Impact of Irrigation Water Salinity on Stomatal Conductance and Chlorophyll Content Index of Tomato Plants Across Phenological Stages

CIHAN KARACA[1*], GULCIN ECE ASLAN[1], AHMET KURUNC[1], RUHI BAŞTUG[1], DURSUN BUYUKTAS[1]
[1]Department of Agricultural Structures and Irrigation, Faculty of Agriculture
Akdeniz University
Pınarbaşı Mahallesi, Konyaaltı/Antalya
TURKİYE
[*]Correspondence: cihankaraca@akdeniz.edu.tr

Abstract: - This study aimed to investigate the effects of different irrigation water salinities on the stomatal conductance (g_s) and chlorophyll content index (CCI) of tomato plants during different phenological stages, at both pre- and post-irrigation. For this purpose, g_s, CCI, and Leaf Area Index (LAI) data were collected from tomato plants grown under four different irrigation water salinity levels. The g_s and CCI data were classified according to the four different tomato phenological stages (vegetative, flowering, early fruit growth, and harvest). Differences in mean g_s and CCI data across different irrigation water salinity levels at various phenological stages were determined using a two-way ANOVA. Differences between phenological stages within each irrigation salinity level and yield parameter were assessed using one-way ANOVA. The results indicated that irrigation water salinity levels of up to 7.5 dS m^{-1} did not affect the CCI at both pre- and post-irrigation ($p > 0.05$). However, significant effects were observed depending on the phenological period ($p < 0.01$) There was a significant decrease in both yield and CCI during the harvest period at each salinity level. The research findings are believed to contribute to optimizing drip irrigation practices using low-quality water in tomato cultivation.

Key-Words: CCI, EC_e, EC_i, g_s, leaf area index, plant physiology, yield

Received: April 29, 2024. Revised: September 20, 2024. Accepted: October 23, 2024. Published: November 7, 2024.

1 Introduction

Optimizing irrigation based on water quality is crucial for agricultural production, particularly for vegetables grown in greenhouses [1]. The quality of irrigation water, particularly its salinity, can significantly affect plant physiology and overall crop yield [2]. Salinity in irrigation water refers to the concentration of dissolved salts such as sodium, chloride, and other ions [3]. Saline irrigation can cause various physiological challenges for plants, including ionic and osmotic stress, which ultimately reduce photosynthesis rates and inhibit growth [4]. Rapid absorption of ions can lead to their accumulation within plant cells, negatively affecting plant-water relationships and reducing relative water content, water uptake, and transpiration rates [5]. The effects of irrigation water salinity on plants vary across different phenological stages [6,7]. During the early growth stages, high salinity levels in the irrigation water can hinder seed germination and seedling establishment [8]. As the plant matures, saline irrigation can reduce vegetative growth, disrupt reproductive development, and decrease the yield. The sensitivity of plants to saline irrigation water typically depends on crop type and cultivar [4].

Different irrigation methods influence salt accumulation because of the varying ways water is delivered to the plant root zone. Irrigation method plays a crucial role in determining the extent and distribution of soil salinity [9]. Various irrigation techniques, such as surface, sprinkler, and drip irrigation, result in different patterns of water application and movement within the soil profile, leading to varying salt accumulation [10]. In surface irrigation method, water is applied to the entire field, often resulting in an uneven distribution and high evaporation rates, leading to the concentration of salts in specific areas of the root zone [11]. Although sprinkler irrigation is effective in removing salt accumulated in the root zone, drip irrigation is often preferred for species highly sensitive to leaf necrosis [11]. Drip irrigation, identified as a potential solution

to soil salinity issues, allows for more precise water application and targeted leaching of salts from root zones. By applying water directly to the plant root zone, drip irrigation minimizes evaporation and promotes localized leaching of salts, preventing their accumulation in the root zone [12]. The drip irrigation method further mitigates the adverse effects that may arise from the salinity of irrigation water. Improved irrigation management strategies, such as selective leaching, use of reclaimed water, and appropriate soil amendments, can help reduce the negative impacts of saline irrigation water on plant physiology and crop production [13].

Plants exhibit physiological changes at both pre- and post-irrigation with saline water. Prior to irrigation, increased salinity can induce stress responses in plants, such as reduced growth and altered biochemical processes [14]. Salinity stress at pre-irrigation can lead to decreased stomatal conductance and chlorophyll content, affecting the plant's ability to perform photosynthesis efficiently. At post-irrigation, plants may experience changes in nutrient uptake, water balance, and other physiological functions, as they adapt to saline conditions [15].

The aim of this study was to investigate the effects of different irrigation water salinities (0.7 dS m^{-1}, 2.5 dS m^{-1}, 5 dS m^{-1} and 7.5 dS m^{-1}) on stomatal conductance (g_s) and chlorophyll content index (CCI) of tomato plants at different phenological stages (vegetative, flowering, early fruit growth, and harvest) at both pre- and post-irrigation.

2 Materials and Methods

2.1 Experimental Area

The study was conducted in lysimeters within a greenhouse at Akdeniz University, Antalya, Turkiye (TR Türkiye, 36°53’ N latitude, 30°38’ E longitude; 12 m above sea level). This Mediterranean-type greenhouse, featured a gothic roof that was 4 m high at the gutters and 6 m at the ridge and measuring 9.60 meters in width and 25 meters in length, the greenhouse was oriented in a north-south direction and made of steel, which was covered with polyethylene. Twelve lysimeters were used in the study, each measuring 2.70 × 1.85 meters and a depth of 0.80 meters and consisted of a top layer of 60 cm soil and a bottom layer of 20 cm gravel.

In the study, four different irrigation water salinity levels were selected: S_0=0.7 dS m^{-1} (control), S_1= 2.5 dS m^{-1} (low), S_2= 5.0 dS m^{-1} (medium), and S_3= 7.5 dS m^{-1} (high). For all salinity treatments, the sodium adsorption ratio (SAR) was maintained as close as possible to that of the tap water source. The desired electrical conductivity values (EC_w) for each treatment were achieved by mixing calculated amounts of calcium chloride, magnesium sulfate, and sodium chloride salts into the irrigation water.

The soil of lysimeters was classified as silty-clay loam (51% silt, 28% clay, and 21% sand, with a bulk density of 1.38 g cm^{-3}). At the beginning of the experiment, saturated soil extract is 0.5 dS m^{-1}. Field capacity and permanent wilting point were determined as 31% and 14%, respectively (%vol). The lysimeter plots were irrigated using a drip irrigation system. This system was configured so that each crop row was serviced by a single lateral line equipped with pressure-compensating drippers, which discharged water at a rate of 2 L h^{-1} under a pressure of 0.1 MPa, with drippers spaced 0.2 meters apart.

Soil moisture content was monitored using tensiometers (SR Series, Irrometer Company Inc, Riverside, USA). The tensiometers were placed close to the lateral pipes, at a distance of 0.10 meters from the drippers, and at a depth of 20 cm. Irrigation was applied to replenish soil moisture to field capacity whenever tensiometer readings reached 20 centibars (cb), indicating a 20% depletion of available water, at a depth of 0.60 meters in the soil profile.

The ÖZKAN F1 variety of tomatoes, commonly cultivated in Antalya, was chosen for this experiment. Tomato seedlings were planted in lysimeter plots spaced at intervals of 0.60 x 0.50 meters. Once the seedlings reached a height of 0.40 meters, they were trained to grow on a single stem and supported using ropes. New side shoots were removed regularly throughout the growing season. After the plants had developed eight clusters, the top shoots were pruned. Leaf pruning followed the method recommended by [16] and local growers' practices.

2.2 Measurements

Leaf area index (LAI), chlorophyll content index (CCI), and stomatal conductance were measured for three selected plants in each replication. The leaf area was determined non-destructively using leaf width and length, following the method described in [17]. The LAI was calculated using the Equation 1:

$$LAI = \frac{n \times LA_{mean}}{A_p} \quad (1)$$

where LAI is the leaf area index (m^2 m^{-2}), n is the number of leaves, LA_{mean} is the mean leaf area (m^2) and Ap is the area per plant (m^2).

Stomatal conductance (mmol m^{-2} s^{-1}) was measured using an SC-1 leaf porometer (Decagon

Devices, Inc., Pullman, WA, USA) at pre- and post-irrigation between 11:00 a.m. and 2:00 p.m., following the manufacturer's instructions. Chlorophyll content index measurements were obtained using a handheld leaf-clip CCM-200 meter (Apogee Instruments, Inc., North Logan, UT, USA) at pre- and post-irrigation.

2.3 Statistical Analysis

The study was designed as a randomized complete block design with three replications, investigating the impacts of four different irrigation water salinity levels (control, low, moderate, and high) and four distinct phenological periods of tomatoes (vegetative, flowering, early fruit growth, and harvest). The mean differences among the data obtained for stomatal conductance and CCI at different irrigation water salinity levels across various phenological stages were evaluated using two-way ANOVA after confirming normality and homoscedasticity using Shapiro-Wilk and Levene tests, respectively. Additionally, one-way ANOVA was conducted to determine differences across phenological stages within each irrigation salinity level and yield parameter. Furthermore, multiple comparisons (LSD) were performed at a significance level of $p< 0.05$ to further explore these mean differences. Statistical analyses were conducted using OriginPro v2024 (OriginLab Corporation, Northampton, MA, USA).

3 Results and Discussion

It is important to note that the interaction between irrigation water salinity and phenological stage on stomatal conductance and chlorophyll content did not show a significant difference ($p> 0.05$). This finding indicates that salinity and phenological stage independently influence stomatal behavior and chlorophyll content. Therefore, the main factor effects were analyzed separately. The effects of different irrigation water salinity levels (0.7 dS m^{-1}, 2.5 dS m^{-1}, 5 dS m^{-1}, and 7.5 dS m^{-1}) on the stomatal conductance (g_s) and chlorophyll content index (CCI) of tomato plants during various phenological stages (vegetative, flowering, early fruit growth, and harvest) at pre- and post-irrigation are given in Table 1.

Table 1. Effect of different irrigation water salinities on seasonal average stomatal conductance and chlorophyll index content of tomato at pre- and post-irrigation in different phenological stages.

Treatments	Stomatal conductivity (mmol m^{-2} s^{-1})		Chlorophyll content index	
	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation
0.7 dS m^{-1}	367.5 a	384.7	51.4	51.4
2.5 dS m^{-1}	355.7 a	355.0	53.0	48.3
5 dS m^{-1}	353.3 a	354.6	50.8	48.9
7.5 dS m^{-1}	323.8 b	327.6	51.5	45.9
$LSD_{Salinty}$	*	ns	ns	ns
Vegetative	365.2 b	367.2 b	63.8 a	63.4 a
Flowering	404.1 a	426.5 a	50.7 b	47.7 b
Early fruit grown	371.1 b	364.9 b	52.3 b	42.4 c
Harvest	259.8 c	263.4 c	39.8 c	41.1 c
$LSD_{Phenological\ stage}$	**	**	**	**

The means indicated with the same letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, and ns, significant at the $p < 0.05$, $p < 0.01$ level, and not significant, respectively.

Regardless of the phenological stage, the pre-irrigation stomatal conductance values ranged from 323.8 to 367.5 (mmol m^{-2} s^{-1}), while the post-irrigation values ranged from 327.6 to 384.7 mmol m^{-2} s^{-1}. However, only pre-irrigation stomatal conductance (g_s) values were significantly ($p<0.05$) affected from irrigation water salinity (Table 1). The highest salinity level of 7.5 dS m^{-1} resulted in lower stomatal conductance values compared to other salinity levels. This showed that plants responded to only high level of salt stress by partially closing their stomata to reduce water loss. Measurements taken at pre- and post-irrigation indicated that changes in stomatal conductance were directly related to plant water uptake and transpiration rate. The closure of stomata likely helps to protect plants against salt stress by reducing water loss [18]. This finding highlights the potential impact of high salinity on plant growth and productivity. However, for post-irrigation, no significant difference in g_s was observed in relation to the irrigation water salinity. This indicates that stomata may reopen after irrigation because of increased water availability, leading to equalized conductance values. Additionally, stomatal control mechanisms are influenced by various factors, including plant water potential, atmospheric vapor pressure deficit, light conditions, and CO_2 concentrations [19].

Regardless of the phenological stage, the effect of different irrigation water salinity levels on the seasonal average CCI was insignificant. In this study, the use of drip irrigation may have prevented the full

impact of salinity on the chlorophyll content from being observed. However, averaged over all irrigation water salinity levels, different phenological stages significantly affected both pre-irrigation and post-irrigation g_s and CCI at $p< 0.01$. The highest stomatal conductance values were obtained during the flowering stage, reflecting the plant's high metabolic activity and water demand during this period. In contrast, the lowest g_s values were observed during the harvest stage, indicating reduced energy requirements and physiological adjustments to limit water loss. During the vegetative and early fruit growth stages, stomatal conductance was lower than that during the flowering stage, but higher than that during the harvest stage ($p< 0.01$). The significant differences between phenological stages were consistent at both pre- and post-irrigation, indicating that stomatal activity for phenological stage averages was influenced by the phenological stage itself, independent of irrigation practices. At both pre- and post- irrigation, the CCI exhibited significant differences at various phenological stages ($p< 0.01$). The highest chlorophyll content was observed during the vegetative stage at both pre- and post-irrigation, reflecting high photosynthetic activity during the active growth phase of the plant. The lowest chlorophyll content was observed during the harvest stage at pre-irrigation, and during both the early fruit growth and harvest stages at post-irrigation. These results indicate chlorophyll loss and, consequently, a decrease in photosynthetic capacity as a part of the plant's physiological aging process. Similarly, Wang et al. (2015) [20] stated that senescence is a highly regulated process characterized by the active breakdown of cells, which ultimately leads to the death of plant organs or whole plants.

To determine the effect of phenological stages on g_s and CCI under each salinity level, a one-way ANOVA was conducted. The results of the analyses for 0.7, 2.5, 5, and 7.5 dS m^{-1} are presented in Tables 2, 3, 4, and 5, respectively.

Table 2. The effect of phenological stages on seasonal average stomatal conductance and CCI under 0.7 dS m^{-1}

Phenological periods	Stomatal conductivity (mmol m^{-2} s^{-1})		Chlorophyll content index	
	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation
Vegetative	359.7 b	364.0	64.2 a	68.0 a
Flowering	423.1 a	465.0	49.3 b	46.5 b
Early fruit grown	386.0 b	400.5	50.7 b	48.4 b
Harvest	301.2 c	309.4	41.5 c	42.5 c
Significant level	**	ns	**	**

The means indicated with the same small letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, and ns, significant at the $p < 0.05$, $p < 0.01$ level, and not significant, respectively.

Table 3. The effect of phenological stages on seasonal average stomatal conductance and CCI under 2.5 dS m^{-1}

Phenological periods	Stomatal conductivity (mmol m^{-2} s^{-1})		Chlorophyll content index	
	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation
Vegetative	360.9 a	363.8 b	66.0 a	62.1 a
Flowering	391.1 a	412.8 a	53.4 b	51.3 b
Early fruit grown	378.7 a	370.4 b	50.4 b	38.0 c
Harvest	291.9 b	273 .0 c	42.1 c	41.8 c
Significant level	*	**	**	**

The means indicated with the same letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, and ns, significant at the $p < 0.05$, $p < 0.01$ level, and not significant, respectively.

Table 4. The effect of phenological stages on seasonal average stomatal conductance and CCI under 5.0 dS m^{-1}

Phenological periods	Stomatal conductivity (mmol m^{-2} s^{-1})		Chlorophyll content index	
	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation
Vegetative	376.2 a	372.5 b	64.7 a	66.0 a
Flowering	431.3 a	460.1 a	47.4 bc	45.1 b
Early fruit grown	377.9 a	340.1 b	51.8 b	43.0 b
Harvest	227.6 b	245.9 c	39.4 c	41.8 b
Significant level	**	**	**	**

The means indicated with the same letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, and ns, significant at the $p < 0.05$, $p < 0.01$ level, and not significant, respectively.

Table 5. The effect of phenological stages on seasonal average stomatal conductance and CCI under 7.5 dS m^{-1}

Phenological periods	Stomatal conductivity (mmol m^{-2} s^{-1})		Chlorophyll content index	
	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation
Vegetative	364.0 a	368.5 a	60.4 a	57.4 a
Flowering	370.8 a	368.2 a	52.9 b	47.7 b
Early fruit grown	341.9 a	348.4 a	56.3 b	40.2 bc
Harvest	218.4 b	225.4 b	36.3 c	38.3 c
Significant level	**	**	**	**

The means indicated with the same letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, and ns, significant at the $p < 0.05$, $p < 0.01$ level, and not significant, respectively.

Under irrigation water salinity of 0.7 dS m^{-1}, stomatal conductivity at pre-irrigation was highest during the flowering period (423.1 mmol m^{-2} s^{-1}) and lowest during the harvest period (301.2 mmol m^{-2} s^{-1}) ($p<0.01$). At post- irrigation, the values increased across all periods, with the flowering period still exhibiting the highest conductivity. Although there was no significant difference between the phenological periods at post-irrigation ($p>0.05$), values increased across all periods, with the g_s still being at the highest level during the flowering period. Under irrigation water salinity of 2.5 dS m^{-1}, stomatal conductivity at pre-irrigation was the highest (391.1 mmol m^{-2} s^{-1}) in flowering period, but there was no significant difference between the vegetative and early fruit-growing periods. The lowest g_s was observed during the harvest period (291.9 mmol m^{-2} s^{-1}) in the same salinity level at pre-irrigation. At post-irrigation, the highest and lowest g_s occurred during the flowering (412.8 mmol m^{-2} s^{-1}) and harvest (273.0 mmol m^{-2} s^{-1}) periods, respectively. Under irrigation water salinity of 5.0 dS m^{-1}, the lowest g_s at pre- and post-irrigation occurred during the harvest periods as 227.6.7 and 245.9 mmol m^{-2} s^{-1}, respectively. Although there was no difference between the other periods at pre-irrigation, the highest g_s at post-irrigation occurred during the flowering period (460.1 mmol m^{-2} s^{-1}). Under irrigation water salinity of 7.5 dS m^{-1}, there was no significant difference between the vegetative, flowering, and early fruit-grown periods at both pre- and post-irrigation. During the harvest period, the lowest g_s was 218.4 and 225.4 mmol m^{-2} s^{-1} at pre- and post-irrigation, respectively. Statistical differences between the periods were the same under all irrigation water salinity treatments except 0.7 dS m^{-1}. These findings indicate that irrigation water salinity exerted a similar influence across different growth stages, with the exception under the control irrigation water salinity treatment. Additionally, the harvest period consistently exhibited the lowest values, suggesting a reduction in metabolic activity as plants matured.

Under irrigation water salinity of 0.7 dS m^{-1} treatment, the highest CCI at pre- and post-irrigation was observed during the vegetative period (64.2 and 68.0, respectively), while the lowest was observed during the harvest period (41.5 and 42.5, respectively). In the control treatment (0.7 dS m^{-1}), irrigation application did not alter the statistical classification of CCI values between the periods. Under irrigation water salinity of 2.5 dS m^{-1} treatment, the CCI was highest (66.0) at pre-irrigation during the vegetative period and lowest (42.1) during the harvest period. At post-irrigation, the CCI was still the highest (62.1) during the vegetative period and similar at pre-irrigation during the harvest period. Under irrigation water salinity of 5.0 dS m^{-1} treatment, the highest CCI was observed during the vegetative period at pre- and post-irrigation (64.7 and 66.0, respectively), while no significant difference was observed between all other periods at post-irrigation. Under irrigation water salinity of 7.5 dS m^{-1} treatment, the CCI at pre-irrigation was highest during the vegetative period (60.4) and lowest during the harvest period (36.3). At post-irrigation, the vegetative period continued to exhibit the highest CCI (57.4), whereas the harvest period remained the lowest (38.3). The vegetative period consistently demonstrated the highest CCI, reflecting a high chlorophyll content and photosynthetic capacity. The harvest period exhibited the lowest CCI, indicating a decline in the chlorophyll content as the plants approached maturity.

Figure 1 illustrates the leaf area index changes for the different salinity treatments during the growing season.

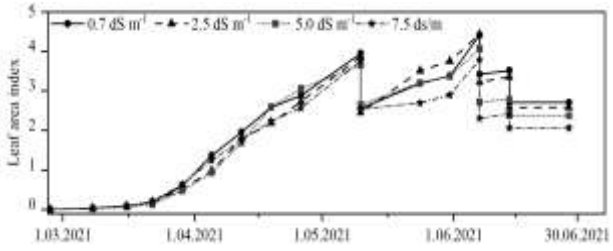

Figure 1. Leaf area index changes of different salinity treatments during the growing season

As shown in the Figure 1, plant development proceeded similarly under all saline water treatment

until the 75th day after planting (DAP) on 7 May 2021. After that day, leaf pruning resulted in LAI values ranging from 3.65 to 3.95. Following the second leaf pruning (DAP 103), the differences between the treatments became apparent. While the highest numerical LAI under 0.7 dS m^{-1} occurred at the end of the growing season, LAI decreased as irrigation water salinity increased. The effects of irrigation water salinity on the yield parameters are presented in Table 6.

Table 6. Effect of irrigation water salinity on yield parameters

Treatment	Total fruit production (g m^{-2})	Marketable fruit production (g m^{-2})	Not marketable fruit production (g m^{-2})
0.7 dS m^{-1}	16353.3	13970.4	2382.9
2.5 dS m^{-1}	15641.0	13371.1	2269.9
5.0 dS m^{-1}	15216.5	13087.4	2129.1
7.5 dS m^{-1}	15506.7	13478.8	2027.9
Significant level	ns	ns	ns

The means indicated with the same letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, and ns, significant at the $p < 0.05$, $p < 0.01$ level, and not significant, respectively.

Although irrigation water salinity had a slight impact on the different yield parameters, the statistical analysis results indicated that the yield parameters of tomatoes grown under drip irrigation were not affected by irrigation water salinity (Table 6). Maggio et al. (2007) [21] investigated the effects of eight distinct salinity levels (EC = 2.5 (non-salinized control), 4.2, 6.0, 7.8, 9.6, 11.4, 13.2; 15.0 dS m^{-1}) applied to tomato plants. They identified 9.6 dS m^{-1} as the critical threshold, beyond which significant alterations were noted in various physiological characteristics, notably affecting stomatal function and overall crop yield. In drip irrigation, daily or near-daily irrigation can be applied at very low rates. As a result of these irrigation applications, soil water is kept close to field capacity. Frequent irrigation using this method, where irrigation efficiency is high, prevents plants from being affected by both matrix and salt-based osmotic stress [11]. Furthermore, although salt accumulation occurs during drip irrigation, as in other methods, these salts accumulate on the soil surface between the drippers and on the outer wall of the wetted area. Consequently, this method has created a more favorable root zone environment for plants in terms of water use than other irrigation methods for low-quality water use. The findings of this study, as evidenced by the results obtained from the LAI and yield parameters, align with the aforementioned information.

4 Conclusions

This study, conducted under different irrigation water salinity levels, revealed a significant impact of phenological periods on plant physiological responses. In greenhouse tomato cultivation using the drip irrigation method, it was found that irrigation water salinity had no significant effect on CCI and yield, regardless of the phenological period, and that differences in plant development were limited. These results support the conclusion that frequent irrigation with low-quality water using the drip method reduces the osmotic effect caused by salt in the plant root zone by transferring salt accumulation to the outer edge of the wetted area. The results indicate that there are differences in the importance levels of stomatal conductance and chlorophyll content before and after irrigation. This finding can be attributed to the increase in the available water in the plant root zone, which leads to physiological changes as the plant rapidly adapts to the prevailing conditions. These findings are expected to contribute to a better understanding of agricultural irrigation strategies and plant physiology, providing valuable insights into optimal irrigation management using low-quality water under greenhouse conditions.

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Contribution of individual authors to the creation of a scientific article (ghostwriting policy)

Conceptualization, D.B. AND A.K.; methodology, C.K. and G.E.A; software, C.K.; validation, C.K.; formal analysis, C.K.; investigation, C.K., G.E.A. and D.B.; resources, D.B. and R.B.; data curation, C.K.; writing—original draft preparation, C.K.; writing—review and editing, A.K., C.K., D.B. and G.E.A A.N.; visualization, C.K. ; supervision, D.B.; project administration, D.B.; funding acquisition, D.B.. All authors have read and agreed to the published version of the manuscript.

Sources of funding for research presented in a scientific article or scientific article itself

This research was funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA) iGUESSmed project, Grant Agreement number: 1916. The PRIMA programme is supported by the European Union. under the Horizon 2020 Framework for Research and Innovation

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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