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ORIGINALARTICLE

Potential of Leaf Extract of Miracle Plant (*Moringa oleifera* L.) As Seed Priming Agent and Foliar Fertilization of Cotton (*Gossypium hirsutum* L.)

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ABSTRACT

Background: A key desire of a farmer is to produce a higher yield with low inputs. Through proper nutrient management, potential yield can be picked but mostly cotton is not grown with balanced nutrition. In this scenario, seed priming and foliar fertilizers have the advantage of quick plant responses. Therefore, a cost-effective, farmers-friendly, and full of nutrients supplement should be considered. Moringa is known as a miracle plant and its role as a seed priming agent and foliar spray has been observed in many other crops.

Objective: In this regard, present study was conducted to evaluate the effect of the leaf extract of a miracle plant (*Moringa oleifera* L.) on the growth and development of cotton.

Methodology: Two cotton varieties CRIS-585 and CRIS-342 were seeded with an experimental design of split-plot repeated thrice. The experiment included i) control ii) foliar spray of tap water iii) seed priming with leaf extract of miracle plant (MLE), iv) seed priming and foliar spray with MLE v) foliar spray with MLE. The extract was 3% solution. Cotton seeds were soaked to complete seed priming for 3 hours. After emergence 30, 60, and 90 days were selected to apply the foliar spray.

Results: It is proved from the application of leaf extract that seed priming, as well as a foliar spray on cotton crops, had significant (p=0.05) effects on its yield and related parameters. The highest boll weight (3.8 g) and seed cotton yield (3844 kg ha^{-1}) were recorded when a combined application of seed priming and foliar spray was done followed by the sole application of the foliar spray. While the minimum values of boll weight and seed cotton yield were found from the control where no seed priming or foliar spray was done.

Conclusion: It is concluded that the application of leaf extract of miracle plants as seed priming and foliar spray in cotton crops significantly affect seed cotton yield and its traits.

Keywords	*Address of Correspondence	Article info.
Cotton, Miracle Plant (Moringa oleifera L.), Seed Priming, Foliar Spray, Phytohormone	*uroojpanhwar@gmail.com*	*Received: August 26, 2021* *Accepted: October 07, 2022*

Cite this article: *Panhwar BU, Keerio A, Panhwar AK, Panhwar RA, Panhwar FH, Channa AR. Potential of Leaf Extract of Miracle Plant (Moringa oleifera L.) As Seed Priming Agent and Foliar Fertilization of Cotton (Gossypium hirsutum L.). 2022; 13(2):148-156.*

INTRODUCTION

The outstanding growth and development of the cotton (*Gossypium hirsutum* L.) plant are likely in the balanced presence of water and dissolved minerals (salts) in their rhizosphere, likewise, the role of phytohormones cannot be denied. Through proper nutrient management, potential yield can be picked.[1,2] In most cases, cotton is not grown with balanced nutrition and needs some supplements. In this scenario, seed priming[3] and foliar fertilizers[4] have the advantages of quick plant responses, and it is predominantly important when the rhizosphere is unable to fully furnish the plant and the plant itself has inadequate root growth. A major wish of a cotton producer is to get a higher yield with low inputs. Here an economical, easy-to-handle, farmers-friendly, and full of nutrients supplement should be considered for foliar spray as well as seed priming. To fulfill these needs, the leaf extract of a miracle plant (*Moringa oleifera* L.) was selected as its role for seed priming and foliar spray fertilization has been observed in numerous studies.[5,6]MLE (Leaf extract of miracle plant) is a good source of a derivative (purine adenine) called zeatin of a known plant hormone cytokinin, phenols, vitamins, minerals, antioxidants and a group of companionable solutes that safeguard cells under stresses.[7,8]

Cytokinins are known to stimulate or inhibit numerous plant growth processes and developmental attributes including seed germination. These physiological processes are regulated by cytokinins endogenously as well as when applied exogenously on the intact plant.[9] In addition to zeatin, MLE is rich in antioxidants, therefore, the defense system of a cotton plant can likely to be improved in response to natural as well as man-made stresses.[10]Therefore, it can be assumed that transmission of zeatin occurs during the paused stage of germination and promotes germination eventually,[11]when these compounds can be absorbed through a foliar spray.[9]

Seed is the first step that affects and reflects the final yield. Seed emergence is a strategic factor in this regard. Therefore, it is important to ensure something better regarding its management. It is important to adopt a suitable (economical and low-risk) approach to grow more for the increasing population of Pakistan with fewer available resources (land and water) in an exceedingly hostile environment. Therefore, seed priming is considered an easy, inexpensive and eco-friendly approach, which was proposed by Harris[12] to enhance the seed performance during seed germination. It improves germination and establishment, during the phases of flowering and maturation, plant population, seedling length, plant height, and the yield of many crop species[13,14] and it also boost tolerance against abiotic stress[3], insect pests[15,16] and disease.[6] Successful seed priming is accomplished through water uptake that process occurs in three steps (uptake of moisture, origination of physiological processes, and end of germination and start of seedling) respectively.[17]

Abundant literature is available showing the importance of foliar fertilization. It is a good addition to soil application. Spraying through leaf extract of miracle plants has proved some prominent effects such as a longer and more vigorous lifetime and more resistance to pests and diseases[6]. This validates its prospective to be used as a foliar spray to hasten plant growth and development of cotton. As synthetic sources of growth-promoting hormones are costly, there is a need to explore natural sources which are reasonable, environmentally defensive farmer friendly, and realistic under natural conditions.[10] Therefore, it was decided to study the response of MLE as a priming agent and foliar supplement.

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 was arranged in a split-plot design replicated thrice. Sowing was done on 15th May 2020 of two approved cotton varieties viz. CRIS-585 and CRIS-342. Recommended doses i.e., 145 kg N ha^{-1}, 56 kg P ha$^{-1,}$ and 62 kg K ha^{-1} were applied to the soil. Potassium and phosphorus were applied at the seedbed preparation for crop husbandry and nitrogen was in the first, middle, and last splits followed by sowing, budding, and peak flowering stages. Weather condition is presented in Fig **1**, **2**, and **3** and soil analysis is presented in Table **1** as well.

All other recommended agronomic practices were done according to the requirements.

Table 1. Soil Characteristics of the Experimental Plot before Sowing of Cotton Crop.

Parameters		Results from various soil depths cm		
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}	Nitrate nitrogen (NO_3-N)	8.6	7.9	6.6
	Phosphorus (P)	4.1	2.2	1.7
	Potassium (K)	120	101	110

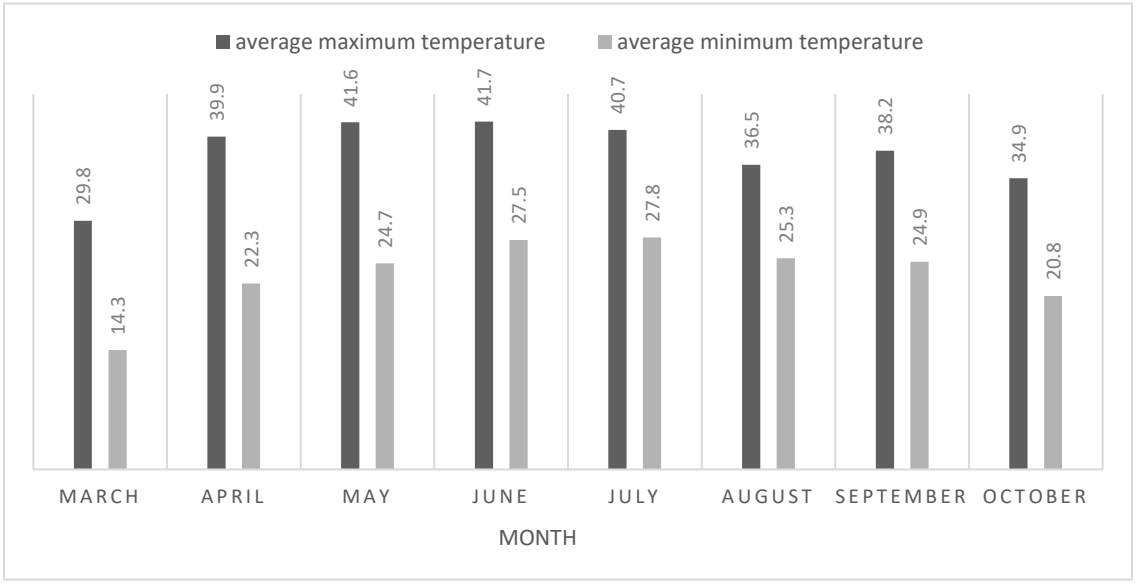

Figure 1. Average maximum and minimum temperature C^0

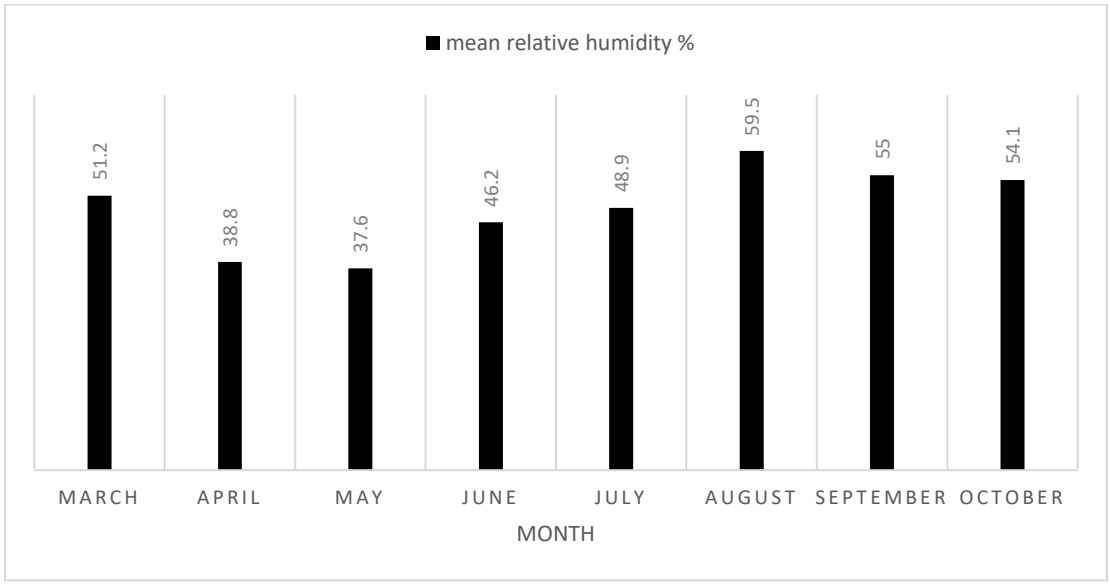

Figure 2. Mean relative humidity%.

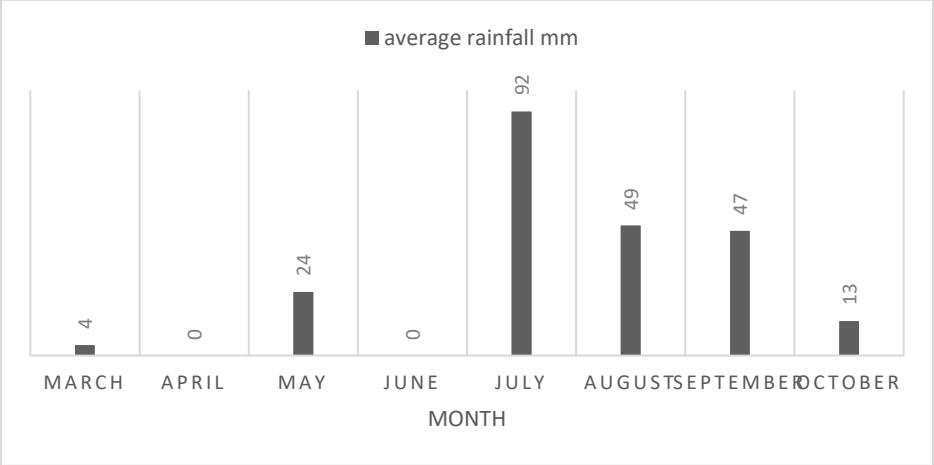

Figure 3. Average rainfall mm

Figures **1, 2,** and **3** followed the average maximum and minimum temperature $C^{0,}$ Mean relative humidity %, and average rainfall mm, respectively, at CCRI, Sakrand in 2019.

The details of the experimental units were set as:

i) Control (without priming and foliar spray),
ii) Foliar spray of simple water used for extract preparation (FSW),
iii) Seed priming with MLE 3% solution (SPM),
iv) Seed priming and foliar spray MLE 3% solution (SPFS),
v) Foliar spray with MLE 3% solution (FS).

Preparation of leaf extract: Zeatin is rich in young leaves and tender branches[18], therefore, young leaves were detached from the tree standing at CCRI, Sakrand. Leaves were rinsed thoroughly and after rinsing 30g of leaves were grounded using a pestle mortar. The ground material was then added to 1L tap water and stirred for some movement thoroughly. Staining was done through a fine cotton cloth to prepare approximately 3% solution.[19,20] Usually, 6-8 tanks (20 L) capacity is used per acre.[6]

Cottonseed priming: Cotton seeds were soaked in a 3% solution of MLE for 3 h at a 1:5 seed-to-solution ratio.[21] After priming, cottonseeds were sown directly.

Foliar spray: Because of the peak nutrient requirement stages of the cotton crop, MLE was sprinkled at 30 (one month after sowing), 60 (flowering), and 90 (boll opening) days after sowing (Figure.5).

Electrical conductivity of seed leachates: For determination of electrolyte leakage, one gram of cotton seeds was soaked in 50 ml of deionized water. Before soaking, seeds were cleaned and air-dried. Electrical conductivity was measured in $\mu S\ cm^{-1}\ g^{-1}$ starting from 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, and finally at 24 hours after soaking.

Statistical analysis: The data was analyzed using the software Statistic. The USA. Ver. 8.1. Mean separations were done by SED, LSD, and Tukey's pair-wise test. The analysis of variance (ANOVA) was done by following a two-factor factorial completely randomized design.

RESULTS AND DISCUSSION

This study observes the potential of leaf extract of miracle plant (MLE) as a natural plant growth enhancer for cotton crops. Its analysis shows that they have substantial quantities of mineral essential nutrients. Furthermore, it is enriched with antioxidants (enzymatic as well as non-enzymatic) that promotes it as a natural growth enhancer.[10]

MLE was evaluated for its potential to act as a seed priming medium and foliar spray solution to improve growth and yield in cotton. The hypothesis was developed that the growth, development, and seed cotton yield constraint can be overcome by seed priming and foliar spray thus exploring the easily adoptable best application for cotton crops. It has been reported to hasten the growth and yield of wheat crops[3] and improve resistance to pests and diseases.[6] Its method of application (seed priming and foliar spray) is also important to affect growth-enhancing characteristics. In former studies, it has been

witnessed that seed priming has increased the seed germination and seedling vigor along with final yield in wheat crops[3] while working with cotton crops its positive effects have been observed on growth and development and contending cotton leaf curl virus disease using foliar spray on cotton crop.[2] In the present study the results regarding plant height (cm) at 75, 90, and 135 days after sowing (DAS) are statistically non-significant (p=0.05) but visually good growth was observed from all the treatments either through seed priming or foliar spray on both varieties as compared to control (Table **2**).

The results are accorded with the findings of Panhwar *et al.,*[3] where both vegetative and reproductive growth was increased with the use of MLE on wheat crops. This also ratifies that MLE has the potential to show long-term effects at later growth stages after germination. As mentioned earlier it is gifted by nature in having a substantial source of zeatin a plant hormone and other bio-stimulating compounds along with essential nutrients, antioxidants, and a complete family of amino acids in ready-to-take form[7,10]therefore exogenously applied MLE through the foliar spray and seed priming improved the plant defense system, secondary metabolites and antioxidative system that is why it may be assumed that these compounds have transferred to the growing seed during the germination and boosted amylase movement that increased starch breakdown[11], eventually improving successive growth and development of a cotton plant. As it has already been processed by grinding hence it may release a higher amount of K^+, Ca^{2+}, Mg^{2+}, and PO_4^{3-} and other nutrients as mentioned earlier when dissolved in water that might have transferred to seed during imbibition. On average, the maximum value of plant height (160.8 cm) at 135 DAS was noted from seed priming + foliar spray of MLE 3% solution (T4) and the minimum value (147.8 cm) was observed from control (T1). Improvement in height of cotton plants that emerged from primed seeds was possibly due to the excellent source of all essential nutrients which are known to promote the growth and development of a plant. A similar trend was observed in the number of bolls counted in plant [1] (Table **3**). On average, the highest number of bolls counted plant^{-1} (65.2) was noted from T4 and the minimum (35.8) was from control (T1) recorded at 135 DAS. In addition, the CRIS-585 produced the highest plant height and the number of bolls counted plant^{-1} as compared to CRIS-342 but statistically non-significant (p=0.05). As stated by Foidl *et al.,*[10]both vegetative as well as reproductive growth were probably increased due to the presence of essential nutrients, ascorbates, zeatine, etc in MLE.

Table2. Plant Height (cm) Recorded at Various Growth Stages.

Treatments	Variety	Plant height (cm) DAS		
		75	90	135
T1(Control)	CRIS-585	112.90b	17.73c	153.47ab
	CRIS-342	111.03b	17.26c	142.20b
T2 (FS W)	CRIS-585	115.77b	19.633bc	158.20ab
	CRIS-342	114.50b	21.933bc	155.67ab
T3 (SP)	CRIS-585	130.40ab	33.067abc	154.87ab
	CRIS-342	131.13ab	31.867abc	151.20ab
T4 (SP+FS MLE)	CRIS-585	137.67a	39.400a	164.60a
	CRIS-342	128.13ab	34.200ab	157.00ab
T5 (FS MLE)	CRIS-585	137.23a	38.033a	156.40ab
	CRIS-342	125.80ab	33.900ab	152.07ab
LSD Values (5%)	Treatment (T)	12.84	9.01	NS
	Variety (V)	4.64	NS	3.45
	T×V	NS	NS	NS

SP = Seed priming, W = Water, FS = Foliar spray, MLE = Leaves extract of miracle plant.

The means sharing the same letters did not differ significantly at p= 0.05.

Table3. Number of Bolls Counted Plant^{-1} At Various Growth Stages.

Treatments	Variety	Number of bolls counted plant^{-1} DAS		
		75	90	135
T1(Control)	CRIS-585	1.93c	17.73c	36.27a
	CRIS-342	3.00bc	17.26c	35.40a
T2 (FS W)	CRIS-585	4.20bc	19.63bc	37.13a
	CRIS-342	1.70c	21.93bc	63.93a
T3 (SP)	CRIS-585	9.50ab	33.06abc	53.80a
	CRIS-342	6.20bc	31.86abc	72.00a
T4 (SP+FS MLE)	CRIS-585	8.60abc	39.40a	97.40a
	CRIS-342	4.20bc	34.20ab	102.93a
T5 (FS MLE)	CRIS-585	15.70a	38.03a	48.27a
	CRIS-342	6.30bc	33.90ab	52.87a
LSD Values (5%)	Treatment (T)	2.10	NS	8.34
	Variety (V)	3.41	10.59	61.2
	T×V	NS	NS	NS

SP = Seed priming, W = Water, FS = Foliar spray, MLE = Leaves extract of miracle plant.

The means sharing the same letters did not differ significantly at p= 0.05.

Table 4. Boll Weight (g) and Seed Cotton Yield (kg ha^{-1}).

Treatments	Variety	Boll weight (g)	Seed cotton yield (kg ha^{-1})
T1 (Control)	CRIS-585	3.52ab	2330b
	CRIS-342	3.11b	2563ab
T2 (FS W)	CRIS-585	3.58ab	2873ab
	CRIS-342	3.13b	3106ab
T3 (SP MLE)	CRIS-585	3.89ab	2796ab
	CRIS-342	3.20b	3339ab
T4 (SP+FS MLE)	CRIS-585	4.10a	4194a
	CRIS-342	3.64ab	3495ab
T5 (FS MLE)	CRIS-585	3.82ab	3029ab
	CRIS-342	3.63ab	3339ab
LSD Values (5%)	Treatment (T)	0.34	NS
	Variety (V)	0.25	NS
	T×V	NS	813.26

SP = Seed priming, W = Water, FS = Foliar spray, MLE = Leaves extract of miracle plant.

The means sharing the same letters did not differ significantly at p= 0.05.

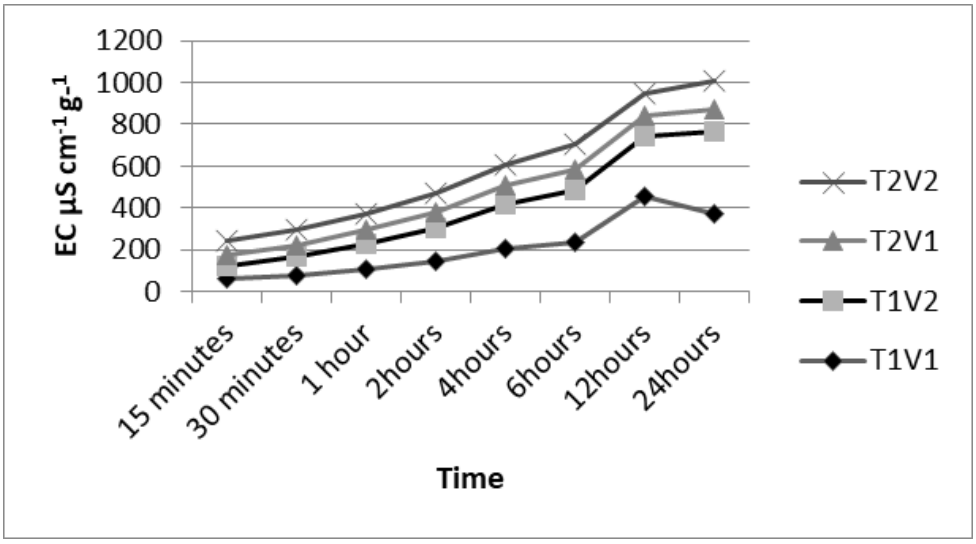

Figure 4. Seed Membrane Permeability Test.
T1= no priming, T2= MLE priming, V1= CRIS-342 and V2= CRIS-585

Figure 5. Pictorial View of Foliar Spray of MLE on Cotton Crop Taken at the Experimental Field of CCRI Sakrand during the Experiment (Used, photographs taken by author during, 2020).

Application of MLE as seed priming or foliar spray in cotton crops significantly (p=0.05) affected seed cotton yield and boll weight g (Table **4**). On average the highest boll weight (3.8 g) and seed cotton yield (3844 kg ha^{-1}) were recorded from T4, followed by T5 (3.7 g and 3184 kg ha^{-1}) and T3 (3.5 g and 3068 kg ha^{-1}), respectively. While the minimum values of boll weight (3.3 g) and seed cotton yield (2446 kg ha^{-1}) were found in the control plot (T1). The increased seed cotton yield of primed seed was possibly the result of the initialization of metabolic

reactions that enhanced embryo growth[14], which eventually hastened cell division. Also as mentioned earlier ascorbate-enriched MLE increases ascorbic acid contents which promotes growth and development and ultimately final yield when applied exogenously.

To assess the vigor and viability of seeds a possible test can be done which is the electrical conductivity (EC) of seed leachates (water that has infiltrated through a seed with some of its constituents). From EC dS/m values we can conclude that lower EC shows high vigor and vice versa. Also, it is negatively correlated to germination and positively correlated to damaged seeds as it shows the integrity of the cell membrane.[22,23] It has been observed from the present study that seed soaking treatment remains effective in decreasing the electrolyte conductivity of cotton seed leachates, which shows plasma membrane strength and stability, therefore, treated seeds has the lowest EC than the control. (Figure **4**).

CONCLUSION

The results indicated that the application of leaf extract of miracle plants as seed priming or foliar spray in cotton crops significantly affected seed cotton yield and its traits. It is observed from the present study that seed soaking treatment is effective in decreasing the electrolyte conductivity of cotton seed leachates, which shows plasma membrane strength and stability.

ETHICAL APROVAL

None.

CONFLICT OF INTEREST

None.

FUNDING SOURCE

None.

ACKNOWLEDGEMENTS

None.

LIST OF ABBREVIATIONS

DAS	Days after sowing
EC	Electrical conductivity
FS	Foliar spray
FSW	Foliar spray of simple water
MLE	Leaf Extract of Miracle Plant
SPFS	Seed priming and spray MLE 3%
SPM	Seed priming with MLE

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