

EFFECT OF MACRO AND MICRO NUTRIENTS ON SEVERITY OF MAIZE LETHAL NECROSIS DISEASE AND MAIZE GRAIN YIELD

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Abstract

To determine the impact of macro and micro nutrients on severity of maize lethal necrosis (MLN) disease and maize grain yield, a greenhouse and field experiments were conducted. In greenhouse experiment, macro and micro nutrient treatments namely N+P+K, N+P (-K), P+K (-N), N+K(-P), N+P+K+ micronutrients, N+P+K+ micronutrients+ secondary elements and control were tested on plants subjected to viral inoculation. Inoculation treatments with sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV), combined SCMV+MCMV, and control was done at 21 days after emergence. The same fertilizer treatments were tested in the field but the plants were left to natural viral infection. Application of NPK+ micronutrients+ secondary nutrients and NPK+ micro nutrients significantly reduced the severity of MLN disease symptoms and increased crop yield. This indicates that integrated use of mineral fertilizers is critical in improving current maize grain yield levels and reducing crop loss due to MLN disease.

Key Words: Grain yield, fertilizer, inoculation, maize chlorotic mottle virus, sugarcane mosaic virus.

1. Introduction

Maize lethal necrosis (MLN) disease poses a serious threat to maize production worldwide (Bockelman et al., 2000). Combined viral infection of maize plants with maize chlorotic mottle virus and sugar cane mosaic virus results in the MLN disease in Kenya and other countries. MCMV and SCMV are both readily transmitted mechanically by maize thrips and aphids, respectively. The viruses are also known to be seed transmitted (Cabanas et al., 2013). Maize Chlorotic Mottle Virus (MCMV) was initially identified in Peru and later in several parts of the United States. It is therefore a new virus in Africa (Niblett and Claflin 1978; Uyemoto, 1983; Xie et al., 2011).

Maize lethal necrosis disease inherently affects all maize varieties culminating into chlorotic mottling of the leaves, severe stunting, necrosis, decreased grain yield of maize and eventually plant death (Wangai et al., 2012). Environmental factors like low soil fertility aggravates insect pest infestations thereby increasing the incidence of insect vector transmitting viral diseases (Csinos et al., 1989). Soil fertility may affect the severity of some infectious diseases of maize like MLN disease. Therefore, fertilizing to maintain soils at highly productive levels produces vigorous plants

that do not die prematurely (Saddiq et al., 2017). Balanced application of secondary and micro nutrients can have significant effects on crop yields in sub-Saharan Africa (Vanlauwe et al., 2014). The secondary and micro nutrients has however received less interest than the macro nutrients like N, P and K as depicted by the fact that most fertilizer subsidy programs primarily focus on N, P and K fertilizers (Siamachira and Mashango, 2015). Significant reduction in maize yields up to 40% resulting from Zinc deficiencies have been previously reported by the International Zinc Association (Landon, 1991)

In Kenya, Bomet is one of the Counties that recently experienced huge losses due to MLN disease, Some farmers have experienced up to 90% crop loss (Wangai et al., 2012). In most parts of Kenya, including Bomet county, farmers tend to use a blanket fertilizer regime that does not take into account varying conditions and agronomic practices within the agricultural landscapes. This results in great variability in yield, locally or within districts (NAAIAP/KARI, 2014). Low fertilizer efficiency, current blanket fertilizer recommendations, inappropriate farming practices such as lack of crop rotation, cultivation down the slope, inadequate knowledge on crop requirements and soil characteristics, and the non-replenishments of nutrients other than N, P and K may aggravate the prevalence of MLN disease and limit crop production (Kebeney et al., 2014). In Bomet county, Sotik Sub county, the inherent soil nutrients are not adequate to supply the required plant nutrition throughout the growth and development phases of maize. This makes supplementary fertilizer inputs, especially P and K absolutely necessary. The Sub County also requires application of fertilizer with micro-nutrients such as copper, zinc, Boron and Manganese (NAAIAP/KARI, 2014). Therefore, the objective of the study was to determine the effect of macro and micro nutrients on severity of maize lethal necrosis (MLN) disease, Sugarcane mosaic virus, Maize chlorotic mottle virus and maize grain yield.

2. Materials and methods

2.1 Green house experiment

2.1.2 Experimental design and treatment

A greenhouse was carried out during the long and short rains in the 2014/2015 growing season at the University of Nairobi's Kabete Field Station. Kabete is located on latitude of 1.2524⁰S and a longitude of 36.3709⁰E and 1550 meters elevation above the sea level. The nutrient management treatments comprised: Control (no nutrient applied), NPK, NP(-K), PK(-N), NK(-P), NPK+ micronutrients, and NPK+ micronutrients +secondary elements. Plants were also subjected to inoculation with MLN disease causing viruses. Virus inoculation treatments were: Control (no viral inoculation), inoculation with Sugarcane Mosaic Virus (SMCV), inoculation with maize chlorotic mottle virus (MCMV) and inoculation with combined SMCV+MCMV viruses (MLN disease). Treatments were laid out in a randomized complete block design with a 7 x 4 factorial arrangement that was adopted for the greenhouse experiment. Nutrient management rates used in the study were 100 kg N/ha, 60 kg P/ha 60 kg K/ha, 10 kg/ha for secondary elements and 5 kg/ha for micro nutrients (AFSIS diagnosis protocol, 2011). The N, P, K, secondary elements and micronutrients were supplied by urea, triple super phosphate (TSP), Muriate of potash (MOP), Mavuno plus and Omex foliar feed, respectively. The crop was inoculated 21 days after planting at 4-6 leaf stage using the CIMMYT procedure (CIMMYT, 2012). Each treatment was administered to 5 maize

plants grown in a 20 cm diameter pot with a volume of 3.8 liters. Pots were equally filled with sterilized, natural forest soil consisting of 70% loam soil and 30% gravel.

2.2 Field experiment

2.2.1 Experimental site

Bomet site is located on latitude of 0.8000⁰S and a longitude of 35.2333⁰E and 1981 meters elevation above the sea level. The rainfall in Bomet county is described as bimodal and ranges between 1100 mm and 1500 mm per annum. The mean monthly temperature in the county is 18 ⁰C. The soils are mainly acidic humic topsoil described as nitosols with moderate to high fertility and are usually somewhat excessively drained, shallow dark brown, very friable, rocky sandy to clay loam (ASDSP, 2014). Soil analysis was performed to determine the pH of the soil and fertility.

2.2.2 Experimental design, treatments and crop husbandry

Treatments were laid out in a randomized complete block design, replicated three times. They comprised: Control (no nutrients applied), NPK, NP(-K), PK(-N), NK(-P), NPK+micronutrients and NPK+micronutrients + secondary elements. The rates and sources were as described in section 2 sub section 2.1. Maize variety H614 was planted with a spacing of 75 cm between rows and 30 cm within rows in a plot of size 1.5 m x 3 m. Number of rows per plot was four and data was collected on the two inner rows. Two seeds per hill were sown and thinning done three weeks after emergence. The trial was surrounded by three continuous border rows. The field was kept clean by weeding manually and harvesting was also done manually. Bulldock insecticide containing Beta-cyfluthrin 0.5g/kg was applied by putting a pinch into each funnel, four weeks after emergence to control stalk borer. The crop was then left for natural infection by the MLN disease causing viruses.

2.3 Data Collection

Data was collected weekly in the greenhouse experiment on the severity of the maize lethal necrosis disease (MLND) two weeks post inoculation for a period of six weeks. And bi weekly in the field experiment until crop maturity. Maize plants were evaluated through visual assessment of symptoms of the infected plants on a 1-5 disease severity scale (CIMMYT, 2015). Other data collected included: number of diseased plants per treatment, plant height in cm (height from soil surface to insertion point of flag leaf), leaf length in cm of 5 longest leaves (mean length in cm) and leaf width in cm for the widest leaves (mean width in cm). Other data from the field experiment collected in each plot containing 22 maize plants were: the number of ears harvested (EH), number of diseased ears (DS) showing symptoms of MLN disease, total weight of cobs harvested and yield in t/ha.

2.4 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using Genstat Statistical package. The differences among the treatment means were compared using the Fisher's protected less significant difference test at 5% probability level. Correlation analyses were done using the same program.

3. Results and Discussion

3.1 Effect of fertilizer application and inoculation on severity of Sugarcane Mosaic Virus (SCMV), Maize Chlorotic Mottle Virus (MCMV) and SCMV+ MCMV (Maize Lethal Necrosis) (MLN) viruses

Fertilizer application, virus inoculation and their interaction had significant effects on disease severity at ($P \leq 0.01$) (Table 1). Plants supplied with fertilizers had significantly lower disease severity than plants that did not receive any fertilizer. Plants supplied with NPK+ micronutrients and NPK+ micronutrients+ secondary nutrients had significantly lower disease severity than plants supplied with NK, NP, PK and NPK when inoculated with SCMV, MCMV and SCMV+MCMV treatments. This can be attributed to the fact that plants with an optimal nutritional status have the highest tolerance to pests and diseases (Dordas, 2008). Studies have shown that maintaining the rigidity and stabilizing the cell wall are ways that elements like Boron (B), Manganese (Mn) and Calcium (Ca) apply to significantly prevent pest and disease attack in plants (Jordan-Meille 2009). Susceptibility rises as nutrient concentrations deviate from this optimum. In the experiment, severity of disease was most observable on plants that lacked K followed by those that lacked N. According to Sarwar, (2012) several studies have shown that susceptibility to infections are mostly reported in plants deficient in K. Optimum Potassium status not only affects plant growth and development, but also plays a major role in plant resistance to diseases (Elmer, 2006) (Huber, 1985).

Generally, symptom expressions were more observable on plants inoculated with combined infection of MCMV+SCMV (MLN) irrespective of the fertilizer treatment, the plants showed extensive systematic necrosis on the leaves. This is in agreement with Miano (2014) who reported that symptoms of MLN are much more severe than the symptoms of either MCMV or the potyviruses alone. Varied soil nutrition, significantly affected the expression of MLN (combined MCMV and SCMV), MCMV and SCMV symptoms on the maize plants. Severities of symptoms were highest where no soil nutrients were applied. Several studies have shown that plants suffering nutrient stress are less vigorous and more susceptible to a variety of diseases than plants given adequate nutritional regimes. This however can be mitigated by balanced nutrient management and other cultural practices like application of chemicals (Kebeney et al., 2014).

3.2 Effect of fertilizer application and inoculation with SCMV, MCMV, combined SCMV+MCMV (MLN) on the growth parameters of maize.

Fertilizer application, virus inoculation and their interaction had significant effects at ($P \leq 0.01$) on plant height, leaf length and leaf width (Figure 1). Plant height, leaf length and leaf width varied in the order NPK+ micro nutrients + secondary nutrients > NPK+ micro nutrients > NPK > NP > NK > PK > no-fertilizer (control). This demonstrates the beneficial effects of combined application of major nutrients (N, P and K), secondary nutrients (Ca, Mn and Mg) and micronutrients (Boron (B), Zinc (Zn) and Sulfur (S)). The positive performance in maize growth can be attributed to secondary elements like Magnesium (Mg) which has a vital role in growth and development of plants Mehmet et al., (2015). The study found that application of PK alone had no significant effect on the plant

height, leaf length and leaf width relative to non-fertilized plants, highlighting the importance of balanced nutrient management. Treatments of NK and NP on the other hand significantly increased plant height, leaf length and leaf width compared to PK because of the role of N in plant growth and development. According to Biradar et al., 2012 nitrogen is critical in enhancing plant's vigor in growth, leaf enlargement and production; thus, its deficiency stunts growth resulting into deformed leaves. Virus inoculation however, significantly reduced plant height, leaf length and leaf width. Under NPK treated plots, inoculation with either, MCMV or combined SCMV+MCMV significantly reduced plant height relative to zero- inoculation. Generally, inoculation with SCMV alone had significantly less effect on plant height, leaf length and leaf width than inoculation with MCMV alone which had significantly less effect on plant height than inoculation with combined SCMV+MCMV. Stunted growth in maize has been attributed to MLN (SCMV+MCMV) leading to yield losses in more than 25,000 ha of maize (Isabirye and Rwomushana, 2016)

3.3 Effect of fertilizer application on the severity of MLN disease, yield and yield components of maize.

In the field experiment where maize was left to natural MLN disease causing virus infection, treatments NPK, NPK+ micro nutrients and NPK+ micro nutrients+ secondary nutrients significantly reduced MLN severity relative to the NP and no-fertilizer (control) treatments at ($P \leq 0.01$) in both seasons at 18 and 20 Weeks after emergence. In a study on the role of nutrients in controlling plant diseases, Graham and Webb 1991 observed that nutrients can affect disease tolerance. They reported that tolerance is measured in terms of the crops ability to maintain its own growth or yield in spite of disease infection. Application of fertilizer also significantly reduced the number of diseased ears per plot in both seasons (Table 2 and Table 3) Treatments NPK+ secondary nutrients+ micronutrients, NPK+ micronutrients and NPK had significantly lower number of diseased ears than NP and control during both seasons. The number of diseased ears was also significantly lower in plots that had received NK and PK compared to plots treated with NP and those that had no fertilizer control treatment during both seasons. There was no significant difference in number of diseased ears for plots that had received NP and plots where no fertilizer was applied in both seasons.

Fertilizer application also had a significant effect on the cob weight and yield in t/ha in plots that had received NPK+ secondary nutrients+ micronutrients relative to PK, NP and control plots. Treatments NPK+ micro nutrients and NPK also had significantly higher cob weight compared to NP and no fertilizer (control) treatments. Unbalanced application of the nutrients on the other hand had low beneficial effects to the yield of maize. This was observed on plots that had singly been treated with NK, NP and PK. Wangai et al 2012 and Adams et al 2013 observed that MLN disease can seriously affect maize yields since the infected plants are mostly barren with deformed ears forming little or no seed set. Application of NPK alone also yielded less than plots that had been treated with additional micronutrients. It has been observed that fertilizer application in Kenya is predominantly in favor of macro nutrients N and P only, while K use is restricted to a few crops like sugarcane and potatoes whose K requirements are excessive (Imtiaz et al., 2010). Manganese (Mn), copper (Cu), boron (B), iron (Fe), molybdenum (Mo), zinc (Zn) nickel (Ni) and chlorine (Cl) are the eight trace elements considered essential for higher plants. Their absence or

inadequacy in the soil leads to reduction in yields and impairment of the quality of crop products (Alloway, 2008). Lisuma et al, 2006 also reported that the use of micro nutrients contributed to significant increase in number of grains per cob, 100 grain weight and yield in t/ha when applied in combination of macro nutrients as compared to conventional fertilization which lack micro nutrients

4. Conclusion

Application of macronutrients, secondary elements and micronutrients significantly decreased the severity of the maize lethal necrosis disease in both experiments at ($P \leq 0.01$) compared to the control plots and sole applications of NK and NP. Addition of secondary and micro nutrients also significantly reduced the number of diseased ears per plot. There was also significant differences at ($P \leq 0.01$) of yield in t/ha in plots that had balanced application of NPK+ secondary +micro nutrients compared to plots where no nutrients was applied (control) and the single treatments of the macronutrients NK, NP, PK and NPK. This is an indication that balanced fertilizer application can mitigate against MLN disease and improve maize yields in maize production

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Tables and figures**Table 1:** Effect of fertilizer application on the severity of MCMV, SCMV and MLN viruses on maize plants at 6 weeks post inoculation, greenhouse experiment in Kabete

Inoculation (I)	Fertilizer regime (F)							mean
	Control	NK	NP	PK	NPK	NPK+M	NPK+M+S	
Uninoculated	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
SCMV	4.0	2.7	3.0	3.0	2.3	1.7	1.4	2.6
MCMV	4.2	2.7	3.3	3.4	2.7	1.9	1.5	2.8
SCMV + MCMV	4.5	3.5	3.9	4.0	3.3	2.0	2.0	3.3
Mean	3.5	2.5	2.8	2.9	2.3	1.7	1.5	
P-value (F)	< 0.001							
P-value (I)	< 0.001							
P-value (F * I)	< 0.001							
LSD _{0.05} (F)	0.3							
LSD _{0.05} (I)	0.2							
LSD _{0.05} (I*F)	0.7							
CV%	17.3							

SCMV- sugarcane mosaic virus, MCMV- maize chlorotic mottle virus, control (no fertilizer added), NPK+M- NPK+ micronutrients, NPK+M+S- NPK+ micronutrients + secondary nutrients.

Disease scale (1-5): No symptoms seen on leaves (1); Very mild chlorotic mottling on 1-2 leaves (2) generalized chlorosis (3); Excessive chlorotic mottling on lower leaves and necrosis of newly emerging leaves (4); Complete plant necrosis (5).

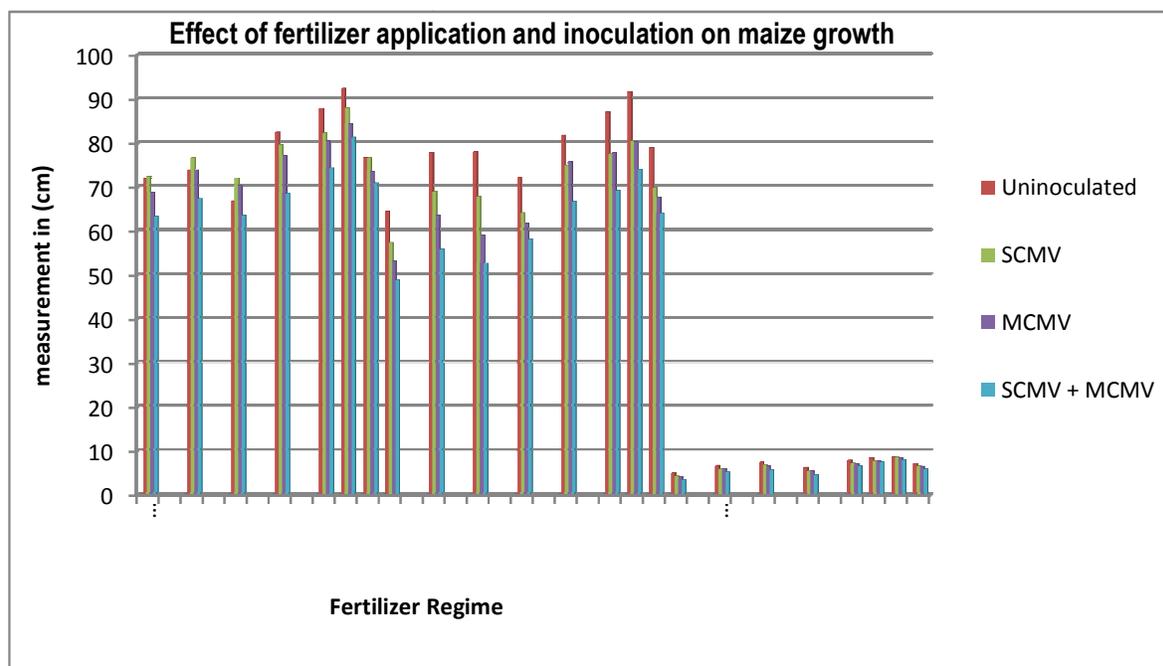


Figure 1: Effect of fertilizer application and inoculation on the growth parameters of maize

Table 2: Effect of fertilizer application on the severity of MLN disease, yield and yield components of maize, field experiment in Bomet county 2013 long rains.

Fertilizer regime	MLN severity			Agronomic traits			
	16WAE	18WAE	20WAE	Ears harvested	Diseased ears	Cob weight(Kg)	Yield (t/ha)
Control	1.3	1.8	2.0	19.3	12.0	2.6	5.8
NK	1.0	1.0	1.0	17.7	6.0	3.7	8.2
NP	1.5	1.5	1.6	17.3	10.4	3.2	7.1
PK	1.2	1.2	1.2	17.7	7.2	2.9	6.5
NPK	1.0	1.0	1.1	19.3	6.7	4.0	8.9
NPK +Micro elements	1.0	1.0	1.0	18.0	5.7	3.9	8.7
NPK+Sec+Micro	1.0	1.0	1.0	19.7	4.7	4.2	9.3
P-value	0.005	0.001	0.001	0.855	0.001	0.011	0.001
LSD_{0.05}	NS	0.3	0.4	NS	3.1	0.9	0.7
CV%	12.9	12.2	12.2	14.2	23.4	13.7	5.2

Disease scale (1-5): No symptoms seen on leaves (1); Very mild chlorotic mottling on 1-2 leaves (2) generalized chlorosis (3); Excessive chlorotic mottling on lower leaves and necrosis of newly emerging leaves (4); Complete plant necrosis (5).

Table 3: Effect of fertilizer application on the severity of MLN disease, yield and yield components of maize, field experiment in Bomet county 2013/2014 short rains.

Fertilizer regime	MLN severity			Agronomic traits			
	16WAE	18WAE	20WAE	Ears harvested	Diseased ears	Cob weight(Kg)	Yield (t/ha)
Control	1.5	2.0	2.0	19.1	10.7	2.9	6.4
NK	1.3	1.5	1.7	17.0	6.7	3.5	7.8
NP	1.5	1.7	1.8	17.3	10.5	2.5	5.6
PK	1.5	1.7	2.0	18.0	8.0	3.2	7.1
NPK	1.3	1.5	1.7	17.7	7.3	3.3	7.5
NPK +Micro elements	1.2	1.3	1.7	18.0	5.0	3.7	8.2
NPK+Sec+Micro	1.0	1.3	1.5	19.6	6.7	4.0	8.9
P-value	0.020	0.001	0.001	0.281	0.001	0.032	0.001
LSD_{0.05}	NS	0.3	0.4	NS	3.1	1.1	0.7
CV%	14.2	12.5	12.4	11.8	23.7	15.1	5.7

Disease scale (1-5): No symptoms seen on leaves (1); Very mild chlorotic mottling on 1-2 leaves (2) generalized chlorosis (3); Excessive chlorotic mottling on lower leaves and necrosis of newly emerging leaves (4); Complete plant necrosis (5).

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