a crucial role in plants' growth and development and is also considered a limiting factor for crop production. This study aimed to determine the critical level of Mn in soil and leaves for improving the quality of mangosteen fruit. The research was conducted in 2015 in several mangosteen production centers, including West Sumatera (Simalanggang and Banja Laweh), West Java (Karacak and Barengkok), Bali (Belimbing and Selamadeg). A well-maintained of fifteen to twenty years old mangosteen plants were used with uniform soil fertility. Purposive random sampling is conducted in each location to select ten plants from each site. Soil samples are taken from under the plant canopy around 0-30 cm in depth at the four cardinal directions. The terminal leaves that collected from the middle part of the plant canopy are used as the leaf samples. Physiological ripe fruits are taken after ripening with index 3-4.

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The parameters observed consist of (1) soil pH of H<sub>2</sub>O, Ca, Mg, Mn-available, (2) levels of Mn, Ca, Mg, and K in the leaves, (3) fruit quality (fruit weight, fruit diameter, fruit skin thickness, aryl weight, seed weight). The results showed the Mn content in the soil ranged from 18.89 to 33.63 ppm. Available Mn is negatively affected by pH, Ca, and Mg exchangeability. Soil Mn is linearly related to leaf Mn with R<sup>2</sup> 0.76. The critical values of Mn in leaf and soil for mangosteen plants in Indonesia were 425 ppm and 22 to 24 ppm, respectively.

**Keywords:** Critical level; manganese; mangosteen; fruit; soil.

## 1. INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) has been developed in several sub-optimal lands in Indonesia. This sub-optimal land is dominated by acid soil, which is characterized by macronutrients deficiency and excessive micronutrients, predominantly Mn. An excessive amount of Mn could suppress the availability of Ca and Mg for plants and decrease fruit quality. Manganese (Mn) is considered a limiting factor for crop production on acid soils and also could interfere in neutral or alkaline soils with poor aeration [1]. As the pH decreases, Mn is mobilized from various forms and increases Mn concentration in soil solution and its availability. At low soil pH (<5.2), mostly Mn was in an available form for plant uptake which is influenced by plant roots [2].

As an essential micronutrient, Mn plays a crucial role in plants' growth and development and contributes to the structure of proteins and enzymes in photosynthesis. Deficiency Mn is harmful to chloroplasts because it affects the water separation system in photosynthesis II (PS II), providing the necessary electrons in photosynthesis. Despite the importance of Mn on photosynthesis and other processes, the physiological relevance of Mn uptake and compartmentation in plants has been underrated [3,4]. Mn deficiency leads to the reduced number of Mn-complexes in PSII cores, leading to destabilization and disintegration of PSII complexes that decrease the clean photosynthesis rate [5]. However, most studies showed a decline in photochemical activity, in particular, that of PSII upon Mn limitation [6]. The result of omission macro and micronutrients for deficiencies in mangosteen leaves show that Mn is deficient if give at 17 and 58 mg Mn kg<sup>-1</sup> [7].

Manganese is easily absorbed by roots in the form of Mn<sup>2+</sup> and accumulated in plant tissues. However, under exceeded concentration, it tends to be toxic for plant growth and development [8].

Manganese toxicity is one of the most important interferes to plant growth on acid soils. Excessive Mn can prevent the absorption and translocation of other essential elements such as Ca, Mg, Fe, and P [9,10,11] and inhibits chlorophyll biosynthesis [12]. Mn toxicity involves a decline in photosynthetic efficiency, a reduction of respiration, and decreased photosynthetic pigment content as physiological effects and tends to increase Mn oxidized and phenolic substance in the apoplast [13,14]. Accumulation of excess Mn in the leaves, its primary sequestration in the photosynthetic tissues, and multiple-layer palisade mesophyll [15].

Recently, the indication of excessive Mn absorptions in several mangosteen production centers has been noticed. A strong indicator revealed that high variation levels of Mn concentration in soils and plant tissues lead to varied the quality of mangosteen fruit. However, it is not yet well-known the sufficient amount of Mn concentration in the soil and the leaves that are required to produce a good quality of the mangosteen fruit. The research to establish the critical level of Mn and its relation to the quality of the mangosteen fruit is required to be conducted. This study aimed to determine the critical limit of Mn and its relationship to fruit quality and leaf nutrient content in several mangosteen production centers in Indonesia.

## 2. METHODOLOGY

### 2.1 Place and Time of Study

The research was conducted in 2015 in three Provinces of mangosteen production centers namely: West Sumatra Province (three locations: Simalanggang, Banja Laweh, and experimental station of Tropical Fruit Research Institute in Solok), West Java Province (two locations: Karacak and Barengkok), and Bali Province (two locations: Belimbing and Salamadeg).

Fifteen to twenty years old of mangosteen plants with uniform soil fertility status was used. These research sites were selected based on the production potential area, and collecting traders at the district and sub-district levels. Purposive random sampling is arranged in each location of the research. Ten plants samples were selected and 100 fruits with physiological maturity at stage 2 [16] were harvested from each plant sample for fruit quality analysis. Fully developed leaf samples were collected from the terminal branch for nutrients analysis.

Parameters observed included soil chemical properties (pH H<sub>2</sub>O, Ca, Mg, and Mn availability), fruit quality (wet fruit weight, fruit skin, fruit flesh, and seeds), levels of leaf chemical properties (N, P, K, Ca, Mg, Fe, Cu, Zn, and Mn).

## 2.2 Soil Sampling and Analysis

Soil samples were taken using a stainless steel auger at a depth of 0-30 cm. Each composite soil sample consisted of 5 sub-samples taken randomly from 4 cardinal directions around the root zone of the sample plants. Soil samples were air-dried, pulverized, ground and sieved through a 2 mm diameter sieve, and then stored for analysis. The fine soil with a diameter of 2 mm was then ground so that it could pass a 0.5 mm sieve for organic carbon analysis. Parameters of soil chemical properties observed were pH H<sub>2</sub>O (1:5), Ca and Mg were interchangeable, and Mn availability.

Soil pH analysis was carried out by diluting it in a ratio of 1:5 (w/v), shaking with a shaker for 30 minutes, and measuring with a pH meter that had been calibrated with pH buffers of 7.0 and 4.0. The exchangeable Ca and Mg were analyzed by NH<sub>4</sub>OAc 1M method (buffer pH 7.0) measured by atomic absorption spectrophotometer. Mn was analyzed by the dithionite-citrate method and measured by atomic absorption spectrophotometer.

## 2.3 Analysis of Leaf and Fruit Skin Samples

Leaf and fruit skin samples were collected from the same location where soil and fruit samples were obtained. The terminal leaves that were fully developed on the outside in the 4 cardinal directions were taken as many as 12 leaves for each tree. Fruit skin samples were taken when the fruit was physiologically ripe (harvest index 4). Prior to stripping, it was cleaned with ionized

water and cut into pieces of 2-3 cm in size. Samples of leaves and fruit skin that have been cleaned were dried at room temperature and oven at 70°C (24 hours) until their weight was stable. After the leaf and fruit skin samples were dried, the samples were ground and stored for analysis.

Leaf and fruit skin samples were analyzed for K, Ca, Mg, and Mn nutrients. Samples of leaves and fruit skin that had been ground were extracted by wet ashing with a mixture of concentrated acids HNO<sub>3</sub> and HClO<sub>4</sub>. Measurement of K, Ca, and Mg was carried out with 1 ml of extract plus 9 ml of 25% lanthanum solution and measured by atomic absorption spectrophotometer. Mn was measured directly from the extract with an atomic absorption spectrophotometer [17].

The critical level of Mn in leaves and soil was determined based on the relationship of Mn concentration in the leaves and soil with the weight of mangosteen fruit. In the graph, vertical and horizontal lines are drawn, the two lines are shifted to the right and left, up and down so that the point in the four-part are relatively equal [18,19].

## 3. RESULTS AND DISCUSSION

### 3.1 Soil Chemical Properties

The result of soil analysis from six sites showed that Karacak and Barengkok were categorized as acid soil with soil pH 4.81 and 4.31 respectively. Meanwhile, Simalangang, Banja Loweh, Belimbing, and Selamadek were classified as slightly acid as 5.61, 5.70, 5.87, and 5.92, respectively (Table 1).

Soil available Ca and Mg were ranged from 5.09 to 12.29 and 0.46 to 2.26 cmol(+) kg<sup>-1</sup> respectively, and both were categorized as low to high [17]. The concentration of exchangeable Ca and Mg exchangeable could affect the soil pH (Fig. 1). The increasing of Ca concentration in soil from 3.59 to 16.18 cmol (+) kg<sup>-1</sup> could increase soil pH from 4.10 to 6.20. The increase of soil pH might be due to the increase of exchangeable Ca in the soil as Ca reacts with H<sub>2</sub>O and donates OH<sup>-</sup> and decrease the ion H<sup>+</sup> concentration in soil solution. Increasing the Mg concentration in soil from 0.29 to 2.18 cmol(+) kg<sup>-1</sup> affected the increase of soil pH from 4.09 to 6.20.

Soil available Mn ranged from 18.89 to 33.63 ppm and was categorized as high. According to

Fageria et al. [2], as the pH decreases at low soil pH (<5.2), Mn is mobilized from various fractions and increases Mn concentration in soil solution and its availability. Soil pH has a dominant effect on solubility, therefore, the availability and potential phytotoxicity of ions (nutrients and toxic elements) [20]. Mn excess results in a sharp decrease in shoot height, biomass accumulation, and total leaf area of a woody species *Populus cathayana* [21].

The present study showed that increasing soil pH from 4.1 to 6.4 was followed by the decreasing of soil available Mn as 35.8 to 16 ppm (Fig. 2). Previous research found that Mn toxicity is a limiting factor for plant growth especially in acid soil with soil pH below 5.6 and low availability of Ca and Mg. Increasing soil pH will be followed by the decreasing of soil available Mn. In general, increasing one unit of pH could decreased Mn concentration by 100-fold [1,22]. There was a positive correlation between Ca and soil pH and Mg and soil pH with R<sup>2</sup> as 0.72 and 0.58, respectively (Fig. 1). Meanwhile, a correlation on Mn with soil pH, Mn and Ca, and Mn with Mg showed a negative correlation with R<sup>2</sup> values as 0.57, 0.46, and 0.36 respectively (Fig 2). Mn toxicity may become a limiting factor

for crop production when mostly available in Mn<sup>2+</sup> form. Mn<sup>2+</sup> is highly soluble and is the thermodynamically most stable form in soils at low pH.

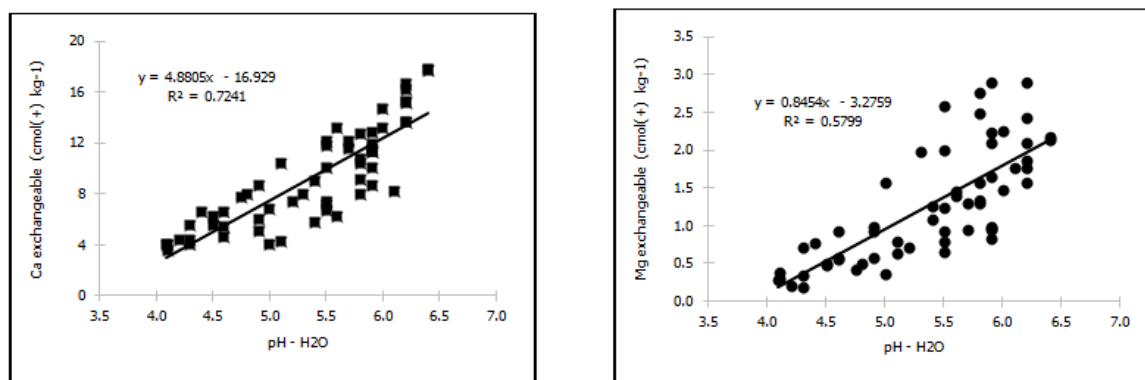
The concentration of Mn<sup>2+</sup> in the soil solution can vary markedly, depending on the soil solution pH. Acidification of the rhizosphere by exudation of H<sup>+</sup> or organic acids increases the availability of Mn<sup>2+</sup> for accumulation into the cell. However, when soils pH decreases (< pH 5.5), Mn<sup>2+</sup> toxicity could be a critical limiting factor to plant growth [1].

The increasing of Mn availability in the soil will be followed by the decrease of Ca and Mg availability at several mangosteens productions centers (Fig. 2). Mn concentration decreased from 35.8 to 15 ppm and followed with the increase of Ca from 3.59 to 17.8 cmol<sub>(+)</sub> kg<sup>-1</sup>, or the Mn content decreased by 1.46 ppm when Ca increased by 1.00 cmol<sub>(+)</sub> kg<sup>-1</sup>. Meanwhile the decreasing of Mn concentration from 32.6 to 17 ppm and Mg concentration increased from 0.2 to 2.58 cmol<sub>(+)</sub> kg<sup>-1</sup>, and the decreasing of Mn concentration as 6.55 ppm followed with increasing Mg concentration by 1.00 cmol<sub>(+)</sub> kg<sup>-1</sup>. This condition occurred in calcareous soil. As

**Table 1. Soil chemical properties from 60 soil samples collected from each site**

Location	pH H <sub>2</sub> O	Ca (cmol <sub>(+)</sub> kg <sup>-1</sup> )	Mg (cmol <sub>(+)</sub> kg <sup>-1</sup> )	Mn (ppm)
Simalanggang	5.61±0.45	9.58±3.86	1.35±0.34	18.89±2.00
Banja Laweh	5.70±0.25	11.79±1.01	1.02±0.25	22.02±2.04
Karacak	4.81±0.25	5.92±1.60	0.58±0.16	31.91±4.45
Barengkok	4.31±0.22	5.09±1.41	0.46±0.26	33.63±3.82
Belimbing	5.87±0.18	11.03±1.59	2.26±0.53	24.81±2.94
Selamadeg	5.92±0.44	12.29±4.77	1.93±0.47	19.42±5.06

Note: data are means ± standard error



**Fig. 1. Relationship between soil pH and soil nutrient concentration of Ca and exchangeable Mg in the soil**

stated by Rutkowska *et al.* [23], liming could decrease Mn and Zn concentration in the soil extracted by 1 mol/l HCl. Recently studied by Martias et al. [24] showed that application of sub-micron dolomite more than 120g/tree decreased Mn concentration in mangosteen leaves.

Mn deficiency could be a serious nutrient disorder in a plant that grows in dry, and calcareous soils, as well as in soils with high organic matter content, where the bio-availability of Mn could reduce below the necessary amount for supporting plant growth [25].

### 3.2 Relationship of Soil Available Mn with Mn in Leaves and the Quality of Fruits

To enhance mangosteen quality, Mn is needed at a certain level. However, under abundant amounts, Mn could decline the quality of fruit (Fig. 3). The present study showed that the critical limit of Mn in the soil affected fruit quality in several parameters, namely the weight of fruit and skin, fruit diameter, and mangosteen rind thickness. The critical level of soil available Mn from this study ranges from 22 to 24 ppm (Fig. 3).

Manganese is a micronutrient that is relatively easily absorbed by the roots of plants, and its absorption even exceeded the requirement. As soil availability, Mn increases, Mn concentration in leaves increases as well, and this proved that these ions are relatively easily absorbed by mangosteen roots and translocated to leaf tissue. This present study showed that the lowest Mn level was 134 ppm and the highest was 1131 ppm in mangosteen leaf tissue (Fig. 4). There is a linear relationship with  $R^2 = 0.76$  between soil available Mn and leaves, indicates that the increasing soil available Mn follows by the increase in Mn concentration in leaves. This finding on Mn behavior in this present study is in agreement with Blamey et al. [26].

Previous results showed that the increasing of Mn concentration from 0.5 to 30  $\mu\text{M}$  Mn in solution, increased Mn concentration in cowpea and soybean leaf tissues from 160 to 1100  $\text{mg.kg}^{-1}$ ; the corresponding increase at 400  $\mu\text{M}$  Mn was from 90 to 6400  $\text{mg.kg}^{-1}$  in sunflower and from 310 to 12 000  $\text{mg.kg}^{-1}$  in white lupin [10,26]. Fernando et al. [27] was the first to show that Mn is evenly distributed in leaf photosynthetic and nonphotosynthetic tissues.

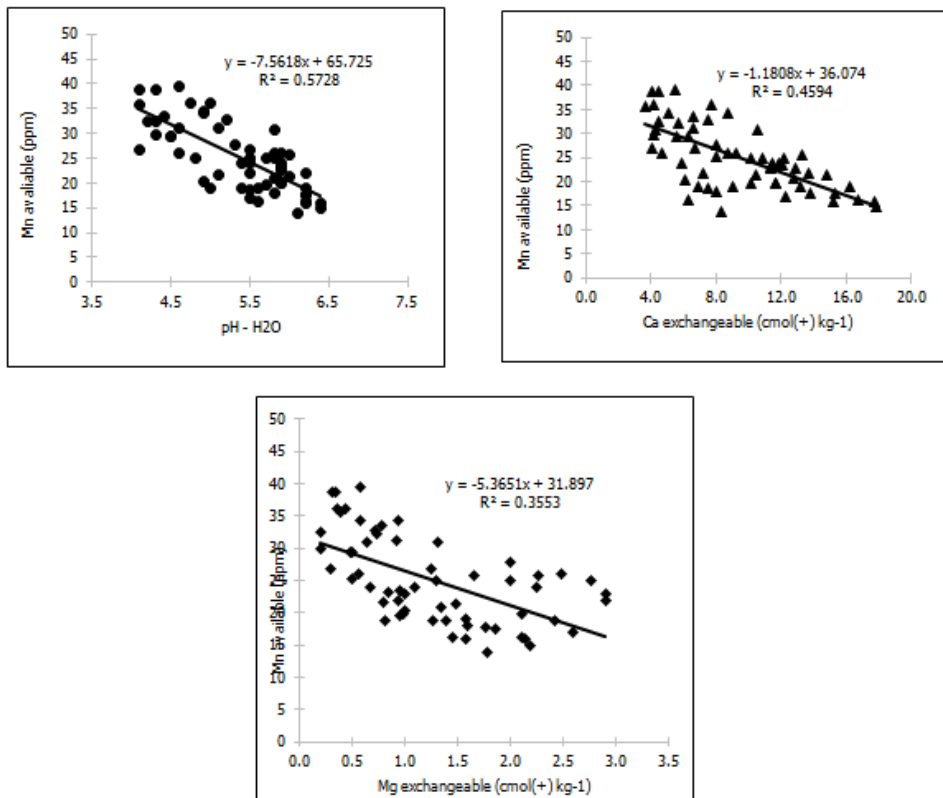
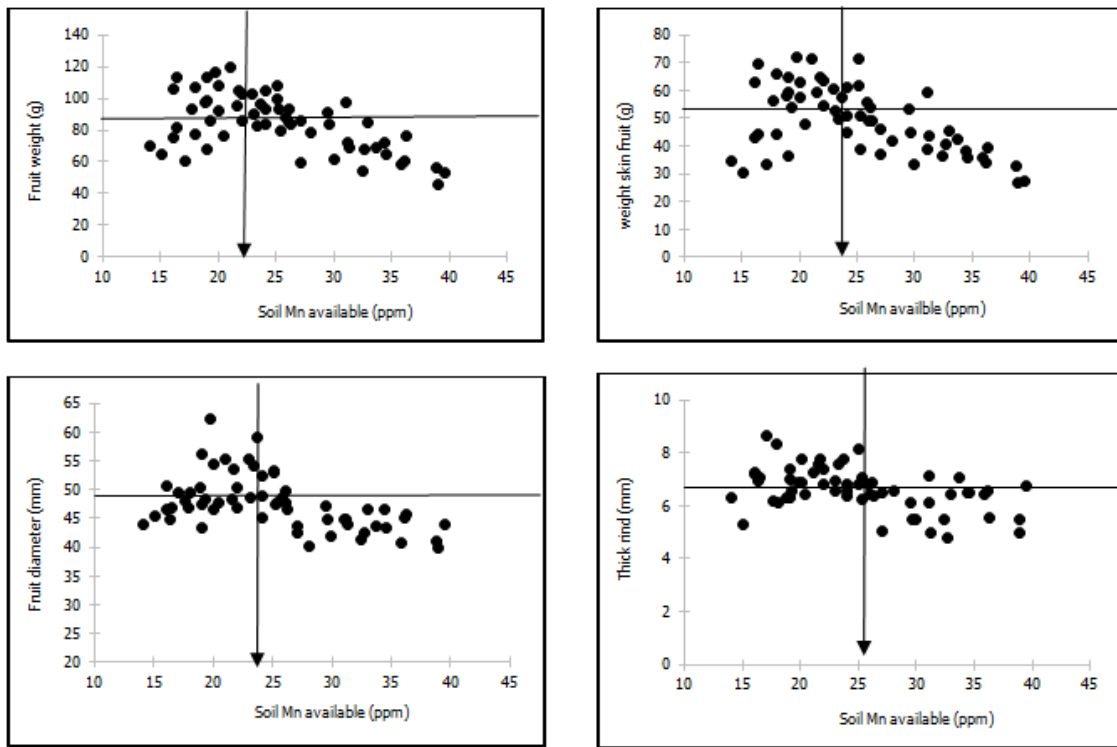
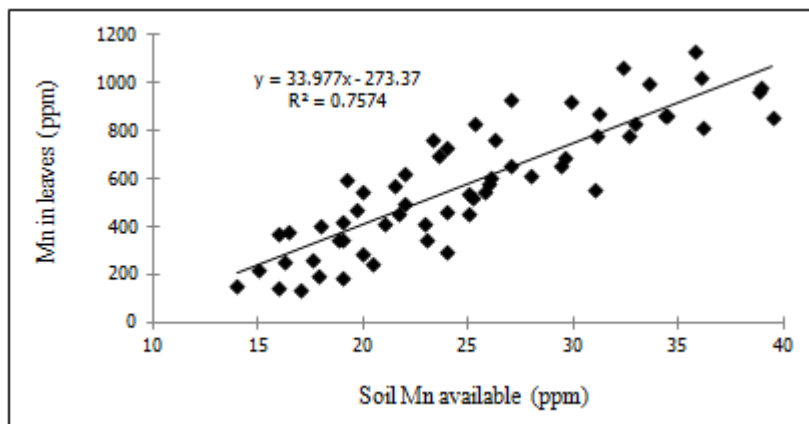


Fig. 2. Relationship between Mn available and soil pH, Ca concentration and exchangeable Mg



**Fig. 3. The critical level of soil available Mn for fruit weight, skin weight, fruit diameter, and mangosteen rind thickness**



**Fig. 4. The relationship of Mn concentration in soil and mangosteen leaf at study sites**

The regression analysis showed a quadratic relationship between the leaf nutrient content and fruit weight (Fig. 5). These results revealed that Mn is required in mangosteen growth as seen in the relationship between the leaf Mn and fruit production. However, the excessive absorption of Mn by the plants is potentially toxic and this circumstance leads to a decrease in the weight of the mangosteen fruit (Fig. 5). The relationship of leaf Mn concentration with mangosteen fruit weight shows the content of Mn

317 ppm to 432 ppm is the level of Mn optimum to produce fruit weight mangosteen.

The symptoms of Mn toxicity vary widely among plant species, with chlorotic leaves and necrotic spots as the most common symptoms [28]. Decreased photosynthesis reduces the weight of mangosteen fruit. The relationship between the Mn concentration in the leaves and the mangosteen fruit weight is quadratic. Mn levels in the leaves < 400 ppm could increase the weight

of the mangosteen fruit, and however > 400 ppm reduces the weight of the mangosteen fruit. Thus, it can be concluded that the critical limit of Mn nutrients in mangosteen leaves is 425 ppm. As Mn concentration in leaves increased from 134 to 400 ppm, the mangosteen fruit weight increase from 61.40 to 120.27 g as well. However, the increase of Mn concentration in leaves above 400 ppm caused the decrease of fruit weight from 117.27 to 46.10 g. The lowest mangosteen fruit weight was 46.10 g with Mn concentration in leaves was reached 976 ppm. Manganese deficiency often occurs as a latent disorder without clear visual symptoms. Latent Mn deficiency substantially increases transpiration and decreases the water use efficiency (WUE) in barley plants [28]. One of the consequences of Mn deficiency in plants is an impaired growth, leading to a decrease in biomass [29]. Mn deficiency caused severe yield and quality reductions in crops [30].

The range of Mn concentration in leaves of mangosteen differs greatly among species as 30-500 mg kg<sup>-1</sup> Mn dry weight. Nonetheless, when it is present in excessive amounts, it is extremely toxic to plant cells [31]. At higher light intensities, Mn toxicity has been causally linked to inhibition of photosynthesis and ensuing oxidative stress [32].

### 3.3 Interaction between Mn with K, Ca, and Mg in Leaves

The concentration of Ca, Mg, and K in the leaves have the effect of increasing the Mn concentration in the leaves. The result showed

that at above 400 ppm of Mn in leaves, the concentration of Ca, Mg, and K in the leaves decreased (Fig. 6). The Mn content in the leaves began to decrease when Ca, Mg, and K reached 2.2, 0.45, and 1.83% respectively. This result showed that the absorption of Mn in high concentrations could disrupt the absorption of Ca, Mg, and K.

The excess Mn interferes with the absorption, translocation, and utilization of mineral elements such as Ca, Mg, and Fe. Potassium, Ca, and Mg is needed for the development of mangosteen fruit. Excessive Mn can prevent the uptake and translocation of other essential elements such as Ca, Mg, Fe, and P [10,11]. The level of K, Ca and Mg had a positive and significant correlation with fruit weight, skin weight, and aril weight, seed weight, and mangosteen fruit diameter (Table 2). This present result proved that these nutrients take a role in improving the quality of the mangosteen.

The correlation between soil and leaf Mn was negative and significantly related to fruit weight, rind weight, aril weight, seed weight, fruit diameter, and mangosteen rind thickness. This means that increasing Mn concentration in soil and leaves at certain levels could give have a negative impact on mangosteen quality. Thus, it is necessary to maintain a sufficient amount of Mn concentration in the soil and leaves. The nutrient efficient plants play an important role in increasing crop yields per unit area as well as improve health and quality of human life [2].

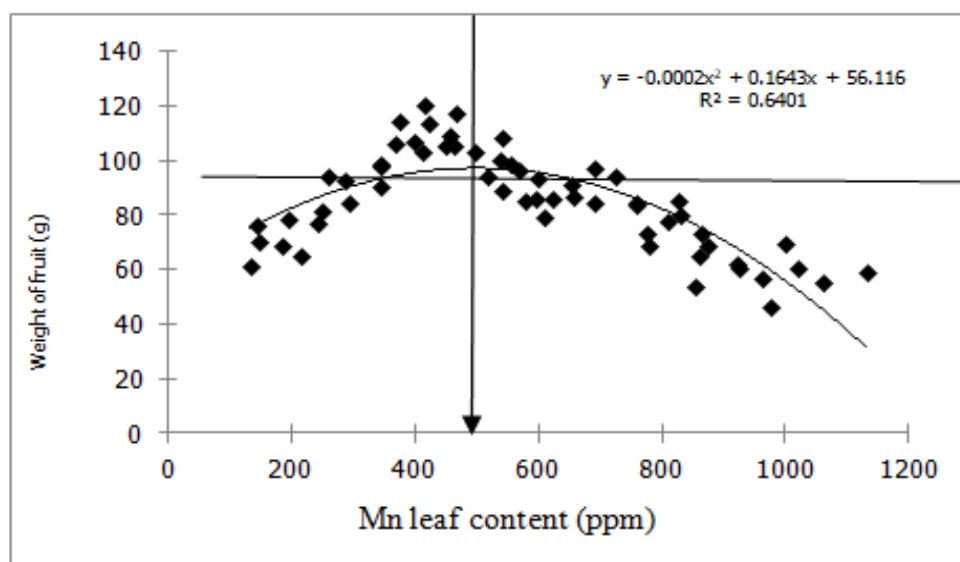


Fig. 5. Relationship of Mn leaf levels with mangosteen weight in study sites



Table 2. Correlation of various types of nutrient to the physical quality components of the mangosteen fruit, n= 100<sup>1</sup>

Components of mangosteen fruit	Exchangeable Ca	Exchangeable Mg	Available Mn-	Leaf-K	Leaf-Ca	Leaf-Mg	Leaf-Mn
Fruit weight	0.431**	0.322	-0.552**	0.613**	0.750**	0.842**	-0.476**
Fruit skin weight	0.383**	0.289	-0.511**	0.554**	0.695**	0.803**	-0.415**
Aryl weight	0.421**	0.304	-0.517**	0.584**	0.702**	0.762**	-0.480**
Seed weight	0.258	0.270	-0.245	0.536**	0.556**	0.552**	-0.243
Fruit diameter	0.458**	0.147	-0.487**	0.633**	0.538**	0.684**	-0.354**
Thick rind	0.540**	0.422**	-0.456**	0.461**	0.325*	0.408**	-0.460**

<sup>1</sup>Pearson correlation coefficient followed by \*, \*\* are significantly correlated at P < 0.05 and 0.01, respectively.

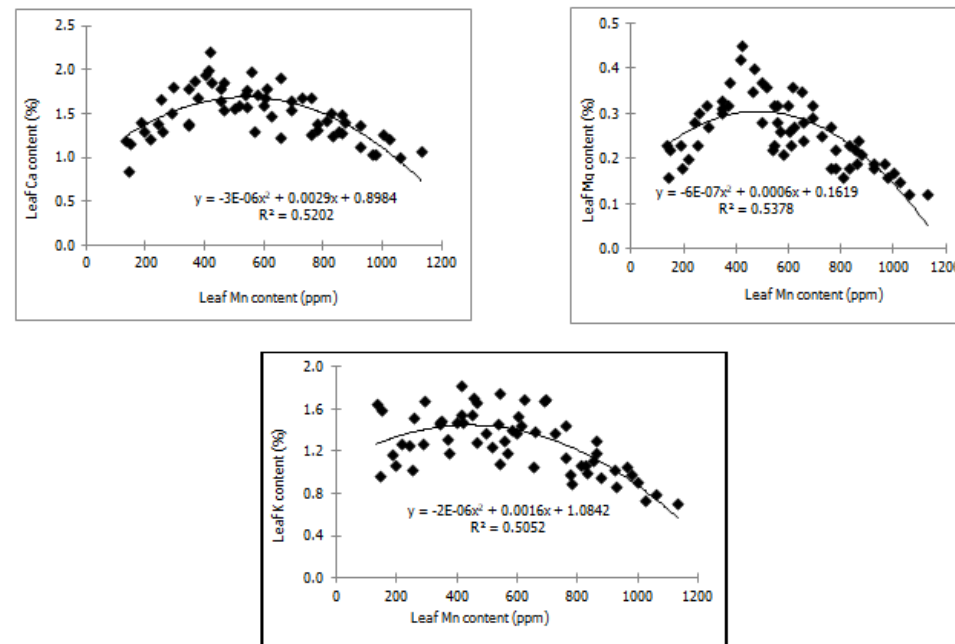


Fig. 6. Effect of Ca, Mg, and K on Mn content in mangosteen leaves



#### 4. CONCLUSIONS

The available Mn in the soil is negatively affected by pH, Ca, and Mg with  $R^2$  0.57, 0.46, and 0.36, respectively. The concentration of Mg, Ca, and K in leaves increased when Mn concentration was below 400 ppm, and decreased when Mn is more than 400 ppm. There was a negative correlation between Mn in the soil and leaves with mangosteen fruit quality. The critical value of Mn in mangosteen leaves is around 425 ppm, while in the soil it ranges from 22 to 24 ppm.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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