Exploring the effect of humic acid on chlorophyll levels in Japanese mint under drip and flood irrigation amidst water and nutrient constraints

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ABSTRACT

A field experiment on Mentha (Mentha arvensis L.) was conducted during the spring seasons of 2022 and 2023 at G.B. Pant University of Agriculture and Technology, Pantnagar. The objective was to investigate the impact of foliar spray of humic acid on SPAD values under conditions of limited water and nutrients. The experiment comprised two drip irrigation levels (80% and 100% of crop evapotranspiration) and four nutrient management practices. These practices included: 25% NPK basal + 75% NPK fertigation, 50% NPK basal + 50% NPK fertigation, No basal + 75% NPK fertigation + three foliar sprays of humic acid at 0.3%, and 25% NPK basal + 50% NPK fertigation + three foliar sprays of humic acid at 0.3%. The experiment was laid out in a randomized block design with three replicates (factorial), and an extra control was included. The control group followed conventional practices, where the crop received recommended doses of fertilizers (N: P2O5: K2O @ 120:60:40 kg/ha) and was irrigated at an IW: CPE ratio of 1.2 with a 5 cm irrigation depth. Significantly higher SPAD values were observed when the crop was irrigated at 100% ETc level of drip irrigation and with nutrient management practices involving foliar application of humic acid, compared to 80% ETc level of drip irrigation and total NPK supply through chemical fertilizers, respectively. At harvest, crops irrigated at 100% ETc level recorded SPAD readings that were 11.1% and 18% higher than those irrigated at 80% ETc level during 2022 and 2023, respectively. The humic acid application helped to maintain the SPAD values even after a 25% reduction in nutrient dosage. SPAD values for control were significantly lower than the combination of best nutrient management practices with either 80 or 100% ET_c level of drip irrigation.

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Keywords: (Japanese mint, Humic acid, SPAD, Drip fertigation)

1. INTRODUCTION

Mentha, belonging to the Lamiaceae family, is an economically vital herbaceous crop due to its essential oil content, finding extensive applications in pharmaceuticals, food, and cosmetics (Singh et al., 2015) [1]. In India, Japanese mint (*Mentha arvensis*) holds a significant presence, solidifying the nation's position as the leading global producer of this mint species. Japanese mint, referred to as menthol mint for its remarkably high menthol content ranging from 75-80%, surpasses other mint species. In India, Mentha cultivation is primarily concentrated in the central regions of the Indo-Gangetic plains, including the Tarai belt of Uttarakhand (Kumar et al., 2001) [2]. In this area, Mentha is cultivated as a spring-season crop, receiving minimal rainfall. Being a shallow-rooted crop that produces a substantial amount of biomass renders it particularly susceptible to water stress. Water deficit in Mentha has, intriguingly, been observed to enhance oil content within the plant. However, this apparent benefit is overshadowed by a significant reduction in herbage yield, ultimately leading to an overall decline in oil yield. This trade-off raises compelling questions about the underlying physiological mechanisms that govern these contrasting responses. One such vital physiological function that comes under scrutiny is photosynthesis, the process driving biomass accumulation in plants. This process is closely linked to the maintenance of chlorophyll structure, an important pigment facilitating the capture of light energy. Disruption in chlorophyll integrity, particularly under drought stress, poses a formidable challenge to normal physiological functions. The SPAD meter, a reading recognized as a reliable indicator of chlorophyll content, has proven instrumental in quantifying stress-related alterations. The findings of Szeles et al. (2023) [3] underscore the significant reduction in SPAD values under water stress conditions, offering a tangible measure of the impact on chlorophyll dynamics. Humic acid is known for its beneficial effects on root growth and expansion;therefore, it has demonstrated its ability to enhance water uptake and improve plant water status, offering a promising remedy to subside the harmful effects of water stress. The application of humic acid has been shown to alleviate the decrease in SPAD values associated with water stress conditions (Matuszak-Salmani et al., 2022) [4]. Apart from this, the humic acid not only enhances nutrient supply but also allows for reduced nitrogen doses, addressing another challenge associated with conventional nutrient management practices that adversely impact SPAD values. The present study was conducted to evaluate the potential of humic acid as an effective strategy to maintain proper chlorophyll content and corresponding SPAD values even under limited nitrogen and water supply.

2. MATERIAL AND METHODS

A field study was conducted during the spring season of 2022 and 2023 at Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar. The research area is located at an altitude of 243.83 m above mean sea level at 29°N latitude and 79.5°E longitude. The soil of the experimental field was sandy loam in texture with a pH of 7.5, medium in organic carbon (0.71%) and low available nitrogen (178.4 kg ha-1), medium available phosphorus (24.8 kg ha-1) and potassium (265.6 kg ha-1). At field capacity and PWP, the soil moisture contents were 20.8 and 8.4 percent, respectively. Total rainfall during the year 2022 was about 48.1 mm and in the year 2023, it was about 233 mm. The study comprised of two levels of drip irrigation (80 and 100% ETc) and four nutrient management practices (25% NPK basal +75% NPK fertigation, 50% NPK basal +50% NPK fertigation, No basal + 75% NPK fertigation + three foliar sprays of humic acid @ 0.3% and 25% NPK basal +50% NPK fertigation + three foliar sprays of humic acid @ 0.3% was laid out in randomized block design (factorial) and one extra control replicated thrice. In control, recommended doses of fertilizers (half of the nitrogen (N) along with the entire quantity of phosphorus (P) and potassium (K) wereadministered as a basal application; while the remaining 50% of nitrogen was split into two equal portions and top-dressed at 40 and 60 DAP through NPK

(12:32:16), MOP and urea fertilizers. Irrigation was applied at an IW: CPE ratio of 1.2, utilizing flood surface irrigation with a depth of 5 cm.

Suckers of the variety “CIM Kranti” were planted 40 cm apart in the second fortnight of February. Fertigation was started at 25 days after planting (DAP). Urea (46% N) and mono potassium phosphate (0:52:34) water-soluble fertilizers were used for fertigation. In drip treatments, fertigation was done in 07 equal splits at 10-dayintervals. In respective treatments, the application of humic acid was performed at 30, 60, and 90 days after planting (DAP). Drip irrigation was applied at two-dayintervals. The amountof water applied was as per the crop evapotranspiration (ETc).

It was calculated by using the following formula (FAO, 1998) [5]:

$$ETc = ET_0 \times Kc$$

$$ET_0 = CPE \times Kpan$$

$$Kpan = 0.7$$

Where,

ETc= Crop evapotranspiration,

ET_0= Reference evapotranspiration, K

Kc = Crop coefficient,

CPE = Cumulative pan evaporation and

$Kpan$ = Pan Coefficient.

Kc values for mint crops at different crop growth stages as given by FAO, 1998 [5] were used to calculate ETc.

SPAD reading and relative leaf water content (RLWC) were recorded at 60, 90 DAP, and at the harvest stage. SPAD readings were taken with the help of SPAD meter (model CCM-200). RLWC values were worked out by using the following formula developed by Barrs and Weatherley (1962) [6].

RLWC(%)=((Fresh weight of leaf sample(g)-dry weight of leaf sample(g)/(Turgid weight of leaf sample(g)-dry weight of leaf sample(g)))×100

Crop was harvested at 120 DAP. Statistical evaluation of the experiment was conducted in two parts. Comparison of drip fertigation treatments was done by analysis of variance (ANOVA) for randomized block design (factorial) as per the statistical program OPSTAT, developed by Sheoran et al., 1998 [7] at CCS Hissar Agricultural University, Haryana. The comparison of the control plot was made with the best combination of nutrient management practice with each 80 and 100% ETc level of irrigation by using an independent sample ‘ t’ test with the help of statistical software packages in 'R' version 1.2.1335 (R Core Team, 2016) [8].

3. RESULTS AND DISCUSSION

SPAD meter reading is an important indicator of chlorophyll content. In both years of the study, the SPAD values for 100% ETc level of drip irrigation were significantly higher as compared to the 80% ETc level of drip irrigation at all the stages of crop growth. At harvest, the increase in SPAD values under 100% ETc level was about 11.1 and 18% for the years 2022 and 2023, respectively (Table 1).The reduction in SPAD values under 80% ETc levels might be due to the decrease in chlorophyll content under this treatment which indicates the possibility of water stress. Under limited availability of water, there is a reduction in photosynthetic electron transport which can potentially reduce the evolution of molecular oxygen, resulting in the production of reactive oxygen species (ROS), which can damage the photosynthetic apparatus (Basu et al., 2016) [9]. Damage in the structure of the photosynthetic apparatus causes a reduction in chlorophyll content. Kapoor et al. (2020) [10] reported that exposure to drought stress causes an increase in the activity of chlorophyllase enzyme and ultimately the photo-oxidation of chlorophyll. Among the nutrient management practices, there was a significant difference in SPAD values at all the crop growth stages for both years of the study. At 60 DAP significantly lower SPAD values were observed for the treatment without basal application of NPK and 75% NPK fertigation and supply of humic acid @ 0.3% by foliar spray (36.42 and 37.07) in the years2022 and 2023, respectively (Table 1). Lower SPAD values in this treatment are the consequent effect of the exclusion of basal nitrogen doses which might have affected the chlorophyll content of plants in the initial stages The SPAD values of all other treatments at 60 DAP were statistically at par during both years of the study. The application of 25% nitrogen as the basal dose was able to maintain the SPAD values at 60 DAP, similar to the treatment with 50% nitrogen application as basal fertilizer.

Treatment	**SPAD Value**					
	60 DAP		**90 DAP**		**At harvest**	
	2022	2023	2022	2023	2022	2023
Drip irrigation level (I)						
I_1: 80% ET_c	35.26	35.08	35.58	35.69	36.81	35.93
I_2: 100% ET_c	40.78	41.83	42.04	42.58	40.88	42.39
SEm±	0.373	0.341	0.274	0.256	0.294	0.351
CD at 5%	1.13	1.03	0.83	0.78	0.89	1.07
Nutrient management practices (N)						
N_1: 25% NPK basal +75% NPK fertigation	38.33	38.95	37.53	38.23	37.92	38.02
N_2: 50% NPK basal +50% NPK fertigation	38.8	39	38.2	38.6	37.23	37.5
N_3: No basal + 75% NPK fertigation + three foliar sprays of humic acid @ 0.3%	36.42	37.07	39.75	40.05	39.87	40.43
N_4: 25% NPK basal +50% NPK fertigation + three foliar sprays of humic acid @ 0.3%	38.53	38.78	39.77	39.67	40.37	40.7
SEm±	0.53	0.48	0.39	0.36	0.42	0.50
CD (P=.05)	1.60	1.75	1.18	1.10	1.26	1.51
Control vs Best I×N						
Control	34.87	35.2	34.47	34.03	35.97	34.83
Under 80% ET_c	36.07	35.67	37.3	37.03	39.14	38.5
t- value	1.25	0.67	4.53	3.23	4.71	4.02
Significance	NS	NS	S	S	S	S
Under 100% ET_c						
t- value	9.26	11.15	12.51	20.71	6.53	7.32
Significance	S	S	S	S	S	S
Interaction (I×N)						
SEm±	0.75	0.68	0.55	0.51	0.59	0.70
CD (P=.05)	NS	NS	1.66	1.55	1.78	2.13

Table 1: SPAD values as affected by drip irrigation levels and nutrient management practice.

At 90 DAP and at harvest, similar trends of variation in SPAD values for different nutrient management practices were emerged. Even with reduction in NPK doses the treatments with humic acid supply were able to maintain the SPAD values at later stages, which suggests that humic acid was able to provide the required nourishment for chlorophyll synthesis. Significantly higher values of SPAD with humic acid supply can be attributed to the regulation of ROS (Hasanuzzaman et al., 2021) [11] which can cause photo-oxidation of the chlorophyll. ROS production is enhanced under drought stress and humic acid can help to alleviate such stress conditions as it is known to maintain the higher plant water status. Relative leaf water content, a parameter that reflects plant water status is improved by the addition of humic acid. By improving root growth humic acid shows its ultimate effect on the water uptake. It was observed that there was a strong correlation between RLWC and SPAD reading as evident by figure 1 and figure 2. In the year 2022, the coefficient of determination between these parameters was about 86.8 and it was about 87.3% in the next year of the study. These high values of the coefficient of determination suggest a strong and reliable relationship between these two parameters.

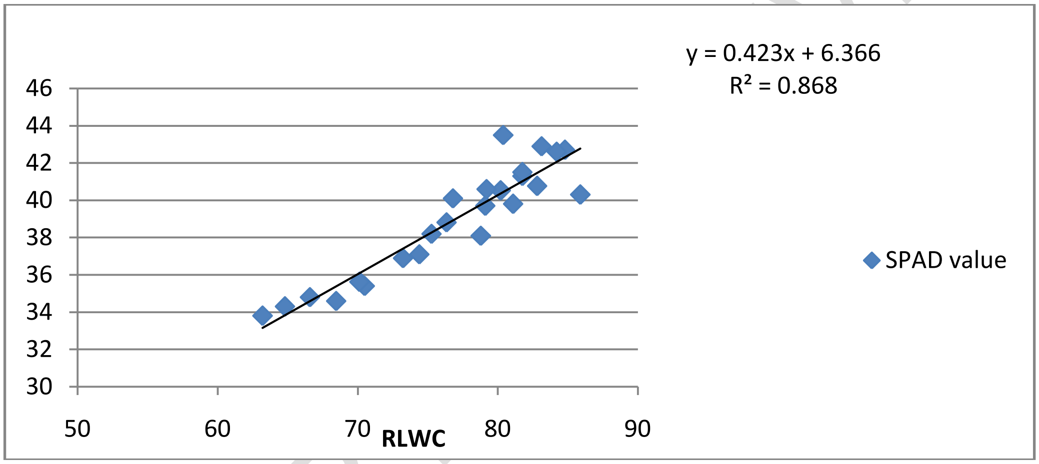

Fig.1.Relationship between RLWC and SPAD values in the year 2022

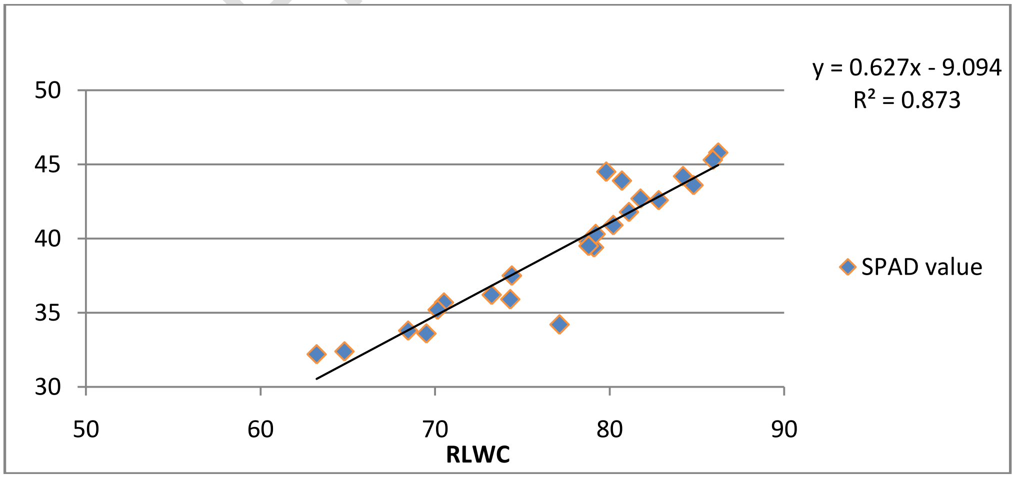

Fig.2. Relationship between RLWC and SPAD values in the year 2023

Comparison of SPAD values under control treatment was made with the maximum values of SPAD in conjunction with both 80 and 100% ET_c levels of drip irrigation (Table 1). During 2022 the highest values of SPAD under 100% ET_c treatment were found in combination with the 25% NPK application as basal and 50% as fertigation along with humic acid application except at 90 DAP. At this stage, the maximum value of SPAD in 100% ET_c level was observed for the nutrient management practice with 75% NPK application as fertigation in combination with humic acid. This combination was able to achieve the highest SPAD values at 90 DAP and harvest for the year 2023. However, the highest SPAD at 60 DAP in 2023 was achieved for the treatment where 25% NPK application was done as basal and 75% as fertigation. At all the stages of crop growth, the highest SPAD values under 100% ET_c level of drip irrigation were significantly higher than the control treatment. Lower SPAD values under control suggest that plants in this treatment might have faced water stress which caused the degradation of chloroplast aperture. At 60 DAP, the highest SPAD value in 80% ET_c level was observed in combination with the treatment with 50% NPK application as basal and 50% NPK application as fertigation (36.07) for the year 2022. For subsequent year the highest value of SPAD (35.67) was observed for the combination of 80% ETc with 25% NPK application as basal and 50% NPK application as fertigation along with foliar applied humic acid. In both years of the study, the highest values under 80% ET_c at 60 DAP were statistically at par with the control treatment. With the progression of crop growth, a significant difference between SPAD values for control and the best combination of nutrient management practices with 80% ET_c level of irrigation emerged. The highest values of SPAD were continuously observed for the treatments with foliar application of humic acid in both years of the study. These values were significantly higher than the control, which might be due to the ability of humic acid to subside the chloroplast degeneration under drought conditions.

The interaction effect of humic acid and irrigation level was found significant at 90 DAP and harvest for both years of the study (Table 2 and Table 3). At 90 DAP and harvest, there was a significant difference in SPAD values between different nutrient management practices under 80% ETc level of drip irrigation. The trend was consistent for both years of the study. It was observed that higher values under 80% ETc level of drip irrigation were observed for the nutrient management practices where foliar spray of humic acid was performed. However, the difference was non-significant for all the combinations of nutrient management practices with 100% ETc level of drip irrigation. Chlorophyll degradation is more pronounced under water stress conditions and humic acid can subside the adverse effect of drought by maintaining chlorophyll ultra-structure (Fan et al. 2014) [12]. A dripirrigation level of 80% ETc imparted the water stress in Mentha and chlorophyll content decreased. A foliarspray of humic acid was able to plummet the effect of drought on chlorophyll content and ultimately on SPAD values.

Drip irrigation levels	Nutrient management practices			
	25% NPK basal +75% NPK fertigation	50% NPK basal +50% NPK fertigation	No basal + 75% NPK fertigation + three foliar spray of humic acid @ 0.3%	25% NPK basal +50% NPK fertigation + three foliar spray of humic acid @ 0.3%
Year 2022				
80% ET_c	33.57	34.40	37.07	37.3
100% ET_c	41.5	42	42.43	42.2
SEm±	0.55	**C.D. (P=.05)**1.66		
Year 2023				
80% ET_c	33.97	35.37	36.4	37.03
100% ET_c	42.5	41.83	43.7	42.3
SEm±	0.51	**C.D. (P=.05)**1.55		

Table 2: Interaction effect of drip irrigation levels and nutrient management practices on SPAD value at 90 days after planting.

Drip irrigation levels	Nutrient management practices			
	25% NPK basal +75% NPK fertigation	50% NPK basal +50% NPK fertigation	No basal + 75% NPK fertigation + three foliar spray of humic acid @ 0.3%	25% NPK basal +50% NPK fertigation + three foliar spray of humic acid @ 0.3%
Year 2022				
80% ET_c	34.6	34.91	38.61	39.14
100% ET_c	41.23	39.55	41.13	41.6
SEm±	0.59	**C.D. (P=.05)**1.78		
Year 2023				
80% ET_c	33.9	33.57	37.77	38.5
100% ET_c	42.13	41.43	43.1	42.9
SEm±	0.70	**C.D. (P=.05)** 2.13		

Table 3: Interaction effect of drip irrigation levels and nutrient management practices on SPAD at harvest

4. CONCLUSION

There is a critical impact of water stress on chlorophyll content in Japanese mint, indicating a decline in chlorophyll levels. However, the application of humic acid through foliar spray proved to be an effective strategy in mitigating the negative effects of water stress on chlorophyll content, consistently yielding higher SPAD values. The findings emphasize that humic acid can be used as a supplement with chemical fertilizers to maintain proper nitrogen supply even after a 25% reduction in the applied dosage.

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