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Short-Term Waterlogging Depresses Early Growth of Sunflower (*Helianthus annuus* L.) on Saline Soils with a Shallow Water Table in the Coastal Zone of Bangladesh

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Abstract: Sunflower (*Helianthus annuus* L.), which is widely grown globally for its high-quality edible oil, is reasonably salt and drought tolerant but it is susceptible to waterlogging. In the saline coastal zone of the Ganges delta, sunflower is often exposed to sudden heavy rainfall during early growth but plant tolerance to such events is not known. Hence, we evaluated the effect of short-term soil inundation (referred to as waterlogging) for 0, 24, 48 and 72 h on sunflower at emergence, 2-leaf, and 4-leaf stages in early- and late-sown crops under field conditions (saline, clay-textured soil, and shallow groundwater). Waterlogging for 24 h did not affect sunflower at any stage but waterlogging for 48 and 72 h suppressed emergence and growth at the 2 and 4-leaf stages. Waterlogging for 72 h completely prevented the emergence for early sowing, whereas emergence was less affected for later sowing. Shoot and root dry weight were most affected at the emergence and 2-leaf stage, not at the 4-leaf stage. In conclusion, waterlogging caused by more than 24 h soil inundation at up to the 4-leaf stage severely depressed emergence and growth, indicating the need for effective drainage at sowing of sunflower in the low-lying coastal saline zone of Bangladesh.

Keywords: emergence; leaf chlorophyll content; shoot and root growth; soil inundation

1. Introduction

In many parts of the world, waterlogging risk for agricultural crops has changed because of less predictable rainfall related to climate variability [1–3]. Around 10–16% of global land is affected by waterlogging, including 10% of agricultural land in Bangladesh, India, Pakistan, and China [4]. Waterlogging due to the combined effect of monsoon rainfall and tidal influence affects large areas of land in the Ganges delta [5]. In the salt-affected south-west coastal region of Bangladesh, dry (Rabi) season crops are often waterlogged due to sudden intense rainfall, flat land, slow drainage, and the shallow water table [6,7]. Numerous studies have mentioned the adverse effect of waterlogging on crop seed germination and emergence, shoot weight, leaf senescence, and root growth [8–11]. Waterlogging creates anoxia and hypoxia in the soil, which limits oxygen diffusion and absorption of available nutrients by plants [12].

Sunflower is emerging as a promising crop for the dry season in fallow areas of coastal Bangladesh because of its adaption to drought and salinity in this environment [13–15]. However, sunflower is susceptible to damage during the germination or seedling stage due

to unexpected post-monsoon rainfall [16]. A recent study that varied the time of sowing for sunflower in this area showed significant yield loss (30%) due to waterlogging from sudden rainfall [16]. The same study reported that adequate surface drainage and appropriate agronomic management were needed to minimize the waterlogging risks. A subsequent field study tested waterlogging and drainage treatments under saline soil conditions in the coastal Ganges Delta and reported 48% lower yield in the waterlogged treatments relative to the combined surface and subsurface drainage treatment [17].

Waterlogging sunflower for four days at establishment time significantly reduced plant height, stem length, disk diameter, stem thickness, leaf number, and root dry weight [18]. By contrast, Orchard and Jessop [19] found that waterlogging for three days at the 6-leaf stage had no apparent impact, but more marked effects were observed when waterlogging was imposed for 6 and 9 days. The same study pointed out that waterlogging at anthesis more severely reduced leaf area, dry matter, and grain yield than at the 6-leaf stage. Similarly, sunflower was more sensitive to waterlogging at the flowering stage than the earlier stage based on the yield decline [20,21]. On the contrary, Singh et al. [22] reported that the waterlogging effect was more prominent at the 6 to 8 leaf stage (50 days after sowing) than at the flowering stage (80 days after sowing). All these studies were conducted in non-saline soils, which may underestimate the effects on sunflower in the Ganges saline coastal zone since waterlogging effects in crops are exacerbated by soil salinity [23].

Previous studies mostly imposed prolonged waterlogging treatments starting from the 6-leaf or at anthesis or grain-filling stages in the glasshouse or in pot experiments. However, little information is available on the effect of short-term waterlogging on the emergence and early-stage (2 and 4-leaf stage) growth of sunflower. Our previous field observations suggest that early stages of sunflower growth on saline clay-textured soil with a shallow water table were sensitive to heavy post-monsoonal rainfall events [16]. This study, therefore, aimed to evaluate the impact of short-term waterlogging on the emergence, and shoot and root development of sunflower at early growth stages, and to determine the most sensitive stage for waterlogging damage and the maximum duration of waterlogging that plants could tolerate under field conditions.

2. Materials and Methods

2.1. Site Description and Experimental Setup

The field experiment was sited in a farmer's field in Dacope in the Khulna district of Bangladesh ($22^{\circ}37'52''$ N and $89^{\circ}50'7''$ E) from November 2018 to January 2019. The area has an elevation of 2–3 m above mean sea level. A wet season rice crop was harvested from the field on 15 November 2018. The experimental area is located in the Ganges Tidal Floodplain [6]. The climate is subtropical with a hot and humid summer (March–June), a cool and dry winter (December–February), and a monsoonal rainy season (June–October). The soil texture was silty clay and clay at 0–30 and 30–60 cm soil depth, respectively. The soil (0–15 cm) had a bulk density of 1.50 Mg m^{-3} ; a pH of 7.7; an organic carbon concentration of 15 g kg^{-1} ; a total nitrogen concentration of 1.7 g kg^{-1} ; an extractable P concentration of 5 mg kg^{-1} ; and an exchangeable K concentration of 360 mg kg^{-1} . The groundwater level varied from 0.8 m in November 2018 to 1.1 m in January 2019. At sowing, soil salinity ($\text{EC}_{1:5}$) was 0.41, 0.29, 0.33, 0.38 and 0.49 dS m^{-1} at 0–15, 15–30, 30–45 and 45–60 cm soil depth, respectively.

2.2. Experimental Design

A factorial treatment combination was arranged in a randomized complete block design with three replications. Treatments comprised:

- a. Standing water duration (standing water above the soil surface) referred to as waterlogging: T1 = no waterlogging (control), T2 = 24 h waterlogging, T3 = 48 h waterlogging, and T4 = 72 h waterlogging;

- b. Growth stage for waterlogging imposition: V1 = waterlogging right after sowing (emergence), V2 = waterlogging when plants developed 2-leaves, and V3 = waterlogging when plants developed 4- leaves.
- c. Sowing dates: S1 = early sowing (1 December 2018) and S2 = late sowing (24 December 2018).

A hybrid sunflower cv. Hysun-33 was used as a test variety. Hysun-33 is promising in the study area for growth after wet season rice. The plot size was 3 m long and 3 m wide with a plant to plant spacing of 40 cm and a row to row spacing of 60 cm. Plots were separated by inserting polythene sheets from the surface to a depth of 50 cm and then by earthen bunds around each plot. The buffer zone between adjacent plots was 1.5 m. Plots were inundated by water to a depth of 5 cm above the ground surface by supplying water from a nearby canal. A perforated PVC pipe was installed in the plots to monitor the water level. Water was added continuously to maintain the desired water level during the waterlogging period. For early sowing, waterlogging was imposed on 2 December (one day after sowing), 15 December (14 DAS) and 25 December (24 DAS) 2018. For late sowing, waterlogging was applied on 25 December 2018, 5 January 2019 and 15 January 2019. The plant emergence, height, and number of leaves, shoot and root dry weight, maximum root length, total root length, and leaf area were measured at 34 DAS and 35 DAS for the first and second sowing, respectively.

2.3. Perched Water Monitoring

A 35 cm long perforated PVC pipe of 10 cm diameter was installed in each plot, leaving 5 cm above the ground surface and 30 cm below the ground surface. After imposing waterlogging at 24 h, 48 h, and 72 h, the plot water level above and below the ground surface was monitored until the water level declined to 30 cm below the ground surface. After the standing water disappeared, the excess waterlogging below the soil surface was estimated by using the waterlogging index SEW_{30} (the sum of excess waterlogging) as suggested by Sieben [24]. That is, the soil was considered to be waterlogged when the water depth below the soil surface remained at less than 30 cm.

2.4. Plant Height, Leaf Area and Leaf Chlorophyll Content

After completion of waterlogging in each sowing period, sunflower emergence in each plot was counted at 15 DAS. In each replication, a 1 m² area was selected to measure plant height, number of leaves, and leaf area. The leaf area of the youngest fully expanded leaf was estimated from the equation: $0.7 * (\text{maximum leaf length} * \text{maximum leaf width})$, at 34–35 DAS [18]. The above-ground biomass from 1 m² was collected and dried in an oven at 65 °C to measure shoot dry weight. The chlorophyll content of the leaves was measured using a hand-held chlorophyll content meter (CCM-200 instrument, Opti-Science, Hudson, NY, USA). Measurements were made at four points from two fully expanded leaves in the two selected plants, and average values were obtained for each treatment.

2.5. Root Measurements

From the selected 1 m² area, five plants were picked randomly at 34–35 DAS to measure total root length. Soils were excavated by removing blocks 10 cm along the row, 10 cm across the row and 20 cm deep. Each block was soaked in fresh water in a bucket for 3–4 h, and then roots were separated from the slurry after which they were washed through a 2 mm sieve. For each block, the total root length was measured manually using a ruler. Root dry weights were measured after oven drying at 65 °C to reach a constant weight.

2.6. Statistical Analysis

Data were analyzed as a three-way analysis of variance using STAR software (Version 2.0.1). The comparison of means was tested using the least significant difference (LSD) at the 95% confidence level. Regression analysis was done for the treatments assessed.

3. Results

3.1. Soil Inundation and Perched Water Depth

The changes in perched water depth after waterlogging are shown in Figure 1. The soil was inundated for 24, 48 and 72 h but thereafter, the water receded slowly below the soil surface in both early and late sowing. The decline in water depth to 30 cm in all waterlogging treatments took more than 102, 126 and 150 h, for 24, 48 or 72 h inundation, respectively (Figure 1).

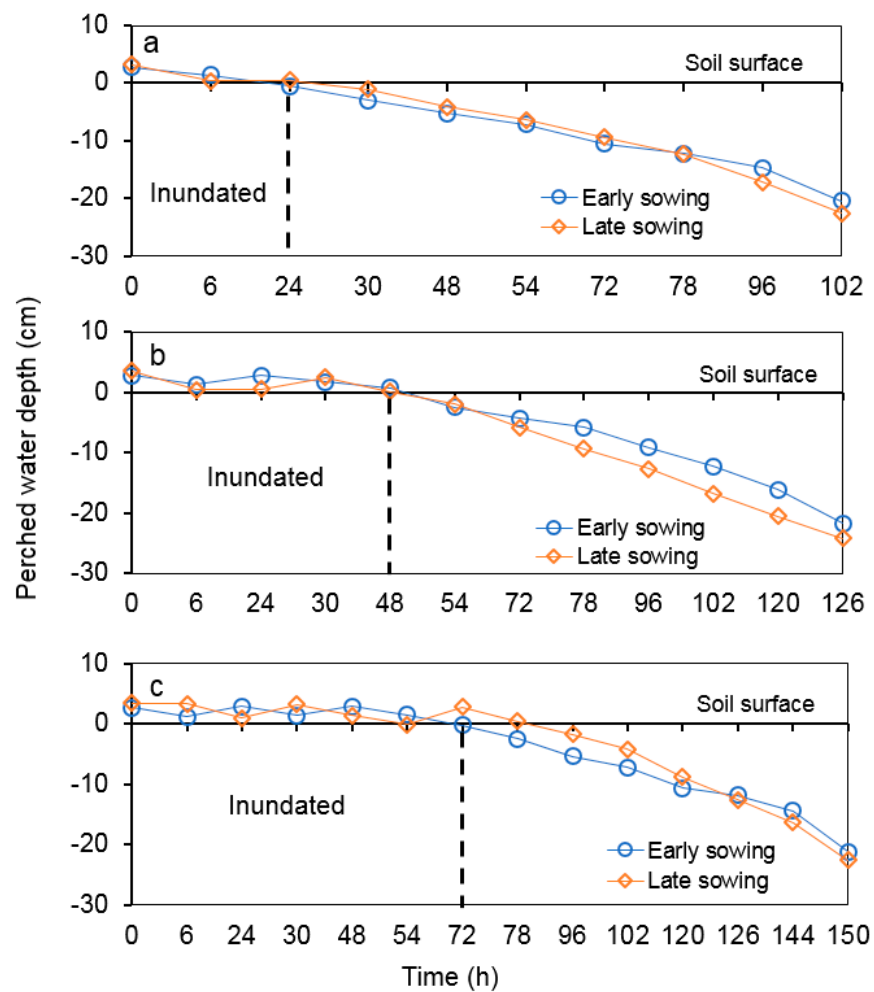


Figure 1. The dynamics of perched water depth above and below the soil surface during and following (a) 24 h, (b) 48 h and (c) 72 h of standing water in early and late sown sunflower. The left side of the dotted lines indicates the period when standing water was maintaining above the soil surface for each treatment and the right side indicates the waterlogging in the upper 30 cm of the soil profile [23].

3.2. Emergence

Emergence was significantly affected by waterlogging ($p < 0.001$), growth stages ($p < 0.001$) and sowing dates ($p < 0.001$) and there was a significant interaction among treatments ($p < 0.001$) (Supplementary Materials; Table S1). Sunflower emergence decreased with increasing waterlogging duration (Figure 2a,b). However, waterlogging for 24 h had a minimal impact (95% and 96% emergence for early and late sowing) compared to that of no-waterlogging (98% emergence) treatment. For early sowing, the emergence was only 20% for 48 h and there was no emergence for 72 h waterlogging. For later sowing, the emergence was satisfactory (67%) for 48 h but was strongly depressed by 72 h (below 40%) of waterlogging.

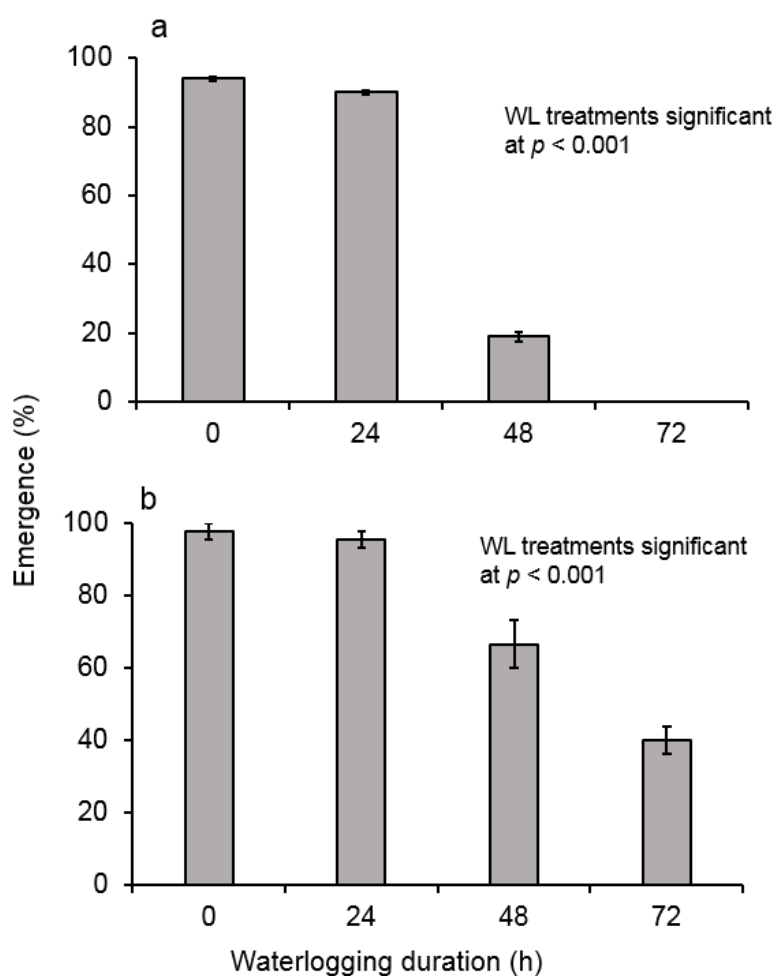


Figure 2. Emergence percentage of sunflower under different waterlogging treatments for (a) early sowing and (b) late sowing. Vertical bars in each graph indicate the standard error of the means. Values are means of three replications. WL indicates waterlogging.

3.3. Leaf Area and Chlorophyll Content

There was a significant effect of treatments ($p < 0.05$) and their interaction ($p < 0.001$) on leaf area and chlorophyll content (Supplementary Materials; Table S1). Waterlogging treatments reduced the leaf area and leaf chlorophyll content at different growth stages for early and late sowing (Table 1). The inundation for 24 h did not affect leaf area at any stage of development. However, a significant reduction in leaf area was observed after waterlogging for 48 and 72 h in all stages, except the 4-leaf stage for late sowing. For both sowing times, control (0 h) and 24 h waterlogging treatments developed higher leaf area (around 40–45% higher) than waterlogging for 48 and 72 h. Water inundation for 24 h had no obvious effect on chlorophyll content. For early sowing, water inundation for 48 and 72 h lowered the chlorophyll content by 8–27% relative to the control. For late sowing, chlorophyll content only decreased at the 4-leaf stage after 48 h inundation. Water inundation for 72 h showed a marked reduction in chlorophyll content in all stages (18–36% lower of control).

Table 1. Effect of waterlogging duration and stage of plant development during waterlogging on leaf area (cm²) per plant and leaf chlorophyll content (CCI) of sunflower in Dacope in the Khulna district of Bangladesh.

Sowing	Waterlogging Duration	Leaf Area (cm ²) at Different Stages			Leaf Chlorophyll Content (CCI)		
		Emergence	2-Leaf	4-Leaf	Emergence	2-Leaf	4-Leaf
Early sowing	Control	28 ^a	32 ^a	33 ^a	18.5 ^a	19.5 ^a	21.3 ^a
	24 h	26 ^a	30 ^a	31 ^a	17.4 ^{ab}	19.2 ^a	20.5 ^a
	48 h	16 ^b	17 ^b	19 ^b	16.2 ^c	18 ^a	16.3 ^b
	72 h	0 ^c	17 ^b	18 ^b	-	14.5 ^b	15.5 ^b
Late sowing	Control	36 ^a	35 ^a	40 ^a	14.3 ^a	17.8 ^a	17.7 ^a
	24 h	33 ^a	32 ^a	29 ^{ab}	14.5 ^a	17.4 ^a	17.9 ^a
	48 h	19 ^b	14 ^b	22 ^b	12.8 ^{ab}	16.5 ^a	13.2 ^b
	72 h	17 ^b	18 ^b	18 ^b	11.7 ^b	12.9 ^b	11.4 ^b

Means with the same letter (a, b, c and ab) in a column are not significantly different. Means are the average of three replications.

3.4. Plant Height

The effect of waterlogging at different stages on plant height for two sowing dates is shown in Figure 3. Plant height decreased after 48 and 72 h of waterlogging for early sown sunflower except at the 2-leaf stage (Figure 3a–c). Waterlogging had no effect on the plant height of late-sown plants at 2-leaf and 4-leaf stages.

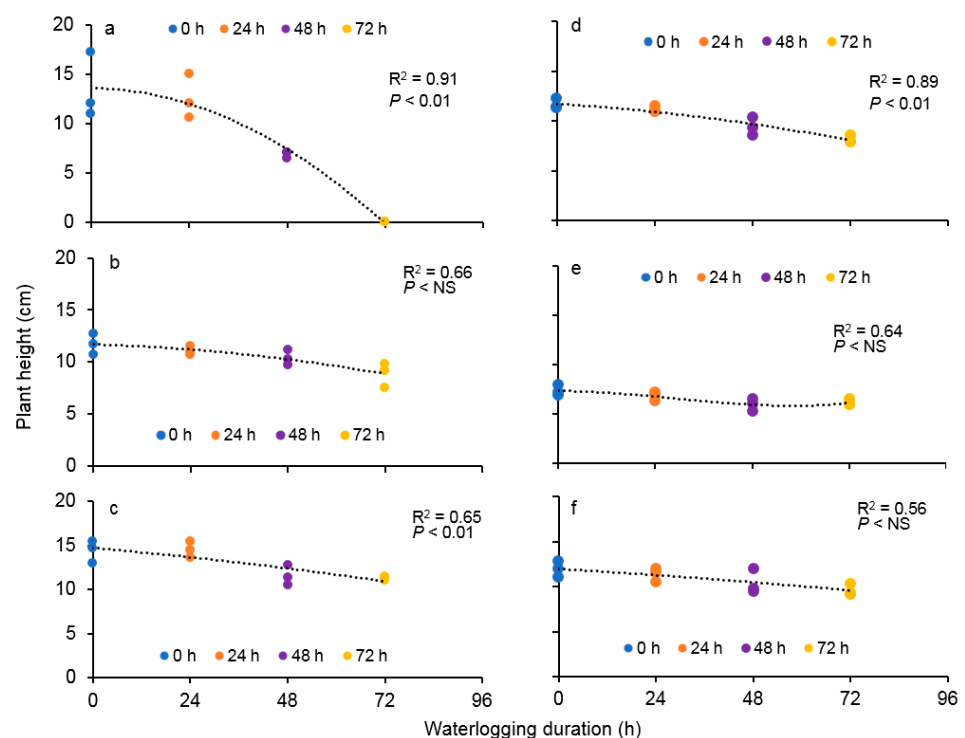


Figure 3. Effect of different waterlogging durations on sunflower plant height for early sowing (a) emergence, (b) 2-leaf stage and (c) 4-leaf stage, and for late sowing (d) emergence, (e) 2-leaf stage and (f) 4-leaf stage. Each value was the mean of three replications.

3.5. Shoot Dry Weight

Shoot dry weight (SDW) was impacted by waterlogging stress ($p < 0.001$) and sowing dates ($p < 0.01$) but not growth stages ($p > 0.05$). Shoot dry weight significantly decreased with soil inundation for more than 24 h at both sowing dates (Table 2). Indeed, waterlogging for 72 h caused more than 50% reduction in SDW relative to no-waterlogging treatment. Waterlogging treatments had similar effects on SDW for plants waterlogged at the 2-leaf

and 4-leaf stages for early and late sowing. There was a higher SDW for early sown plants compared to the late sown plants.

Table 2. Effect of waterlogging duration and stage of plant development on shoot dry weight (g) per square meter in Dacope in the Khulna district of Bangladesh.

Sowing	Waterlogging Duration	Shoot Dry Weight (g)		
		Emergence	2-Leaf	4-Leaf
Early sowing	Control	5.9 ^a	4.9 ^a	4.5 ^a
	24 h	5.8 ^a	4.7 ^a	4.4 ^a
	48 h	2.2 ^b	2.3 ^b	2.3 ^b
	72 h	0 ^c	1.4 ^c	2.1 ^b
Late sowing	Control	4.2 ^a	3.9 ^a	4.9 ^a
	24 h	3.8 ^a	3.8 ^a	3.9 ^a
	48 h	2.4 ^b	2.3 ^b	2.7 ^b
	72 h	1.8 ^b	1.9 ^b	2.1 ^b

Means with the same letter (^a, ^b and ^c) in a column for each sowing date are not significantly different. Means are the average of three replications.

3.6. Root Dry Weight

The root dry weight was significantly reduced by waterlogging treatments except at the 4-leaf growth stage (Figure 4). Soil inundation at emergence and 2-leaf stages for 24 h slightly affected root dry weight but waterlogging for 48 or 72 h markedly decreased root dry weight. Water inundation for more than 48 h reduced the root dry weight by about 60% relative to no-waterlogging treatment in both early and later sowing.

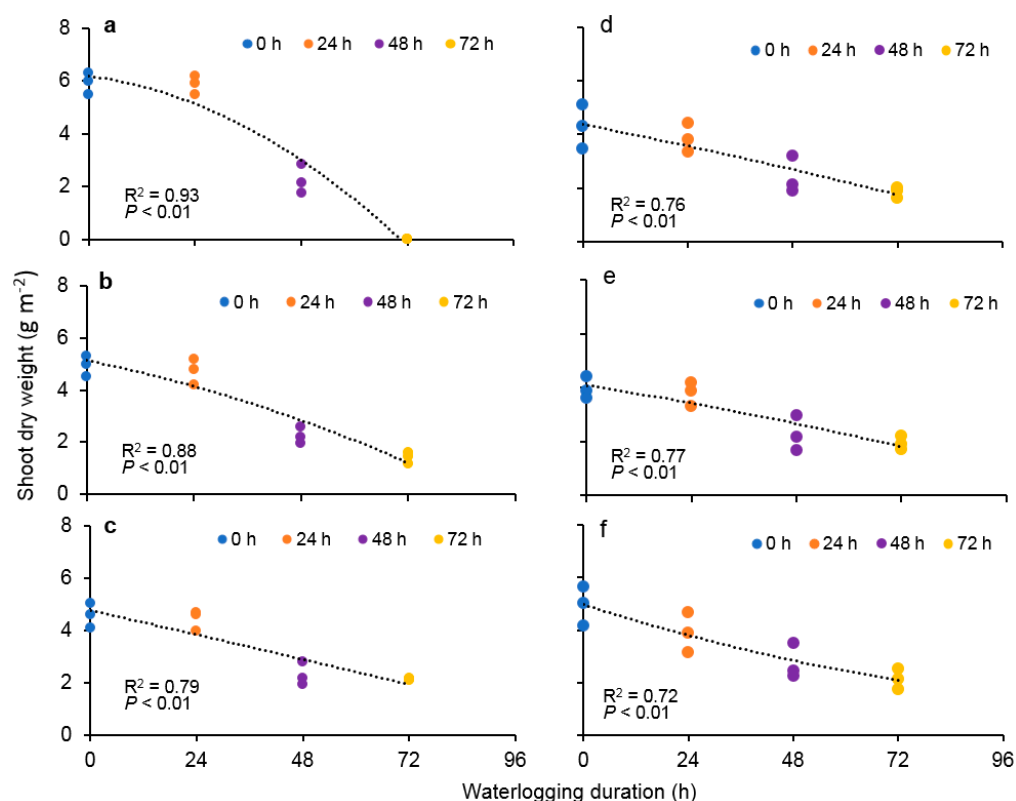


Figure 4. Effect of waterlogging duration on sunflower root dry weight for early sowing (a) emergence, (b) 2-leaf stage and (c) 4-leaf stage, and for late sowing (d) just after sowing, (e) 2-leaf stage and (f) 4-leaf stage. Each value was the mean of three replications.

3.7. Total Root Length

Waterlogging treatments for 48 and 72 h at emergence severely reduced the total root length at both sowing times (Figure 5a,d) when plants were waterlogged at emergence but not when waterlogged at the 2-leaf and 4-leaf stages (Figure 5b,c,e,f). With and without waterlogging, at late sowing plants maintained comparatively higher total root length compared to early sowing.

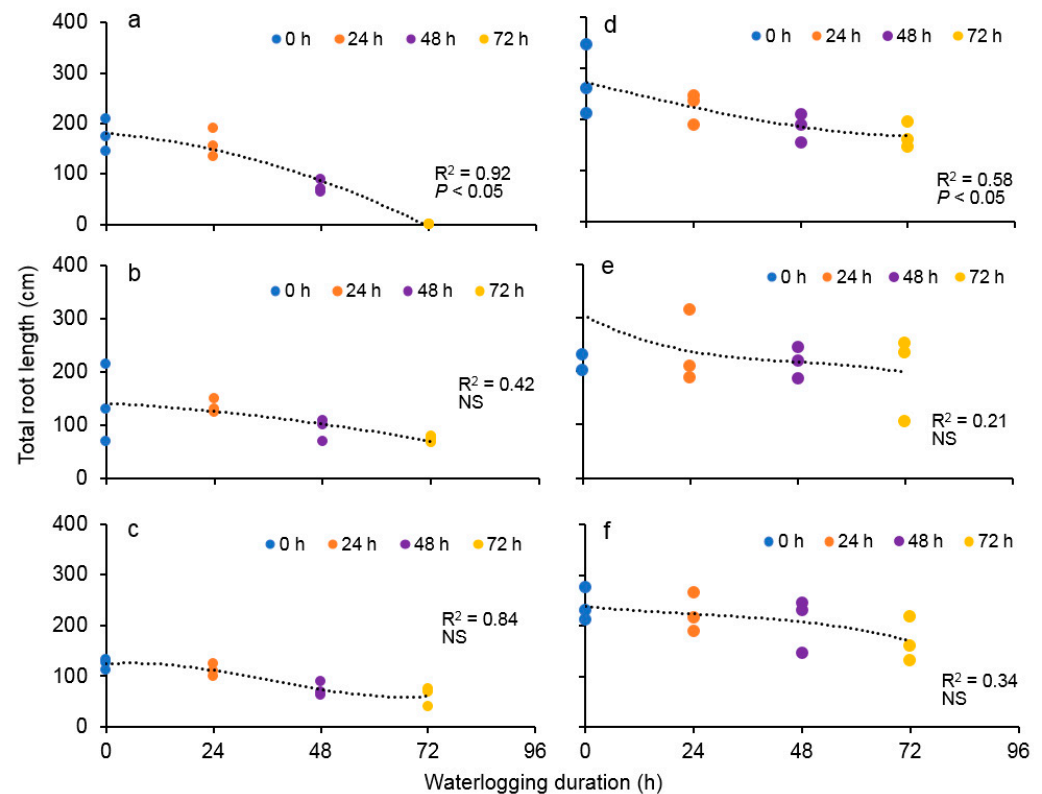


Figure 5. Effect of waterlogging duration on sunflower total root length for early sowing (a) emergence, (b) 2-leaf stage and (c) 4-leaf stage, and for late sowing (d) just after sowing, (e) 2-leaf stage and (f) 4-leaf stage. Each value is the mean of three replications.

4. Discussion

Sunflower tolerance of waterlogging differed with the stage of plant development, the time of sowing, and the duration of water inundation. In general, plant tolerance to waterlogging is associated with the genotype, development stage, the extent of soil drainage, environmental conditions, the interaction of waterlogging and soil salinity and the depth of the groundwater table [18,25,26]. In this experiment, the slow decrease in perched water depth may partly be due to the shallow water table (0.85–1.10 m) and to the soil type (clay-textured) and land shape (flat and low land) in the study area. This slow decrease in perched water extended the waterlogging duration, which may exacerbate waterlogging effects on plant growth and development. Similarly, the experiment conducted by Cox and McFarlane [27] in southwestern Australia reported that the intensity of waterlogging was increased by a shallow water table and subsoil clay.

In the present study, soil inundation for 24 h did not affect emergence, plant height, shoot weight and root growth. However, the inundation for 24 h continued to produce soil waterlogging in the 0–30 cm soil layer for up to 102 h (Figure 1), indicating that sunflower *cv.* Hysun-33 has the ability to adapt to waterlogging. Although the adaptation mechanism was not examined in this study, a previous study reported that sunflower cultivars have the ability to form aerenchyma for better root aeration under waterlogging stress [28].

In addition, sunflowers developed adventitious roots after exposure to waterlogging (observations only), resulting in better oxygen supply to roots tips to maintain root function under waterlogging [24]. These adaptive responses may have been sufficient to provide tolerance to the 24 h surface inundation and subsequent root zone waterlogging. Nevertheless, our results suggest that farmers only have a 24-h window following heavy rainfall to implement effective drainage. Drainage can either be implemented as a rapid response to heavy rainfall [16], or pre-installed as surface or sub-surface drains [17]. While 48 h inundation and subsequent waterlogging in the root zone was damaging to shoot and root growth, sunflower plants showed some evidence of recovery. By contrast, when inundation was extended to 72 h, sunflower emergence was completely inhibited for early sowing with only 40% emergence for later sowing.

Sunflower are more highly sensitive to waterlogging at the sowing (emergence period) and 2-leaf stage than at the maturity stage [29]. In this study, the major reduction in seedling emergence appeared for inundations of more than 24 h duration. Similar results were reported by Loose et al. [30] and Albuquerque and de Carvalho [31], who demonstrated that waterlogging for 48 h and water potential in excess of 0.0001 MPa sharply depresses sunflower emergence. However, waterlogging caused a more severe reduction in seed emergence for early sown than later sown sunflower. This was probably related to higher soil water content (48%, w/w) during the early sowing than during the later sowing (34%, w/w). In addition to waterlogging, for early sowing, some plant root rot was visible due to wet soil and cloudy weather.

The 72 h of inundation and the following root zone waterlogging inhibited leaf expansion, leaf chlorophyll content and plant height of sunflower. A previous study showed that waterlogging for 3 days at the 6-leaf stage had no obvious effect on plant growth at the early stage [18], which differed from the present study where more than 24 h inundation of the saline, clay-textured soil with a shallow water table reduced shoot dry weight and root dry weight. The reduction in shoot dry weight due to waterlogging is commonly related to shorter plant height and stem length, smaller leaf area and lesser total root length [17,19]. These effects are attributed to very low energy production (2 mole ATP instead of 36 from a mole of hexose) [32] by waterlogged roots along with depressed nutrient uptake due to oxygen deficiency for root function [33,34]. There was no consistent effect of waterlogging on total root length and root weight at the 4-leaf stage. A similar result was observed for sunflower where root dry weight and the length of taproots were less affected by waterlogging for 72 h at the 6-leaf stage [23]. The reason for lower root dry weight at emergence and the 2-leaf stage due to water inundation can be attributed to the soil limited oxygen supply to roots due to excess water, which constrains subsequent root growth and development [35]. However, sunflower has the potential to adapt to waterlogging stress by producing adventitious roots at later stages (4–6 leaf stage). Previous studies have also noted that plants produce adventitious roots for survival in waterlogged conditions [10,17,36]. Another adaptation of plants following inundation is the acceleration of ethylene production, which inhibits the growth of roots but induces aerenchyma formation to enhance the oxygen supply to root tips for their survival [37].

Although waterlogging stress has been shown in most species, the effect can be more aggravated in landscapes that are affected by soil salinity [38]. The present study took place in the low-lying, salt-affected coastal zone of the Ganges Delta. Hence, during the dry season when sudden heavy rainfall creates waterlogging, it also causes an additional soil constraint where salinity and waterlogging occur together. These combined stresses exacerbate the uptake of toxic ions from the root zones into leaf tissues [22]. Previous studies have addressed short-term waterlogging for 4–6 days and the results varied with the stage of plant development [18,20]. However, in the present study, short-term waterlogging for 48 and 72 h affected sunflower emergence, leaf expansion, plant height and root growth. This can be attributed to the combination of waterlogging and salinity in the root zone, which is more damaging for crop growth and yield than waterlogging alone [39]. Moreover, in field conditions, plants often experienced intermittent episodes of inundation rather than

permanent waterlogging. Hence, plants may recover in part from the damaging effect of waterlogging when re-aeration of the root zone occurs after a period of stress. Nevertheless, the risk of crop damage can be decreased by installed surface and/or sub-surface drainage to reduce waterlogging effects and improve plant growth and yield [16,17].

5. Conclusions

The effect of waterlogging duration on sunflower was investigated at three stages of early sunflower development (emergence, 2-leaf and 4-leaf) and with early and late sowing. In the present study, sunflower response to waterlogging varies with duration of waterlogging, development stage, and sowing time. Water inundation for 24 h had no detrimental effect at the early stage (emergence to 4-leaf stage) but waterlogging for 48 and 72 h significantly depressed emergence and growth at the 2-leaf and 4-leaf stages. For early sowing, emergence was entirely inhibited by waterlogging for 72 h, although this effect was minimal for later sowing when the impact of waterlogging (72 h) was less severe. Shoot dry weight was significantly reduced at all development stages because of waterlogging stress, but this effect was more marked at the emergence and 2-leaf stages. We conclude that waterlogging for more than 24 h adversely affects the early stage of sunflower growth in saline soils, but this effect can be minimized if waterlogging happens at the later stage of sunflower growth and with a delayed sowing date. A further study could be carried out to document the changes that occur in the soil due to waterlogging and soil salinity and the resultant root adaptations, including the effect on ion uptake and toxicity in plant shoots and roots.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/soilsystems5040068/s1>, Table S1. Significance (p-values) of effects sowing dates, growth stages and waterlogging on sunflower growth parameters at Pankhali, Dacope, Khulna in 2018-19.

Author Contributions: P.L.C.P.: Concept, fieldwork, data collection and analysis, preparation of tables and figures and writing the original manuscript; R.W.B., E.G.B.-L. and E.K.: Assisted with the concept, supervised fieldwork, reviewing and editing the manuscript; M.M.: Resources, reviewing and editing the manuscript; K.K.S.: Assisted with the statistical analysis. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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