Effect of moringa extract on the leaf anatomy and yield potential of tomato infected by *Alternaria solani*

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Abstract

The study evaluated responses of *Alternaria solani* infected (IN) and non-infected (NIN) tomato (*Solanum lycopersicum*) leaf anatomy to moringa leaf extract (MLE) application and correlated responses to fruit yield. IN and NIN relates to the infection status of the plants used in the study. MLE concentrations of 0.5, 0.75 and 1.5 kg L^{-1} (w v^{-1}) were prepared. Controls were distilled water (negative) and 50 mg L^{-1} benzylanimopurine (BAP) cytokinin (positive). Significant interactions were observed for stomatal density ($P < 0.001$). MLE increased lamina thickness, stomatal density, stomatal size and yield. There was a strong positive correlation between yield, stomatal density, stomatal size and lamina thickness. Stomatal density had the greatest correlation (*P* value, r = 0.7979) with fruit yield.

Keywords: moringa leaf extract, leaf anatomical responses, fruit yield, correlation

Introduction

Tomato (*Solanum lycopersicum*) is often exposed to hormones and diseases which affect the crop's leaf anatomy and fruit yield (Seki et al. 2003).

An active synthetic cytokinin (CK) hormone, commercially used as Benzylanimopurine (BAP) influences tomato leaf anatomical characteristics, and functions (Sosnowski et al. 2016). The use of synthetic CKs to manipulate tomato leaf anatomy for the purpose of increasing productivity is possible but adds costs to the grower. The use of CK has therefore been shunned by tomato smallholder farmers in Africa South of the Sahara. Low tomato fruit yield (9 tons ha^{-1}) under farmers' practice in parts of Africa have been obtained, yet potential yields go over 120 tons ha^{-1} (Desta and Yesuf 2015). Low yield is caused by diseases and insufficient crop production practices. Higher productivity, through manipulation of stomatal density, has been reported (Farber et al. 2016).

Among leaf diseases, early blight, caused by *Alternaria solani,* negatively affect leaf anatomy which may reduce fruit yield. Since moringa leaves have zeatin (an active form of cytokinin) (Fuglie 2000), moringa leaf extract (MLE) could be a helpful treatment impacting tomato leaf anatomy and yield.

Materials and methods

Responses of stomatal width and length, guard cells, stomatal density, palisade and spongy mesophyll thicknesses, and lamina thickness of *A. solani* IN and NIN tomato leaves treated with MLE, were evaluated. IN and NIN in this paper refers to the nature

of plants. Stomatal density, lamina thickness and stomatal size were then correlated to fruit yield.

The study was carried out in 2016 at Africa University (AU), of coordinates 18°53′51″S, 32°36′04″E. The soil type was loamy orthoferralitic, 7E.

Young moringa leaves (45 d old) were harvested from trees (7 years old) from AU farm and pounded to a paste form. The pounded material was placed on a cloth strainer, which was stretched over a container to capture the pure extract (moringa concentrate). The MLE concentrate was mixed with distilled water to give three concentrations (0.5 kg L^{-1}, 0.75 kg L^{-1} and 1.5 kg L^{-1} (w v^{-1}). Controls were distilled water (negative) and 50 mg L^{-1} BAP cytokinin (positive). Treatment application was done 5 d after inoculation. Treatments were first applied 5 d after inoculation with *A. solani*, and thereafter every week until physiological maturity.

Treatments were arranged in a randomized complete block design (RCBD), 2 x 5 factorial arrangement. The 2 (IN and NIN tomatoes) x 5 (MLE and controls) factorial experiment was arranged in a randomised complete block design. The treatments were replicated 5 times.

Isolates of *A. solani* were supplied by Kutsaga Research Station, Harare, Zimbabwe (17°55′09.42″ S,31°07′19.6″ E). University of Zimbabwe, Department of Crop Science, Pathology Clinic, authenticated the organism. Isolates were sub-cultured on 30 petri dishes of potato dextrose agar and incubated at 25 ^{o}C for 7 d.

Distilled water (10 mL) was added to each of the 30 plates and spores were carefully scraped with a sterile needle for preparing conidial suspension, with slight modification of the spore density. Spore suspensions of 10 mL were made from each plate and

adjusted using a haemocytometer to conidia density of 1.8×10^4 spores mL^{-1}. For each suspension, 1 mL of spore suspension L^{-1} of distilled water was prepared, placed on ice and taken into the field. Inoculation, at 2 weeks after transplanting 4-week old seedlings of Rodade cultivar. Plants in the infected plots were inoculated by spraying the spore suspension onto the plants at a pressure of 0.2 Mpa.

At the start of the flowering stage, when leaves were fully grown, whole tomato leaves were sampled at 10 o,clock in the morning, when stomata are expected to be fully open. The leaves were and taken to the laboratory for anatomical studies. Stomatal density was determined on the abaxial leaf surfaces as number of stomata per unit of leaf area (mm^{-2}). An epidermal strip was peeled off, mounted in water on a slide and then placed under the microscope. The micrometric observations were recorded according to Kulkarne and Deshpande (2006). Lamina thickness was determined by adding together palisade mesophyll and spongy mesophyll (Kulkarne and Deshpande 2006).

Abaxial epidermal strips were peeled off the leaves with forceps and the detached layers were incubated in the MES/KOH buffer in petri dishes for 2 h, for equilibration. After reaching equilibrium, measurements from the abaxial side were taken with an ocular micrometer (model XY11, Erma Japan). Stomatal sizes were determined as measured products of lengths and widths of guard cells. Stomatal length was calculated by measuring the vertical pore space between the two guard cells of the stomatal apparatus, while the horizontal pore space between the two guard cells at the middle region was taken as stomatal width.

Mature, marketable fruits were harvested and their measurements taken once per week. The fruits were weighed and averaged. Measurements of both the leaf anatomy and fruit yield from the two blocks of IN plants and NIN plants were taken once in particular season, done in four seasons, and averaged.

Analysis of variance (ANOVA) was carried out using GenStat 14th Edition to detect significant differences between means. Where significant differences were found, means were further compared and separated using Fisher's protected least significant difference ($LSD_{0.05}$). Correlation and regression analysis were done to assess how yield was related to stomatal density, stomatal size and lamina thickness.

Results

The NIN tomatoes had a significantly ($P < 0.001$) higher yield of 37.6 tons ha^{-1} than the IN tomatoes which managed to produce a yield of 25.4 tons ha^{-1}. Moringa leaf extract at 1.5 kg L^{-1} produced the highest fresh yield (46.5 t ha^{-1}), while the control (no extract) and the lowest (18.9 t ha^{-1}) (Table 1).

The leaf lamina of the NIN tomatoes were significantly ($P = 0.003$) thicker than that of the IN tomato leaves. The NIN tomatoes had thicker laminae and were significantly different from the IN tomatoes which had thinner laminae. Application of 1.5 kg L^{-1} gave the thickest laminae, whilst the negative control had the least thickness.

There was significant interaction between nature of plants and moringa treatments on the stomatal density. The IN tomato leaves treated with 1.5 kg L^{-1} had the greatest stomatal density (the negative control had the least) (Table 1). The same concentration also showed the greatest stomatal density for the NIN tomato leaves, while the negative

control had the least. The IN leaves had comparably higher stomatal density than the NIN leaves at a MLE concentration of 1.5 kg L^{-1}.

Table 1: Means of fresh fruit yield (kg ha^{-1}), stomatal density (mm^{-1}) and stomatal width (µm) for infected and non-infected plants after treatment with moringa leaf extract (MLE, kg leaves L^{-1} distilled water). Means within a column followed by different superscript letters indicate a significant difference using Fisher's protected least significant difference ($LSD_{0.05}$). Negative (–ve) control = 50 mg L^{-1} benzylanimopurine (BAP) cytokinin), positive (-ve) control = distilled water (*n* = 5)

Moringa leaf extract (MLE)	Fresh fruit yield	Stomatal density (mm^{-1})		Stomatal width (µm)
		Infected (IN)	Non-infected (NIN)	
-ve control- water	18.86[e]	100[d]	190.0[c]	1.50[d]
0.50 MLE	26.65[d]	210[c]	247.2[b]	3.25[c]
0.75 MLE	37.71[b]	265[b]	276.2[b]	4.50[b]
1.50 MLE	46.49[a]	333[a]	312.0[a]	6.55[a]
+ve control	32.71[c]	208[c]	275.6[b]	5.75[a]
P value	<0.001	<0.001		<0.001
$LSD_{(0.05)}$	3.81	32.5		1.03
CV(%)	13.3	10.5		26.40

There was a significant difference ($P < 0.001$) between IN and NIN tomatoes for stomatal width. The NIN MLE treated tomatoes had wider stomatal width; IN tomatoes had a comparably smaller stomatal width. Tomatoes treated with 1.5 kg L^{-1} had the least stomatal length, while tomatoes treated with the same concentration had the widest stomata (Table 1). The negative control had the greatest stomatal length.

There was a significant difference ($P < 0.001$) between IN and NIN tomatoes for stomatal size. The NIN tomatoes having larger stomata than the IN tomatoes. MLE treatment at 1.5 kg L^{-1} had the largest stomata.

Fresh yield was significantly and positively corrrelated with lamina thickness, stomatal size and stomatal density (Figure 3–5). The Pearson's correlation coefficients were 0.643, 0.675 and 0.798 respectively.

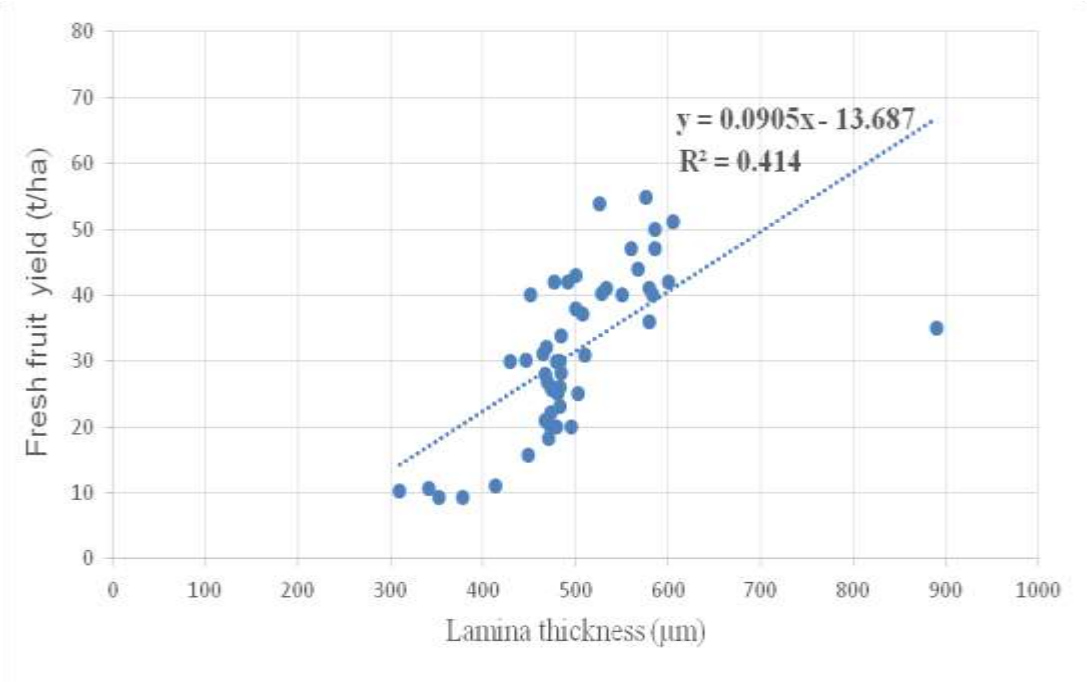

Figure 1: Scatter plot showing the relationship between lamina thickness and fruit yield

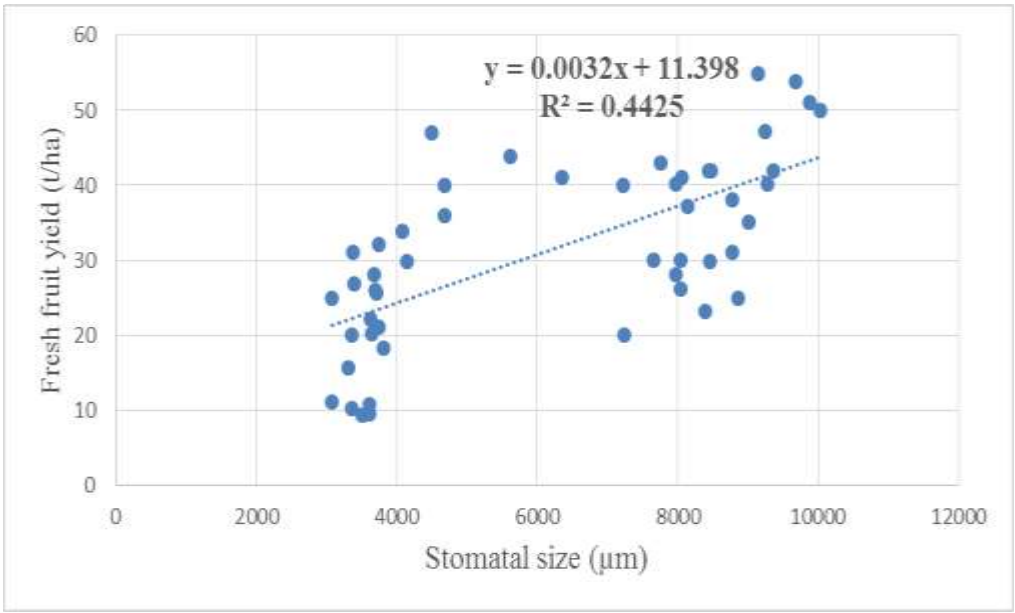

Figure 2: Scatter plot showing the relationship between stomatal size and fruit yield

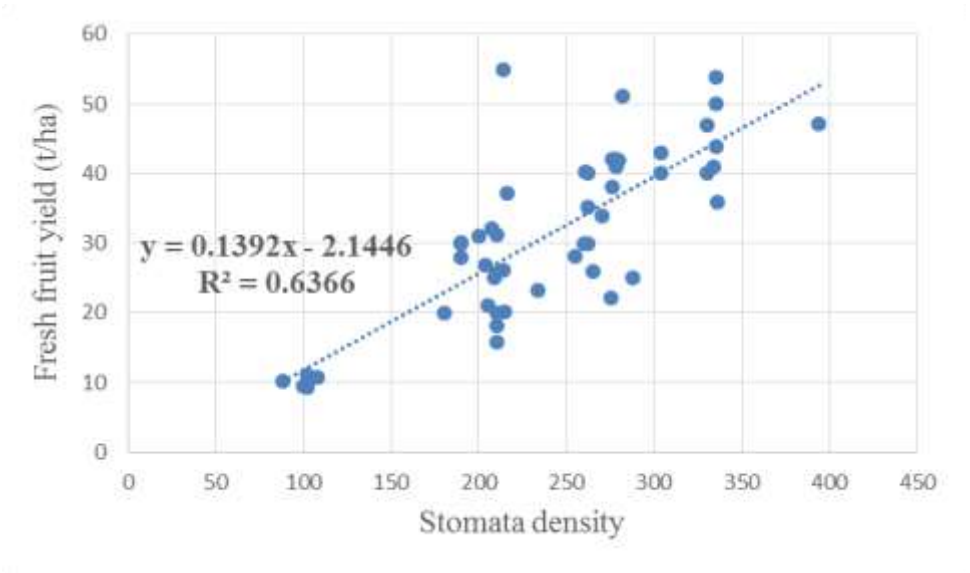

Figure 3: Scatter plot showing the relationship between stomatal density and fruit yield

Discussion

Moringa leaf extract (MLE) increased tomato fruit yield. Moringa leaf extract contains zeatin (Fuglie 200) which could explain the increase in tomato fruit yield in this study. Treatment (1.5 kg L^{-1}) produced the highest fruit yield, while the control treatment (no extract) produced the lowest. The difference in moringa treatments and the control confirmed that MLE increases tomato fruit yield (Mvumi et al. 2012). Although *A. solani* NIN plants gave higher yields than the IN crops, treatment with MLE concentration of 1.5 kg L^{-1} resulted in the highest fruit yield in both crops. This implies that MLE could effectively control *A. solani.*

MLE promoted lamina thickness. The thicker laminae of NIN MLE treated plants than IN MLE treated plants could have been due to lack of infection in NIN plants, whereby whole leaf photosynthesis was taking place, leading to improved stomatal conductance,

and increase palisade mesophyll height (leaf thickness) and stomatal density in tomato leaves.

MLE increased stomatal densities of tomato leaves. Stomatal density is closely associated with leaf development (Yang et al. 1995). Stomatal densities were lower in diseased plants than in healthy ones except for the IN tomatoes which received 1.5 kg L^{-1} MLE treatment, which showed the greatest stomatal density. This implies that MLE has a revitalizing effect from zeatin and could probably effect cell division which result in growth of plant organs such as leaves (Siddhuraju and Becker 2003; Yasmeen et al. 2012) of even IN plants, causing them to greatly recover.

Increase in stomatal size with increase in MLE concentration applied to leaves was also noted. Stomatal reduction, as was in the negative control of IN leaves, causes a decrease in the palisade mesophyll size. This is signaled by a decrease in photosynthesis, metabolic disruption of photosynthetic processes (Farquhar and Sharkey 1982), and consumption of assimilates by the pathogen. Adding this up, it leads to decrease in tomato yield. Increase in stomatal size has vice-versa effects.

The strong positive correlation between yield and lamina thickness, stomatal density and stomatal size shows that enhancing stomatal size or density increases fruit yield. These types of significant correlations between stomatal features and crop yield were also observed and reported in different crops (Yousufzai et al. 2009; Aminian et al. 2010). Results of the current experiment agree well with a previous report by Al Afas et al. (2005).

Conclusion

The study revealed that moringa leaf extract has potential to increase fresh fruit yield, lamina thickness, stomatal density and stomatal size, thus influencing tomato productivity. Furthermore, it showed potential to improve productivity of leaves infected by pathogenic organisms (such as *A. solani*) as portrayed by increase in the stomatal density.

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