J. appl. Res in Plant Sci. Vol. 3(2), 229-235, 2022 eISSN 2708-3004; pISSN: 2708-2997
www.joarps.org https://doi.org/10.38211/joarps.2022.3.2.28

Considering Leaf Extract of Miracle Tree (*Moringa Oleifera* L.) and Potassium Nutrition for Contending Cotton Leaf Curl Virus (CLCuV) Disease of Cotton (*Gossypium Hirsutum* L.)

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Article Received 27-01-2022, Article Revised 25-04-2022, Article Accepted 16-05-2022

Abstract:

Cotton leaf curl virus disease (CLCuV) is a devastating biotic factor that imposed crop productivity a huge loss. To battle this issue this study was assessed in 2019 for the evaluation of the response of leaf extract of miracle tree (*Moringa oleifera* L.) and potassium (K) nutrition to mitigate the plague of CLCuV disease in cotton variety CRIS-129. Treatments have consisted of the various K dosses Viz., 0, 100, and 150 kg K_2SO_4 $ha^{-1,}$ and 3% leaf extract of moringa was sprayed at 30, 60, and 90 days after emergence. Results regarding yield components i-e boll weight (g) and seed index (g) were increased by increasing K application and foliar spray of moringa leaf extract but statistically non-significant ($p<0.05$). However, seed cotton yield and K contents in cotton leaves were significantly increased with all the treatments. The maximum seed cotton yield (2620 kg ha^{-1}) was achieved with 150 kg K_2O ha^{-1} application and it was increased by about 52% over the control treatment. In the case of incidence % of CLCuV disease, both K nutrition as well as foliar spray played a significant role and CLCuV intensity reduced with increasing rate of K doses and foliar sprays.

Keywords: Cotton (*Gossypium hirsutum* L.), Potassium (K) Nutrition, Miracle tree (*Moringa oleifera* L.), CLCuV, Seedcotton yield

Introduction

In Pakistan among the most terrible devastating biotic factor imposed on cotton crop productivity is cotton leaf curl virus disease (CLCuV) accompanied by begomovirus components (Amrao *et al*., 2010). Which has done huge losses to the cotton crop, a coinage of our economy (Abbas *et al*., 2020). It first appeared in Multan, Punjab in 1967 (Nawaz *et al.*, 2019), and huge losses were recorded in the cotton crop. Sindh was well-thought-out to be free from this pandemic malady until 1996 when it was reported from Kamo shaheed, Taulka Obaro, District Ghotki, Sindh (Panhwar *et al*., 2002; PCCC, 1996; Mansoor *et al*., 1998). After its recognition in Sindh, many surveys were conducted to confirm its presence (Saif *et al*., 1997; Panhwar *et al.*, 2001; Mansoor *et al*., 2006). Whitefly (*Bemisia tabaci*) is the known trajectory for its transmission (Akhtar *et al.*, 2014). Treasured exertions have been done by different scientists to clash this disease, but this problem is unsolved even after three decades and needs further dynamism (Iqbal *et al*., 2021). It can be decreased with the use of impervious varieties (Farooq *et al*., 2011), and management practices (Panhwar *et al.,* 2018*)*. A major concern is to induce the capability of plants to face effectively the unfavorable situation. In this scenario, plant nutrition can play a treasured role (Mansoor *et al*., 1998; Marschner, 1995). Crop nutrition can minimize the incidence of disease which in turn can improve the plant establishment and yield (Usherwood *et al.*, 2001). It can be a best management practice to grapple with CLCuV as it is an eco-friendly approach (Zafar *et al*., 2010). In plant nutrition for cotton production potassium (K) is considered the most imperative plant nutrient ((Marschner, 1995; Kubar *et al*., 2016). Moreover, cotton is an extra sensitive crop in the sense of K deficiency than other major crops (Oosterhuis *et al*., 1997; Hassan *et al.*, 2014). Per plant resistance and vigor potassium (K) is known to be an effective nutrient (Usherwood *et al.*, 2001; Oosterhuis *et al*., 2013; Panhwar, 2022). Its deficiency can cause the stomata to open for a long time that may welcome viruses to penetrate from injectable sites as in K deficient plant wound healing is slow (Amtmann *et al*., 2008; Pervez *et al*., 2007). Amazingly, the need for cotton for nitrogen and potassium is alike (Zia-ul-Hassan and Arshad, 2010). However, the farmer community does not put consideration to include K in soil fertilization and they mostly prefer to do with N due to the lack of availability and high purchasing cost of potassium fertilizer (White, 2013). A Higher N rate with ignorance of recommended rate may appeal to whitefly that causes greater disease problems in cotton including CLCuV (Zafar *et al*., 2010) because an increased N level raises the ammonia, free amino acid, and soluble carbohydrates (sugars). Moreover, energy for amide and protein formation decreases which may provide food to attract more whitefly (also other sucking pests) for feeding and reproduction and also for viruses (Athar *et al*., 2011). However, modern cotton cultivation requires more potassium than preceding cultivars moreover, cotton removes more K from soil that has auxiliary added that's why we should not be sure of natural potassium fertility of the soil (Pabuayon *et al*., 2020). In addition, another approach that can be suitable in this connection is a foliar spray of leaf extract of miracle tree (*moringa oleifera* L.). Moringa is a multipurpose tree that why is known as a miracle tree (Sabina and Bhusal, 2020). Abundant literature is available showing the importance of foliar fertilization. It is a good appendage to soil application. Leaves extract of miracle tree (MLE) contains plant growth promoter zeatin a cytokinin (Afzal *et al.*, 2019) whereas cytokinins are known to stimulate or inhibit numerous plant growth processes and developmental attributes including seed germination. These physiological processes are regulated endogenously as well as when applied exogenously in the intact plant (Fageria *et al*., 2009). In addition to zeatin it is rich in antioxidants also therefore the defense system of the cotton plant is likely to be improved in response to natural as well as man-made stresses (Foidl *et al*., 2001). Therefore, it can be assumed that transmission of zeatin can happen to the upward germ during the paused stage of germination and promote the activity (Farooq *et al.,* 2010), eventually improving germination and likewise, these possessions can be absorbed by the leaf as a foliar spray (Fageria *et al*., 2009). In addition, leaf extract of the miracle, tree is a good repellent to insect pests (Panhwar *et al.,* 2021a). In the understanding of this scenario, it was expected that the application of K nutrition and foliar spray of leaf extract of miracle tree could be considered to alter the various functional processes of the cotton plant resulting in reducing the incidence of CLCuV. Thus, the main purpose of the present tryout was to evaluate the role of potassium nutrition and foliar spray of leaf extract of miracle tree in decreasing the impairment of this plague in cotton.

Material and Methods

Experimental site and conditions: An experiment was established at Central Cotton Research Institute (CCRI), Sakrand, Sindh, Pakistan (Latitude 26.099693^0, Longitude 68.299645^0). The experimental trial plan was arranged in a randomized complete block design (RCBD) replicated four times. The cotton cultivar CRIS-129 was selected. Various K doses viz. 0, 100, and 150 kg K_2O ha^{-1} were applied through sulfate of potash (K_2SO_4) comprising 50% K_2O and full phosphorus in the form of diammonium phosphate (DAP) comprising 46% P_2O_5 and considered the rate as 60 kg P_2O_5 ha^{-1}. Both were incorporated thoroughly into the soil before sowing the cotton crop. Nitrogen was provided to the plant in a triad manner in equal parts. One part was given at the beginning stage, the second dose at blooming, and the final remaining part at the flowering stage. The delinted seed was planted on 19th May 2019 ensuing the bed and furrow method. Nearly all recommended practices were followed. Weather condition is presented in Figure. 1 (A, B and C).

Miracle plant leaf extract for foliar spray: Fresh and young leaves of miracle plant (moringa) were detached from the tree. Leaves were rinsed thoroughly, and the sap from the leaves was extracted using a pestle mortar. Then straining was done through a fine cotton cloth and diluted with tap water used for irrigation to the crop to prepare a 3% solution (Panhwar *et al.*, 2021b).

Soil characteristics: Composite soil samples from the surface up to 15cm deep, 15cm to 30cm, and 30cm to 60cm deep were taken before initiation of sowing and analyzed for designated Physico-chemical characteristics using standard protocols (Ryan *et al*., 2001). The soil was silty clay loam and classified as Pacca soil series (fine, mixed, hyperthermic Ustollic Camborthids). The soil was alkaline in reaction (pH 7.9-8.1), free from surplus salts (EC 1.47-1.48 dSm^{-1}), moderately calcareous (12.0 % of $CaCO_3$), and low in organic matter (0.78 %). Results are summarized in Table.1.

Growth and yield parameters: For the sake of meaningful data regarding the growth and yield plant height cm and the number of fruits (bolls $plant^{-1}$) at flower initiation (60 DAS), boll formation stage (90 DAS), and crop maturation (120 DAS). A total of ten plants were selected from each treatment randomly for taking the data. The crop was harvested distinctly and recorded the yield parameters of boll weight (g), seed index (g^{-100} seed weight), and seed cotton yield (kg ha^{-1}).

Plant analysis: Leaf K concentration was diagnosed using a standard procedure (Ryan *et al*., 2001).

Incidence of CLCuV disease: Incidence of CLCuV disease attack during the growth period was observed from each treatment on monthly basis. CLCuV disease severity was measured through a disease rating scale (Table.2) described by Akhtar *et al.,* (2010); Farooq *et al.,* (2011).

Statistical analysis: The data were analyzed using the software Statistix. USA. Ver. 8.1. Mean separations were done by SED, LSD, and Tukey's pairwise test. All the data were subjected to the analysis of variance (ANOVA) technique for an RCBD as defined by Steel and Torrie (1980). The means for each parameter were further separated and compared by using the least significant difference (LSD) test at a 5% level of probability.

Results and Discussion

CLCuV incidence: Generally nutrient deficient plants are less vigorous and more susceptible to diseases under unbalanced fertilizer practice. In this regard, all nutrients are important to combat plant diseases but the impact is specific concerning the certain nutrient. In this study, potassium nutrition found a better approach to reducing the incidence of CLCuV in the cotton crop with well-balanced fertilization. The peak rank of CLCuV was perceived in control and the bottom-most was measured where the potassium content of 150 kg K_2O ha^{-1} was applied (Table. 5).

The disease incidence was recorded following the criteria described by Panhwar *et al*., (2002); Akhtar *et al.,* (2010), and Farooq *et al.*, (2011). It was found that CLCuV disease tolerance was increased with increasing levels of K might be due to lower K supply and shortened the asset of the epidermal wall (Datnoff *et al*., 2006) as described earlier. In K deficient plants the loss of cell wall power may be an issue helping penetration of parasites being as a barrier to facilitating penetration of the pathogens. In these plants, stomata are unbolted for an extensive period than needed resulting in the increased chances of disease perception. It indicates that cell wall habituation and opening of stomatal patterns are linked to diseases. While Amtmann *et al*., (2008) state that changes in enzymatic activities and metabolite concentration in cells facilitate pathogen penetration but the connection between plant nutrition and disease confrontation is quite unclear. Marschner, (1995) further reported that the K scare plants had reduced protein production. Therefore, they accrue amides that are diet for invading pathogens so N and K balance is important in disease exposure (Usherwood *et al.*, 2001). Leaves extract of miracle plant (MLE) contains plant growth promoter zeatin a cytokinin (Yasmeen *et al.*, 2016) whereas cytokinins are known to stimulate or inhibit numerous plant growth processes and developmental attributes that enhances resistance against pest and diseases. These physiological processes are regulated endogenously as well as when applied exogenously in the intact plant (Fageria *et al*., 2009). In addition to zeatin it is rich in antioxidants also therefore the defense system of the cotton plant is likely can be improved in response to natural as well as man-made stresses (Foidl *et al*., 2001). Spraying through leaves extract of miracle plant has proved some prominent effects such as a longer and more vigorous lifetime and more resistance to pests and diseases (Panhwar *et al*., 2021a), this validates that it has hastened the resistance of the cotton plant. In addition, MLE is rich in potassium content also which is known as stress defending nutrient (Basra *et al*., 2011).

Growth parameters: Growth traits of cotton plants and data regarding plant height and boll formation per plant at 60, 90, and 120 days after sowing (DAS) were recorded (Table 3). The results indicated that the plant height was increased with all the treatments at 90 to 120 DAS. A higher amount of potassium (T4 -150 kg K_2O ha^{-1}) significantly increases plant height at 90 DAS and then is statistically non-significant. Whereas boll formation was augmented significantly excluding 90 DAS with entirely treated units reaching up to 120 DAS. The maximum (5.77, 29.31, and 36.53 $plant^{-1}$) values were recorded from T_4, and minimum (2.03, 20.91, and 27.13 $plant^{-1}$) values were observed from control (T_1). The increased growth parameters are attributed because potassium nutrients and foliar spray play a significant role in the growth and development of the cotton plant. K deficiency markedly hinders the growth of the cotton plant (Zia-ul-Hassan *et al.*, 2021). Likewise, MLE is rich in potassium content (Basra *et al*., 2011).

Yield parameters: In this finding application of potassium and MLE foliar spray in cotton crops showed a significant effect on seed cotton yield. It was increased from 1721 to 2610 kg ha^{-1} and 5.2, 19.7, and 52.3 percentage higher yields when applied @ 3% MLE, 100 and 150 kg K_2O ha^{-1}, respectively above control (Table.4). Yield-related components such as boll weight and seed index were also increased as well but were statistically non-significant (Table.4). Potassium nutrition is an important prerequisite for producing a high-yielding and improved quality cotton crop. Therefore, K deficiency will depressingly influence cotton yield and lint quality.

Plant analysis: The results indicated that the concentration of potassium in leaves significantly increased with the increasing rate of potassium application from 2.15 % (T_1-0 kg K_2O ha^{-1}) to 3.54 % (T_4 -150 kg K_2O ha^{-1}) (Figure. 2).

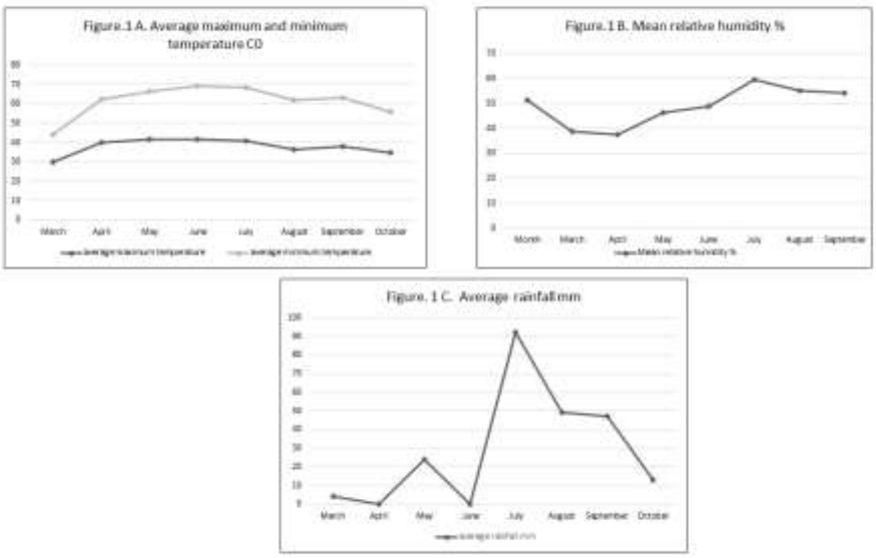


Figure. 1 A, B, and C followed average maximum and minimum temperature $C^{0,}$ Mean relative humidity %, and average rainfall mm, respectively, at CCRI, Sakrand during 2019.

Table 1. Soil characteristics of the experimental plot before sowing of cotton crop

Parameters		**Soil depth cm**		
		0-15	15-30	30-60
pH		8.1	7.9	8.0
Conductivity, dSm^{-1}		1.47	1.46	1.48
Available nutrients, mg kg^{-1}	**NO_3-N**	8.6	7.9	6.6
	P	4.1	2.2	1.7
	K	120	101	110

Table 2. Disease rating scale (symptoms rating) for cotton against CLCuV

Severity level	Visual viewpoints	Comments
0	No symptoms (completely absent)	Resistant
1	Small, scattered vein thickening	Highly tolerant
2	A large group of veins thickened and curled on top of the plant affected.	Tolerant
3	All veins thickening, enation, and severe curling or half of the plant affected.	Susceptible
4	All vein thickening, severe curling, enation, and stunted plant or whole of the plant affected and stunting	Highly susceptible

Table 3. Influence of potassium nutrition and MLE on plant height and boll formation

Treatments	**Plant Height (cm)**			**Boll Formation $Plant^{-1}$**		
	60 DAS	**90 DAS**	**120 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
Control	77.0 c	105.5	120.4	2.03 c	20.91	27.13 b
3% MLE	79.2 bc	108.6	123.2	3.43 b	23.64	29.92 b
100 (Kg K_2O ha^{-1})	84.7 ab	116.1	126.3	4.46 b	27.49	32.76 ab
150 (Kg K_2O ha^{-1})	86.0 a	117.8	128.7	5.77 a	29.31	36.53 a
LSD (p<0.05)	**6.15**	**NS**	**NS**	**1.25**	**NS**	**5.88**

DAS = days after sowing, NS = non-significant (p<0.05), MLE= miracle tree leaves extract

Table 4. Influence of potassium nutrition and MLE on boll weight, seed index, and seed cotton yield

Treatments	**Boll Weight (g)**	**Seed Index (g)**	**Seed Cotton Yield (kg ha^{-1})**
Control	3.1	6.7	1721 c
3% MLE	3.2	7.1	1810 c
100 (Kg K_2O ha^{-1})	3.3	7.7	2059 b
150 (Kg K_2O ha^{-1})	3.4	7.8	2610 a
LSD (p<0.05)	**NS**	**NS**	**195.5**

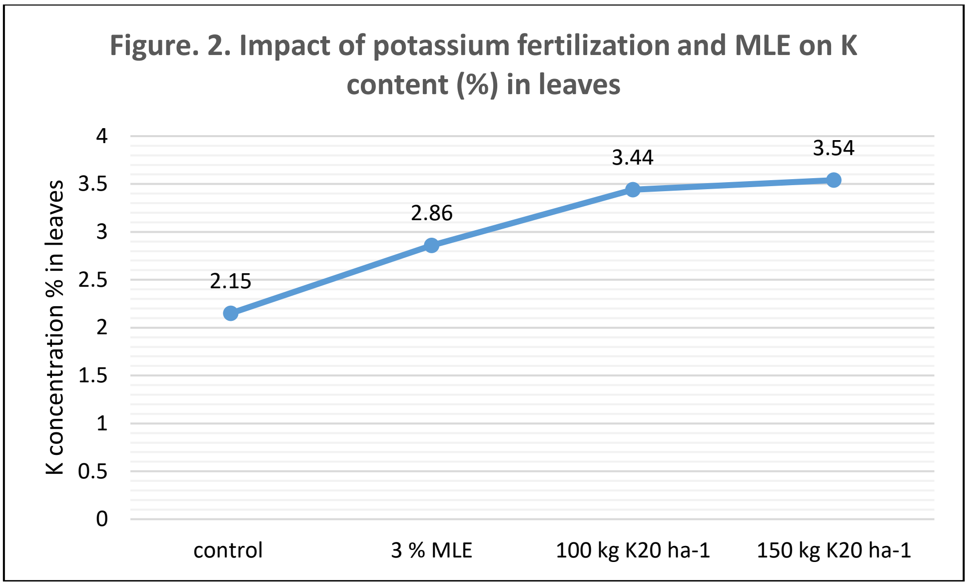
NS = non-significant (p<0.05), MLE= miracle tree leaves extract

Table. 5. Influence of potassium nutrition and MLE on Cotton Leaf Curl Disease (CLCuD)

Treatments	**CLCuV**		
	Disease (%)	**Severity***	**Index****
Control	43.36a	2.72a	29.48a
3% MLE	36.88b	2.36b	21.75b
100 (Kg K_2O ha^{-1})	29.98c	2.16c	16.18c
150 (Kg K_2O ha^{-1})	22.80d	1.74d	9.69d
LSD (p<0.05)	**0.29**	**0.01**	**0.01**

*Disease severity = Disease rating scale

**Disease index = Disease % X Disease severity / Maximum severity value (4)



MLE= miracle tree leaves extract

Conclusion

This study reports that potassium nutrition and foliar spray of moringa leaves proved a miracle in reducing the plague of CLCuV disease and increased seed cotton yield.

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