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RESEARCH ARTICLE

Heavy metals and microplastics derived from laboratory effluents enhance toxicological risks to the ecosystems of canals in Bangladesh

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Abstract

The occurrence of chemical effluents in different water bodies is an emerging concern. However, the effect of laboratory effluents on the canal ecosystem in Bangladesh is largely unknown. In this study, we collected 10 components of the canal ecosystem including sediments, water, fish, crabs, snails, phytoplanktons, and weeds specifically from canals that directly receive laboratory effluents. We examined the occurrence of both the essential (Fe, Mn, Cu, and Zn) and toxic (Pb, Cd, Cr, Co, Ni, and As) metals as well as microplastics (MPs). Results explored that laboratory effluents strongly interact with the components of the canal ecosystem and enhance the abundance of toxic metals in the canal hydrosystem. Furthermore, diverse types of MPs including fibers, fragments, and microbeads were observed in all components of the canal ecosystem. Remarkably, our results unveiled that the co-occurrence of pollutants was more severe in benthic organisms like snails or crabs might be due to their omnivorous food habits. The cumulative pollutant accumulation was much higher in all components of the canal ecosystem indicating a greater ecological risk. Although the potential risk index (RI) or hazardous index (HI) from fish ingestion was found within acceptable limits, the biomagnification of pollutants due to repeated ingestions may have strong ecotoxicological impacts even at very low concentrations.

Keywords Laboratory waste · Heavy metal · Microplastic · Co-occurrence · Ecotoxicity

Introduction

Over the years, toxic pollutants from laboratory effluents have become an increasing concern globally due to their significant impact on environmental health and ecosystem integrity (Martins et al. 2019; Valavanidis and Vlachogianni 2015). In accordance with the developed countries, scientific disciplines alike biology, biotechnology, ecology, and toxicology, have greatly advanced in the past decade in Bangladesh with groundbreaking discoveries and new developments (Hashem et al. 2019). Despite research innovations, the groundswell concern is that after the application of laboratory reagents, agrochemicals, pharmaceuticals, radionuclides, and solvents

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are directly or indirectly released into the sewerage system due to poor treatment systems and/or ignorance. Such a widespread laboratory discharge reaches the urban sewer network and finally to the environmental compartments including sediment, water, and biota (Rakodi 2002; Valavanidis and Vlachogianni 2015). As water bodies receive the discharges of sewage evacuated precariously and without prior treatment, it leads to the degradation of the overall ecological status of the fluvial hydrosystem and surrounding environment (Liu et al. 2021).

Considerable existing evidence demonstrated that toxic heavy metals present in laboratory chemical effluents can destroy the ecological balance by affecting the components of the canal ecosystem. Moreover, metals are merely metabolized to nontoxic compounds, hence once enter the hydrosystem, they are redistributed into the ecological components and aggregated by consumption (Sonone et al. 2020; Li et al. 2025; Yuan et al. 2025). Consequently, the discharge of laboratory chemical effluents or wastes not only triggers pollution but also causes a decline in biodiversity in receiving hydrosystems. Every day a large quantity of laboratory waste is produced that comprises not only chemical effluents but also rubber, cotton, glass, and plastic materials, and most shockingly most portions of this waste are not recycled (Sahu et al. 2021). Despite the practice of recycling and awareness campaigns, over eight million tons of single-use plastic enter the ecosystem every year stimulating the environmental crisis throughout the globe (Häder et al. 2020). Upon entrance into the environment, large-size plastic degrades to microplastics (MPs) over time with various natural or anthropogenic processes including microbial activities, weathering, and oxidation or hydrolysis (He et al. 2018).

Additionally, the special characteristics of MPs like surface area, porosity, and hydrophobicity provide intrinsic potential to adsorb and accumulate toxic metals. Hence, the abundance of MPs in aquatic environments greatly influences the migration of toxic heavy metals to the ecological components and food web (Lin et al. 2022). Therefore, the occurrence of laboratory chemical effluents in fluvial water bodies and their scaled-up toxicological effects on biotic organisms is a global concern (Ford et al. 2021; Sahu et al. 2021). Besides, crop irrigation with polluted water not only impacts agricultural production but also creates a considerable threat to human health (Singh 2021). Although the availability of heavy metals or even MPs in aquatic environments has been extensively studied, their concurrence in the components of the canal ecosystem remains largely unknown (He et al. 2018). Moreover, around the world countries are struggling to arrive at an effective regulatory regime to control the environmental discharge of chemical effluents, which threatens the sustainable structure and functionality of the receiving hydrosystem (Rajaram and Das 2008). Therefore, we conducted an ecotoxicological study of heavy metals and MPs and their co-occurrence to assess the potential risk from discharged laboratory chemical effluents on the components of the canal ecosystem including sediments, water, fish, and benthic organisms like snails, crabs, and even phytoplankton. These findings may play an important role in regulatory decisions in environmental management practices aimed at minimizing the toxicological impacts of laboratory waste on the environment and human health.

Materials and methods

Selection of study area and sampling

In the present experiment, the study area was selected based on a preliminary survey, specifically the canals in Sylhet city of Bangladesh that directly receive laboratory discharges from two leading science and technology universities (Supplementary Fig. S1). Notably, there was no documented wastewater treatment system currently in practice in those laboratories. The geographical location of the canals are 24° 54′ 33.12″ N, 91° 54′ 7.2″ E, and 24° 54′ 43.2″ N, 9 1° 49′ 55.92″ E which are directly linked to a major river of Bangladesh named “Surma” (25° 1′ 0″ N, 91° 21′ 0″ E) that ends to the ocean Bay of Bengal (Fig. 1). After selection of the location, we collected water, soil sediment, and small indigenous fish including *Channa striata*, *Mystus Tengara*, *Cyprinus rubrofuscus*, *Amblypharyngodon microlepis*, *Trichogaster fasciata*, and *Fenneropenaeus indicus*, non-indigenous fish including *Ctenopharyngodon idella* and *Oreochromis niloticus*, crab (*Geothelphusa dehaani*), snail (*Bithynia tentaculata*), phytoplankton, duckweed (*Lemna minor*), waterhyacinth (*Eichhornia. crassipes*), and water lily (*Nymphaea nouchali*) samples from the study area in September 2022 as the components of the canal ecosystem. Hand-net sampling method was used to collect water samples. Approximately 50 L of the top 20 cm of surface water was manually collected using a metal bucket with a 20-cm diameter covering both the upstream and downstream (Dong et al. 2021). To avoid plastic contamination metal sieves and glass bottles were used during water collection. However, separate plastic bottles were used to store the water samples specifically intended for heavy metal measurement. Soil sediment (~ 5 kg in total) was collected from 10 selected spots using a 0.1-m^2 EK man stainless steel grab and the soil sediment from the top (~ 5 cm) was sampled by a metal scoop and homogenized as representative samples (Wu et al. 2020). The sediment samples (~ 250 g) were placed into porcelain Teflon containers and stored at – 25 °C before undergoing analysis in a laboratory. The snail, crab, and fish as biota specimens were dissected to separate their shell and tissue, and then freeze-dried. The quarter methods were used for plankton, duckweed, water hyacinth, and water lily to reduce the sample volume and then stored at – 25 °C for further analysis.

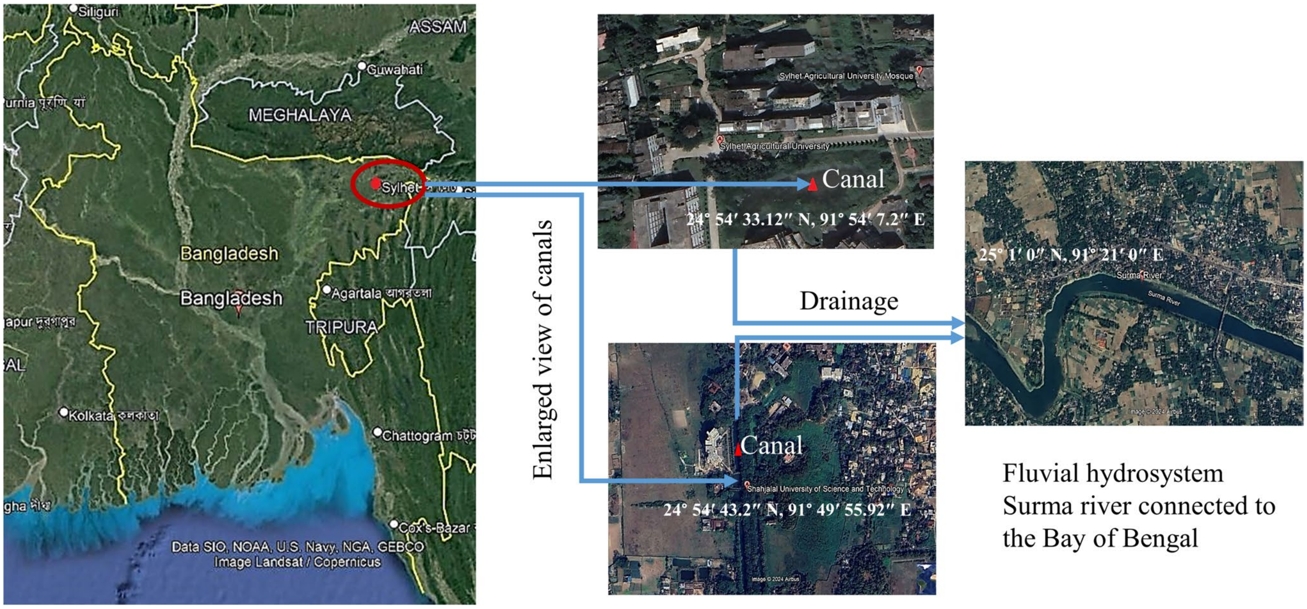

Fig. 1 Geographical locations of canals receiving laboratory discharges and connected to the major river (Surma River) in Bangladesh

Analysis of heavy metals

To assess the ecological risk of trace metals, we examined the abundance of Fe, Mn, Cu, Zn, Cd, Pb, Cr, Co, Ni, and As in all the 10 collected canal ecological samples. In brief, after oven drying at 65 °C for 36 h all samples were ground to powder using an agate mortar and pestle and pass through a 180-mesh nylon sieve to remove large particles. The screened sample was collected in acid-rinsed glass vials and stored in desiccators. Then 0.5 gm of each sample (dry weight) was put into an acid-washed PTFE vessel and digested with the method of di-acid mixture as previously discussed (Hasan et al. 2015). After digestion, samples were diluted to the desired volume with Milli-Q water and analyzed with an atomic absorption spectrometer (model: AA7000, Shimadzu, Japan).

Extraction of microplastics

Microplastics in water samples were extracted by following the previously described method (Alam et al. 2019). In brief, the water samples were treated with 40 mL of 30% H_2O_2 for 24 h at room temperature to digest other organic particles. The soil sediment samples, soft tissue from various organisms (i.e., the digestive tract of fish, the stomach of crab, and flesh of snails), duckweed, water hyacinth, water lily, and phytoplankton were collected and dried in an oven at 60 ◦C for 72 h. Then the samples (1 g per 15 ml acid) were digested using a mixture of 65% HNO_3 and 68% perchloric acid ($HClO_4$) in a 4:1 v/v ratio. This mixture was left overnight at room temperature, followed by 10-min boiling which eradicated biogenic material, as previously described (Lusher et al. 2017). The samples were filtered using a vacuum pump (VE115N, New Delhi) through a glass microfiber filter (GF/C, Whatman, 1.2 µm pore size, 47 mm diameter, China). The filter papers were dried at 60 °C for 10 min and stored in petri dishes for further microscopic observation.

Isolation and characterization of microplastic through microscopic observation

The Whatman GF/C filters were observed thoroughly in a z-shaped pattern from left to right under a stereomicroscope (B-193PL, OPTICA, Poteranica, Italy), whereas images were taken by an OPTICA C-B5 digital camera. The abundance and physical characteristics of the microplastics were visually identified and described according to color, shape, and size (Kasamesiri et al. 2023). The morphotypes of microplastics were classified into fibers, fragments, and microbeads. The particles were measured along their longest dimension using ImageJ software. The overall abundance of microplastics in water was estimated per liter, and sediment and organisms were estimated per gram (g), as well as the total abundance of fibers, fragments, and microbeads was calculated by percentage (%) for all samples.

Assessment of pollution load index

Heavy Metal pollution index (HMPI) is a trustworthy index that assesses the HMs contamination in aquatic ecosystems. HMPI values were calculated as the geometrical mean of values for HMs in all ecological sample components (Töre, Y et al. 2021); HMPI (mg kg^{-1}) = (C1 × C2 × ⋯ × Cn) $^{1/n}$, Where C = the mean concentration of HMs in samples (mg kg^{-1}), n = number of element. Again, to assess the risk of MPs, we also calculated the MPs pollution load index (MPLI) using the following equations according to the previous discussion (Kasamesiri et al. 2023). CFi = Ci/C$_0$, PLI = $\sqrt{\text{CFi}}$, Where, Ci = the abundance of heavy metals or MPs at each sample, and C$_0$ = the lowest MPs abundance in the reservoir based on the literature (Supplementary Table S2). The risk of pollution was categorized into low, moderate, high, and extremely high (Supplementary Table S3) pollution as previously discussed (Wang et al. 2021).

Investigation of EDI (estimated daily intake), hazardous quotient (HQ), and risk index

According to the guidelines of the United States Environmental Protection Agency (USEPA), the EDI values of heavy metals and MPs were determined for Bangladeshi people through the consumption of canal fish by using the following equation as previously discussed (Hussein et al. 2023). EDI (mg kg^{-1}, day^{-1}) = Cm × FIR BW^{-1}, where Cm is the metal concentration at g gm^{-1} wet basis; FIR is the per capita daily intake of fish and BW body weight of an adult. The FIR value was set at 62.6 gm and the BW was 55 kg average for an adult of Bangladesh (Rifat et al. 2023). To calculate the EDI for MPs, after filtration, parts of adsorbent filter paper were placed on a non-absorbent aluminum foil surface and dried at 60 °C for 12 h for complete removal of moisture and then stored in an air-tight container with silica gel as discussed previously (Knott 2005). Afterward, the abundance of MPs was determined using the formula, MPab = (SA$_F$-BSA$_F$)/AD$_S$. Where SA$_F$ indicates the weight of samples adsorb filter paper, BSA$_F$ is the weight of blank samples adsorb filter paper and AD$_S$ indicates the amount of digested samples and the unit expressed as mg kg^{-1} of samples.

Similarly, following the USEPA rules, the hazardous quotient (HQ) of toxic heavy metals was evaluated using the following formula: The hazard quotient (HQ) HQ = EDI/RfD × 10^{-3}, where the RfD is the oral reference dose (Hussein et al. 2023). The RfD values of Pb, Cd, Cr, Zn, Cu, Mn, Fe, and MPs were 0.004, 0.001, 0.0003, 0.3, 0.001, 0.14, 0.7, and 0.2 mg kg^{-1}, day^{-1} respectively (Hussein et al. 2023; Namira et al. 2023). Moreover, for Pb and Cd, we calculated the risk index (RI) due to their high abundance in fish by using the formula, RI = EDI × SF; where SF is the cancer slope factor which sets 0.0085 and 0.038 mg kg^{-1}, day^{-1} for Pb and Cd, respectively (OEHHA, 2008). The risk of cumulative toxic metal uptakes was calculated by using the hazardous index (HI) formula HI = THQ (Pb) + THQ (Cd) + THQ (Cr) based on their abundance according to the previously discussed method (Hussein et al. 2023). According to USEPA, when the value of HQ or HI is above one, it is considered a pollutant that has associated human health risks (Ahmadi-Jouibari et al. 2023).

Quality assurance and quality control

In the present study, all the plastic materials were avoided during sample processing. All equipment was carefully cleaned with deionized water three times before use and wrapped in foil when not in use. During sampling and laboratory analysis, all researchers wore nitrile gloves and cotton aprons. Blank tests were used to evaluate potential contamination from the laboratory. To avoid contamination, all the liquid used in the experiments (i.e., distilled water, H_2O_2, HNO_3, and $HClO_4$) were filtered through a washed GF/C (1.2 µm pore size, 47 mm diameter) glass microfiber filter (Whatman, China).

Statistical analysis

The mean was calculated by averaging the values of three replications and then the standard deviation was calculated. The ANOVA analysis was performed to test the differences among the variables. If significant effects were found, means were separated by least significant differences (LSD). All the statistical analysis was performed using R software (version: R × 64 3.6.2, Auckland, New Zealand). Principal component analysis (PCA) was accomplished using Origin software-2018 (OriginLab Inc., Northampton, MA, USA) as discussed (Jahan et al. 2022).

Results

The abundance of heavy metals in canal ecological samples

To understand the abundance of heavy metal pollutants in the canal hyrdosystem, we sampled 10 components of the canal ecosystem including sediment, water, small indigenous fish (≤ 20 cm in size), non-indigenous fish (≥ 20 cm in size), crab, snail, phytoplankton, duckweed, water hyacinth and water Lily (Fig. 2), specifically from canals those directly receiving laboratory discharges contain different types of organic, inorganic acid and salts (Supplementary Fig. S1). To investigate the toxicological abundance of metals, we categorized metals into two groups, the first group comprised essential metals such as Fe, Mn, Cu, and Zn, and the second group comprised the toxic heavy metals including Pb, Cd, Cr, Co, Ni, and As. Results explored that among the essential metals Fe accumulation was higher in all the ecological samples, followed by Zn, Mn, and Cu, respectively. Again, among the different ecological samples, the highest accumulation of Fe was obtained in crabs followed by water lily, small indigenous fish (S.I. fish), and sediment (Table 1). For example, the highest 467.87 mg kg^{-1} Fe accumulation was found in crabs and the lowest 79.2 mg kg^{-1} Fe was obtained in phytoplankton, exceeding the WHO recommended level (20 mg kg^{-1}) (Nazir et al. 2015). Likewise, the canal water contained above WHO recommended level of 1 mg L^{-1} Fe (Supplementary Table S1). The Mn and Cu content remained below the permissible limit, i.e., 2.06–2.44 mg kg^{-1} and 0.03–3.1 mg kg^{-1} (or mg L^{-1} for water only) of Mn and Cu, respectively in all the different samples. In contrast, Zn content significantly varied among the samples and was fairly found at a low concentration in surface water, e.g., 0.41 mg L^{-1} (Table 1). Among the samples, the lowest 2.34 mg kg^{-1} Zn was found in water lily and the highest 12.98 mg kg^{-1} in sediment which remains below the WHO recommended limit of 50 mg kg^{-1} of samples (Nazir et al. 2015).

Correspondingly, the accumulation of toxic heavy metals precisely Pb, Cd, and Cr significantly varied among the canal ecological samples. Although, in canal water, Pb and Cd concentrations were found in 0.055 and 0.003 mg L^{-1}, the presence of Cr, Ni, Co, and As was below detectable limits (Table 1). The highest 0.91 mg kg^{-1} Pb accumulation was found in sediment followed by snail, phytoplankton, and water hyacinth. Surprisingly the accumulation of Pb was also found in fish muscle samples ranging between 0.035 and 0.11 mg kg^{-1} on a dry weight basis whereas the maximum permissible limit is 0.1 mg kg^{-1} as per FAO and WHO (Islam et al. 2015). Similarly, the maximum permissible limit of Cd was found to be exceeded in all the crab, fish, sediment, and plant or phytoplankton samples of canals. For example, the highest 0.24 mg kg^{-1} was found in sediment followed by water hyacinth (0.157 mg kg^{-1}), snail and duckweed (0.151 mg kg^{-1}), crab (1.47), phytoplankton (0.141 mg kg^{-1}), and fish samples (0.03–0.05 mg kg^{-1}), respectively. In contrast, the Cr content was below the detectable limit in the water while a very significant amount was recorded in sediment (2.83 mg kg^{-1}) and crabs (0.6 mg kg^{-1}) followed by the phytoplankton, duckweed, and water hyacinth (Table 1).

Metal pollution index in the component of canal ecosystem influenced by laboratory discharges

To further understand the loading concentration of different metal pollutants in canals, we calculated the metal pollution index (MPI) in addition to calculating the sum concentration of essential and toxic heavy metals (Fig. 3). Results explored that among the ecological samples, the highest MPI value ranked in sediment (42.3) followed by crab (13.3), duckweed (4.6), water lily (3.4), and water hyacinth (1.7). Among the fish samples, the highest MPI values were found in non-indigenous (N.I.) fish (1.3) compared with small indigenous (S.I.) fish (Fig. 3a). These results indicate a greater cumulative metal accumulation in most of the canal ecological samples which means a greater ecological risk. Again, among all the essential metal elements Fe accumulation was found most in all the samples even at the toxic level in water (Fig. 3b). However, the accumulation of other essential elements (Cu, Zn, and Mn) was obtained at safe levels. In contrast, the sum accumulation of toxic heavy metals was mostly found in sediments (3.2 mg kg^{-1}) followed by crab or plant samples (0.3–1.0 mg kg^{-1}) above expected limits (Töre et al.

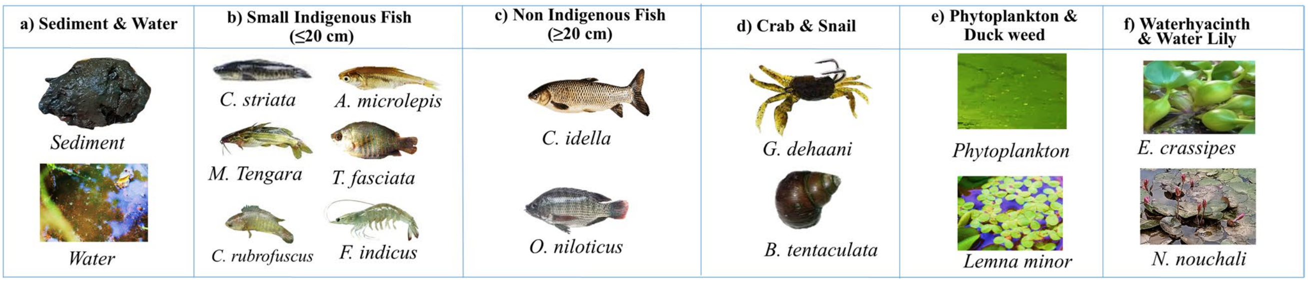

Fig. 2 Overview of the collected samples from canals receiving laboratory discharges; **a** soil sediment and water, **b** small Indigenous Fish ≤ 20 cm in size, **c** non-indigenous fish ≥ 20 cm in size, **d** crab and snail, **e** phytoplankton and duckweed, and (**f**) water hyacinth and water lily

2021). Surprisingly, among the toxic metals Cr accumulation ranked first then Pb and Cd in all the samples indicating their associated risk in the canal ecosystem receiving laboratory discharges (Fig. 3c).

Table 1 The mean concentrations of essential nutrients and toxic heavy metals in different components of the canal ecosystem

Samples	Fe (Iron)	Mn (Manganese)	Cu (Copper)	Zn (Zinc)	Pb (Lead)	Cd (Cadmium)	Cr (Chromium)	Co (Cobalt)	Ni (Nickel)	As (Arsenic)
Water (mg L^{-1})	13.81 ± 0.00 f	3.44 ± 0.00 a	0.41 ± 0.00 c	0.41 ± 0.00 g	0.055 ± 0.00e	0.003 ± 0.00 g	0.0002 ± 0.00 g	ND	ND	ND
Crab (mg kg^{-1})	467.87 ± 0.09 a	2.12 ± 0.00 b	0.61 ± 0.00 c	9.92 ± 0.00 b	0.224 ± 0.01 c	0.147 ± 0.00 c	0.603 ± 0.02 b	ND	ND	ND
S.I.fish (mg kg^{-1})	323.07 ± 0.11 b	2.12 ± 0.00 b	0.19 ± 0.01 d	5.97 ± 0.00 d	0.105 ± 0.00 d	0.054 ± 0.01 e	0.0002 ± 0.00 g	ND	ND	ND
N.I fish (mg kg^{-1})	259.27 ± 0.01 c	2.11 ± 0.00 b	3.11 ± 0.00 a	6.37 ± 0.00 c	0.035 ± 0.00 e	0.030 ± 0.00 f	0.0001 ± 0.00 g	ND	ND	ND
Sediment (mg kg^{-1})	311.93 ± 0.03 b	2.09 ± 0.00 c	1.14 ± 0.00 b	12.98 ± 0.00 a	0.904 ± 0.00 a	0.237 ± 0.01 a	2.827 ± 0.01 a	0.01 ± 0.001 a	ND	ND
Snail (mg kg^{-1})	226.27 ± 0.00 c	2.11 ± 0.00 b	1.64 ± 0.01 b	12.96 ± 0.00 a	0.684 ± 0.01 b	0.151 ± 0.00 b	0.01 ± 0.00 f	ND	ND	ND
Phytoplankton (mg kg^{-1})	79.20 ± 0.01 e	2.10 ± 0.00 b	0.10 ± 0.00 e	5.99 ± 0.00 d	0.574 ± 0.01 b	0.141 ± 0.00 c	0.252 ± 0.02 c	ND	ND	ND
Duck weed (mg kg^{-1})	189.13 ± 0.04 d	2.13 ± 0.00 b	0.22 ± 0.00 d	7.98 ± 0.01 bc	0.384 ± 0.01 c	0.150 ± 0.00 b	0.152 ± 0.01 d	ND	ND	ND
Waterhyacinth (mg kg^{-1})	177.20 ± 0.05 d	2.06 ± 0.00 c	0.03 ± 0.00 e	4.94 ± 0.00 e	0.524 ± 0.01 b	0.157 ± 0.00 b	0.1 ± 0.00 e	ND	ND	ND
Water lily (mg kg^{-1})	366.40 ± 0.01 b	2.07 ± 0.00 c	0.08 ± 0.00 e	2.34 ± 0.00 f	0.264 ± 0.01 c	0.064 ± 0.00 d	ND	ND	ND	ND
P-value	**	*	**	**	**	**	**	ns	ns	ns

Means with the same letters are statistically similar

**Significant at 1% level ($p < 0.01\%$)

*Significant at 5% level ($p < 0.05\%$), *ns* = non-significant

The pervasiveness of microplastics (MPs) in canal ecological sample components receiving laboratory discharges

Plastic pollution has emerged as an additional ecological problem in recent years. Hence to understand the effect of laboratory discharges on the abundance of microplastics (MPs), we determine the morphology (shape and color) and distribution of MPs in all the 10 canal ecological sample components as laboratory wastage promotes MPs pollution by stimulating plastic degradation (Kasamesiri et al. 2023). The demographic results obtained from the microscopic study explored that different types of MPs including fibers, fragments, and microbeads were observed in all the ecological samples except fiber in sediments (Fig. 4). Although the fiber was the lowest, the other fragments and microbeads were higher in the sediment similar to other samples. Most surprisingly, all three categories of MPs were observed in both the fish samples that have augmented associated human health risks. Similarly, a large amount of all types of MPs were found in crabs and snails which might be due to their detritus food habits. As a hyperaccumulator, a large amount of all kinds of MPs also were observed in water hyacinth and duckweed followed by water lily (Fig. 4), which was strongly supported by the numerical data (Fig. 5). Our numerical data also explored that among the three categories of MPs, a large number of accumulated fractions were fragmented in all the ecological samples followed by fibers and microbeads except water hyacinth. In water hyacinth, above 40% fractions of accumulation were observed in the case of microbeads and fragments (Fig. 4b).

To further understand the MP pollution severity, we calculated the microplastic pollution load index (MPLI) by counting the total numbers of different types of MPs (Fig. 5a). Our results explored that the highest MPLI in water is more than 150 which is extremely high. Similarly, the sediment of the laboratory discharge receiving canal was around 120, as because of severe MPLI in sediment and hydro-system all the other ecological samples of canal phytoplankton to plants or fish to carbs or snail showed exceeding threshold limits of MPLI (Fig. 5a). For example, in the photic zone, living organism phytoplankton showed a maximum (129) score of MPLI followed by water hyacinth as a hyperaccumulator. Similarly, the MPLI was also above the threshold limits for the S.I. fish and N.I. fish which is a direct human health concern. In

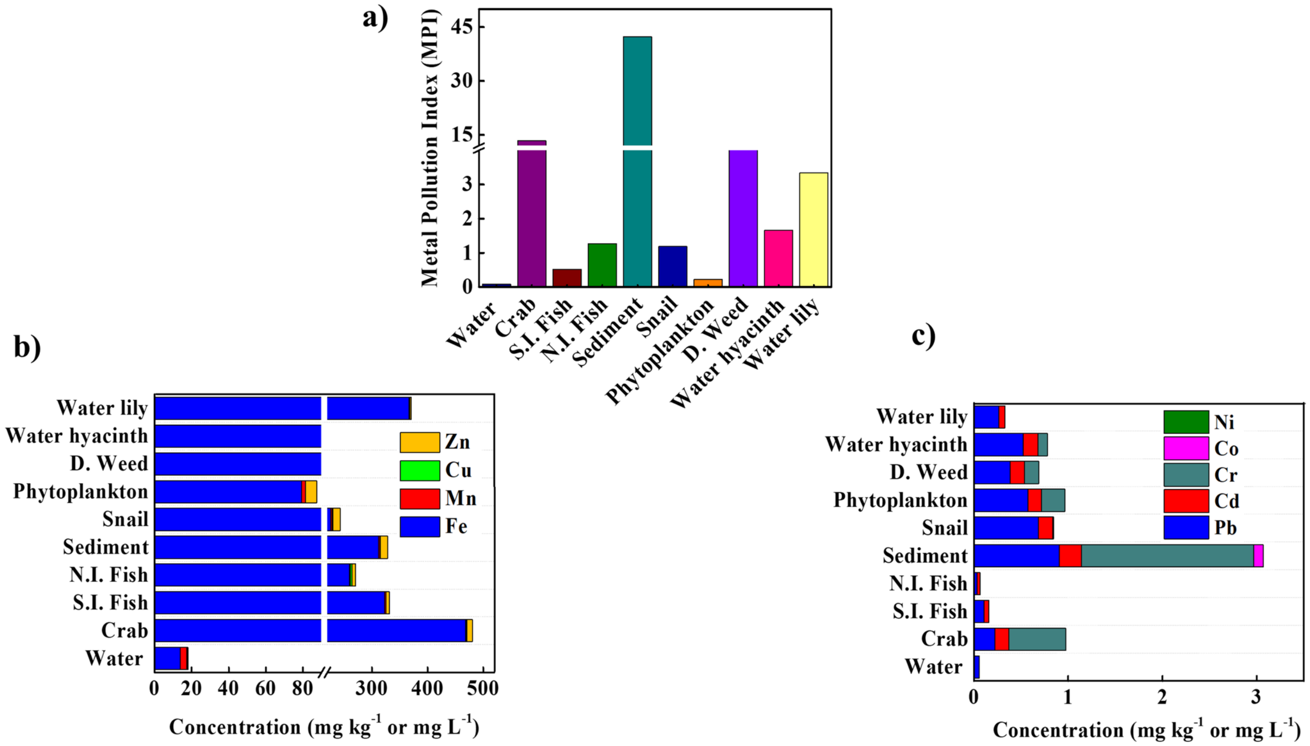

Fig. 3 Metal pollution index and sum concentration of essential and toxic metals in different components of the canal ecosystem: **a** metal pollution index, **b** sum concentration of essential metals Fe, Mn, Cu, and Zn as mg kg^{-1} samples in dry weight basis, and (**c**) toxic heavy metals Pb, Cd, Cr, Co, and Ni in sediment, water, small indigenous fish ≤ 20 cm in size, non-indigenous fish ≥ 20 cm in size, crab, snail, phytoplankton, duckweed, water hyacinth and water Lily samples collected from canals receiving laboratory discharges

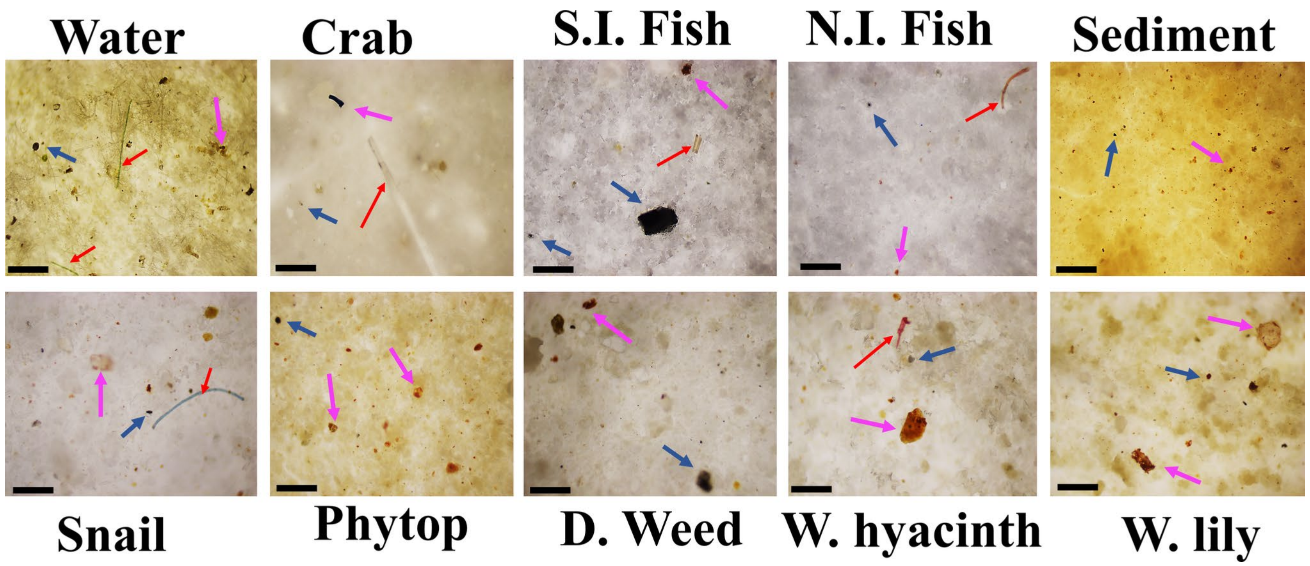

Fig. 4 Microscopic images of different types (fibers, fragments, and microbeads) of microplastic observed in water, crab, small indigenous fish (S.I. fish), non-indigenous fish (N.I. Fish), sediment, snail, phytoplankton, weed, water hyacinth, and water lily. Scale bar = 100 µm, the red color arrow indicates the fiber, pink color arrow indicates segments and the blue color arrow indicates microbeads

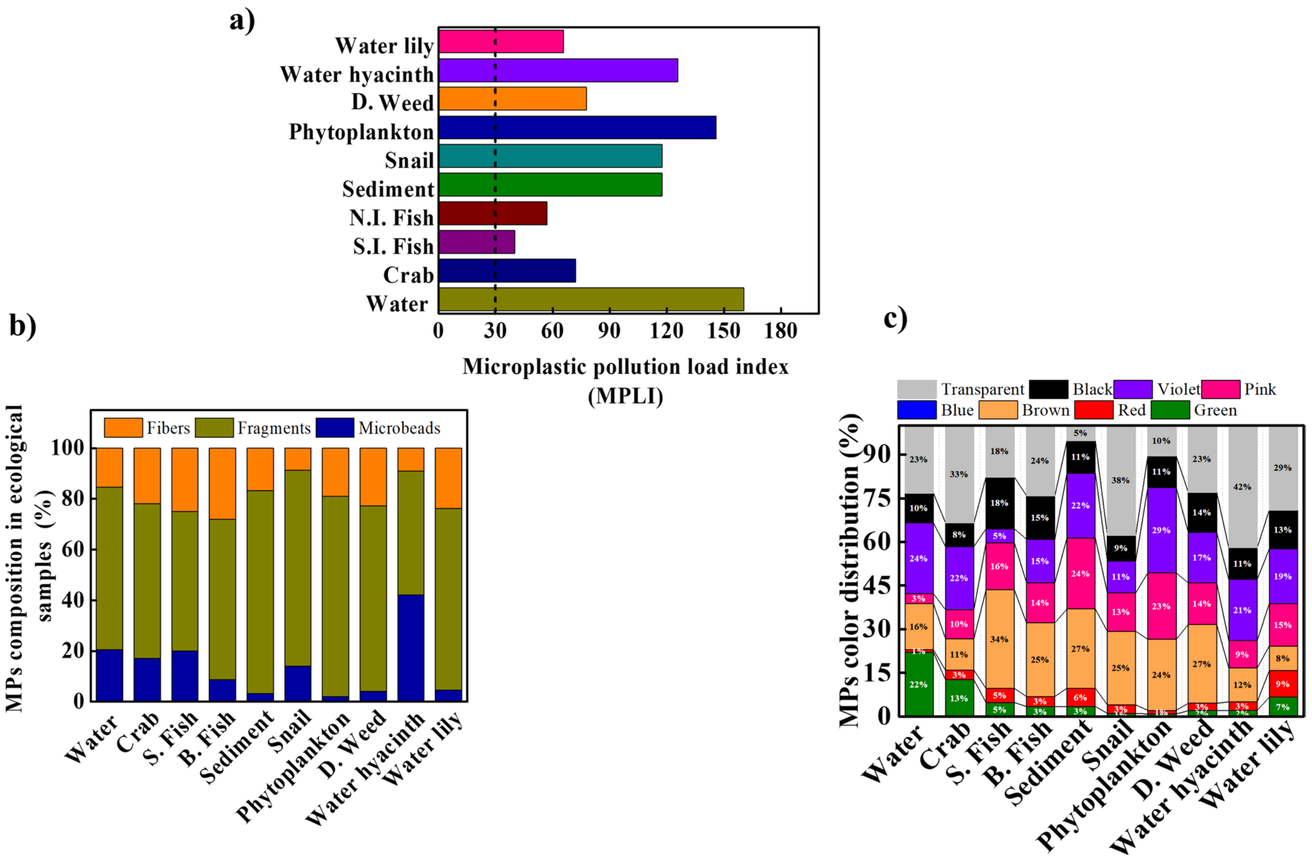

Fig. 5 Microplastic pollution load index, composition, and color distribution in different components of the canal ecosystem: **a** Microplastic pollution load index, **b** items composition of different types of microplastics and (**c**) color distribution percentage of microplastics in sediment, water, small indigenous fish ≤ 20 cm in size, non-indigenous fish ≥ 20 cm in size, crab, snail, phytoplankton, duckweed, water hyacinth and water lily samples collected from canals receiving laboratory discharges

the present experiment, we also calculated the color distribution of MPs. Remarkably, results explored that a total of eight color types of MPs were found to accumulate in canal ecological samples. Among the different color types, transparent color was found in maximum in most of the samples, while the highest 42% distribution of transparent MPs was observed in water hyacinth and the lowest (10%) was found in phytoplankton. Brown color MPs ranked second which were distributed in ranges from lowest 8% to highest 34% in a water lily and S. I. fish, respectively (Fig. 5c). In water, all seven colors of MPs were found to distribute very evenly except the red color. Similarly, the distribution of green color MPs was found lowest in the sediment while brown, pink, and violet colors were mostly abundant, indicating the divergence of MPs pollution and risk in components of the canal ecosystem. These might be augmented by the chemical degradation of plastic in canals receiving laboratory wastage.

Non-carcinogenic health risk of laboratory discharges

To understand the potential health risk level, we estimated the human health hazards index (HI) by calculating estimated daily intakes (EDIs) and hazardous quotient (HQ), specifically for fish (S.I fish and N.I. fish species) consumption (Fig. 6 and Supplementary Table S1). Results explored that the EDI values of all the toxic metals and MPs remain below the risk level except Cd, specifically for small indigenous fish (S.I. fish) consumption. For example, the EDI value of Pb was found 0.12 mg kg^{-1} day^{-1} below the contamination level (0.3 mg kg^{-1} day^{-1}). However, the EDI value of Cd was obtained at 0.062, which ranked above the critical limit (0.05 mg kg^{-1} day^{-1}) in accordance with the regulation (Hussein et al. 2023). In contrast, though the toxic metal was noticed in non-indigenous (N.I.) fish the

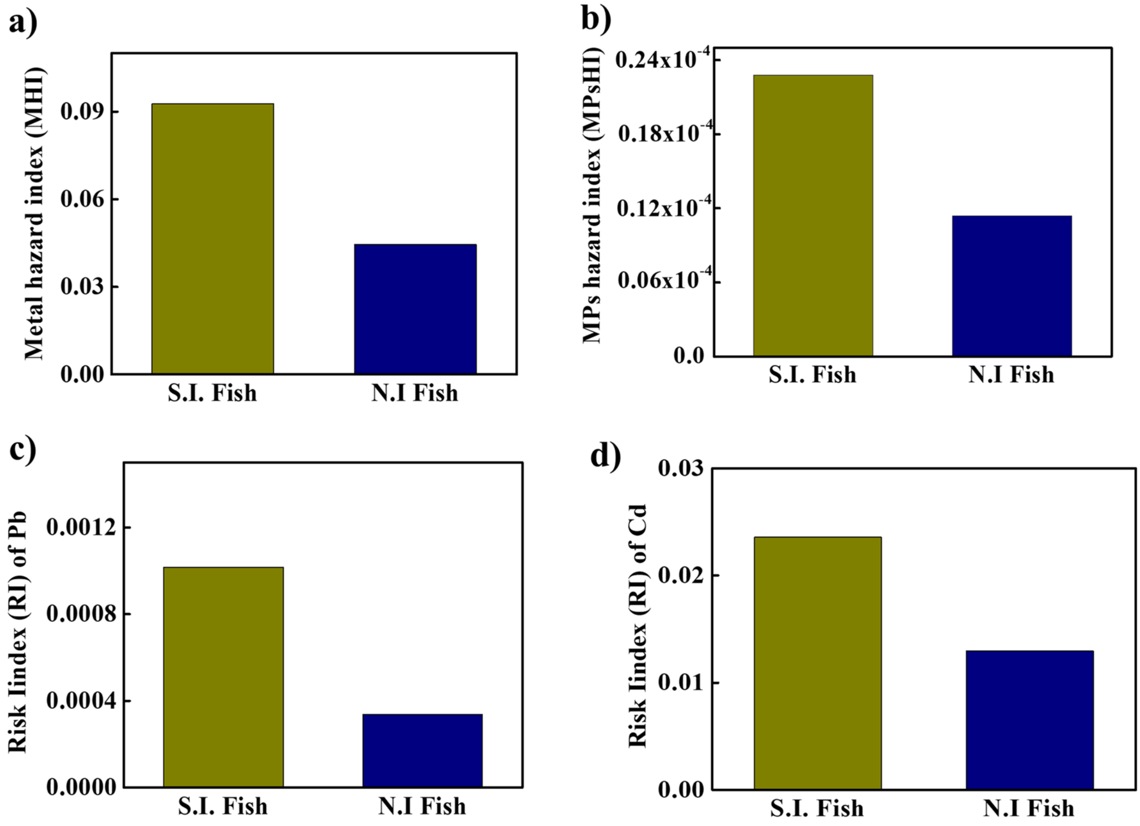

Fig. 6 Health hazards index of metals and microplastics (MPs) in canal fish of Bangladesh receiving laboratory discharges. **a** Metal hazard index (MHI), **b** MPs hazard index (MPsHI), **c** target health risk index (RI) of Pb, and (**d**) target health risk index (RI) of Cd calculated upon consideration of average body weight 55 kg and per capita daily intake 62.6 gm fish

EDI values remain below the risk issues. Similarly, the obtained target HQ found a value below one for the toxic metals (Supplementary Table S4; Fig. 6a, b). Likewise, the hazardous index of MPs (MPsHI) remains below the average risk level (0.0006) for the S.I. fish and N.I. fish indicating no health hazard (Namira et al. 2023). Here, we also calculated the potential risk index particularly for Pb and Cd as they most amply accumulated in both the fish samples and have a number of toxicological effects. Results explored that the average risk index (RI) of Pb and Cd exposure was below the cancer slope factor (SF) by 0.0085 and 0.38, respectively for both the S.I. fish and N.I. fish ingestion (Fig. 6c, d). These results indicate that although the HI or RI values of toxic metals or MPs remain within acceptable limits, the biomagnification due to repeated ingestion of them even at low concentrations may have severe toxicological effects.

Principal component analysis (PCA) for statistical interpretation

In the PCA study, the first two PCs explained 65.12% (PC1 = 45.86% and PC2 = 19.26%) of the total variation (Fig. 7a). The PCA results showed that sediment, crab, water hyacinth, phytoplankton, duckweed, and snails were located in the right quadrant of the PCA score plot on the positive direction of PC1 and positively associated with heavy metals (Fe, Cu, Cd, Pd, Cr, Co and Zn) and microplastics (MPs) (Fig. 7a). In addition, water, N.I.F, W. Lily, and S.I.F were positioned in the opposite direction of PC1 and showed a high concentration of Mn. On the other hand, the PC2 was positively interrelated with Mn, Cu, Co, and Cr whereas MPs, Fe, Zn, Cd, and Pb were located on the negative side of PC2 on the lower-right quadrant of the PCA score plot (Fig. 7a). Similar results

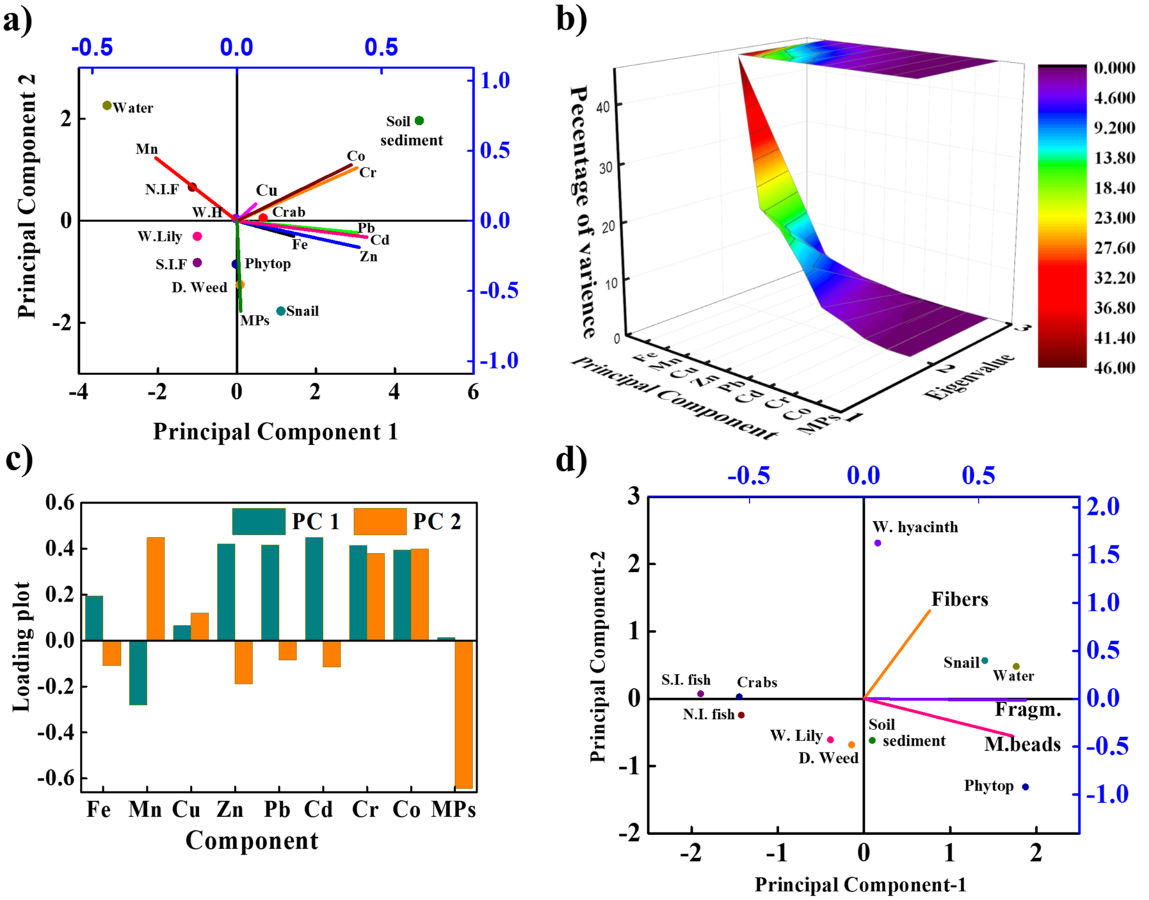

Fig. 7 Principal component analysis (PCA) of loaded metals and microplastics (MPs) in different components of the canal ecosystem. **a** PCA of loaded pollutants. **b** Percentages of variance and eigenvalues. **c** Loading plot of metals and MPs and (**d**) PCA of different types of MPs distributed in sediment, water, small indigenous fish ≤ 20 cm in size, non-indigenous fish ≥ 20 cm in size, crab, snail, phytoplankton (Phytop), duckweed (D. weed), water hyacinth (W.H) and water lily (W. lily) samples of canals receiving laboratory discharges

were also found on the loading plot of bar graph analysis (Fig. 7c). The Eigenvalues and the principal components were presented from left to right direction in the 3D plot along with the percentage of variance from the bottom to the upper direction (Fig. 7b). The highest percentage (%) of eigenvalues and the variance were found in component 1 (eigenvalue 4.12762, variance 45.86). Also, the percentage (%) of eigenvalues and the variance in components 2 (eigenvalue 1.73367, variance 19.26) and 3 (eigenvalue 1.46525, variance 16.28) were closely related (Fig. 7b). However, it slopes downward with changing both the eigenvalues and variance stopped at component 9 (Fig. 7b). For microplastic types distribution, first 2 principal components explained 94.05% (PC1 = 61.11% and PC2 = 32.94%) of total variation (Fig. 7d). Figure 7d demonstrated that fibers, fragments, and microbeads were strongly and positively interrelated to water hyacinth, water, snail, sediment, and phytoplankton. in PC1. On the other hand, the PC2 was positively correlated to fiber contents and negatively associated with fragments and microbeads. The overall result indicates that the laboratory discharges augmented the heavy metals and the availability of microplastics, which adversely affected the canal-ecological samples.

Discussion

A prerequisite harsh reminder of sustainable development is sound environmental conditions. Throughout the world, the scientific disciplines of biology and ecotoxicology have dramatically advanced in the last decade with groundbreaking discoveries (Valavanidis and Vlachogianni 2015). However, the alarming issue is that after the application of the laboratory reagents, agrochemicals, pharmaceuticals, radionuclides, and solvents are directly or indirectly released into the sewerage system due to the inefficient treatment system or ignorance, which is mostly common in developing countries like Bangladesh. Afterward, those chemical effluents reach the urban sewer network and finally to the environmental compartment such as sediment, water, and biota, and raise important health and ecological concerns (Corradini et al. 2019; Rakodi 2002; Valavanidis and Vlachogianni 2015). In the present experiments, we collected 10 components of the canal ecosystem including sediment, water, small indigenous fish (≤ 20 cm in size), non-indigenous fish (≥ 20 cm in size), crab, snail, phytoplankton, duckweed, water hyacinth and water Lily specifically from canals those directly receiving laboratory discharges (Fig. 2). We analyzed both the essential (Fe, Mn, Cu, and Zn) and toxic (Pb, Cd, Cr, Co, Ni, and As) metals in addition to microplastics (MPs). Surprisingly, our results explored that laboratory discharges enhanced the toxicological abundance of metals specifically Fe, Pb, Cd, and Cr in the canal hydrosystem (Table 1 and Fig. 3). In contrast, the abundance of MPs was augmented in all the canal ecological samples, thus creating potential health risks (Figs. 5, 6 and 7). Importantly, the bioaccumulation and biomagnification processes of repeated ingestion of the toxic metals and MPs even at low concentrations may be the footing of much ecological crisis (Namira et al. 2023).

Living organisms in an ecosystem often interact with a cocktail of toxic metals, a major environmental issue that is aggravated to alarming levels by differential discharges of pollutants to the ecological compartment like sediment or water (Li et al. 2022; Wu et al. 2016; Kabir et al. 2024). In accordance with other countries, in the last one decade in Bangladesh, the scientific disciplines of biology, ecology, and toxicology have remarkably advanced with groundbreaking discoveries and new developments (Rahija et al. 2019). However, the most distressing aspect is that after the target use of laboratory reagents, the chemical effluents are directly or indirectly released into the sewerage system due to the ignorance that finally reaches the environmental compartment (Valavanidis and Vlachogianni 2015). To unveil the toxicological risk, in the present experiment, we collected and analyzed canal ecological samples of those receiving laboratory discharges (Fig. 2). Surprisingly, experimental results explored that the canal ecosystem was severely hampered by receiving the chemical effluents released from the chemical laboratories. Results displayed that among the essential elements only Fe accumulation exceeded the WHO recommended level in all the cases while the other Cu, Mn, and Zn remained below the fair levels (Table 1 and Fig. 3a). For example, the highest 467.87 mg kg^{-1} Fe accumulation was found in crab which was twenty times higher than the WHO recommended dose of 141 mg kg^{-1} samples in dry weight basis (Song et al. 2021). In other ecological samples, Fe accumulation ranked in the following order, water lily > small indigenous fish > sediment > non-indigenous fish > snail > duckweed > waterhyacinth > phytoplankton and water, respectively. Although Fe has many important functions from plankton to plants or animals, the excess amount of Fe not only influences the distribution of ecological species but also causes cell structural damage upon excess accumulation (Rasheed et al. 2020; Saaltink et al. 2017) Such availability of Fe in the components of the canal ecosystem might be due to the receive of laboratory chemical effluents, as chemical effluent has the potential to change sediment chemical properties, microbial activity and chemical speciation of Fe leading to the changes in its availability in the ecosystem (Kunhikrishnan et al. 2012; Rasheed et al. 2020).

Although the toxic heavy metals have no metabolic functions, the amount of toxic metals such as Pb, Cd, and Cr concentrations significantly varied among the components of the canal ecosystem (Table 1). Most surprisingly, results explored that Pb and Cd accumulated in all the components of the canal ecosystem with various extents. For example, the foremost Pb accumulation was observed in canal sediment (0.9 mg kg^{-1}) which may lead to higher (0.22–0.68 mg kg^{-1}) accumulation in omnivorous snails or crabs (Rae et al. 2019). Results also showed that Pb and Cd accumulated in both the small indigenous and non-indigenous fish muscle above the FAO or WHO recommended level which has strong human health concerns (Moiseenko and Gashkina 2020). For instance, as per WHO and FAO, the maximum permissible dose of Pb and Cd is 0.3 mg kg^{-1} and 0.05 mg kg^{-1} dry weight, respectively (Nazir et al. 2015; Zafarzadeh et al. 2018), while in the present experiment, we observed above the permissible limits 0.11 mg kg^{-1} of Pb and 0.054 mg kg^{-1} of Cd in canal fish samples (Table 1 and Fig. 2), which might be due to the bioaccumulation or biomagnification processes (Bhat et al. 2019). It has been reported that in addition to geochemical processes, chemical waste may form complexes with heavy metals thereby increasing bioavailability in the environment as we observed in the present study (Ali et al. 2019). Moreover, laboratory discharges may provide additional chemical ions that might compete with heavy metals for binding sites of sediment particles or in water hence, reducing the toxic metals retention capacity of sediment and influencing their toxicity in canal hydrosystem (Saaltink et al. 2017). Besides, laboratory wastes contain toxic metals that could contaminate any of the environmental components such as soil sediment, water, or biota. Eventually, toxic metals may travel through the food chain, one of the main routes of human exposure (Anderson et al. 2022).

Additionally, the pervasiveness of microplastic (MPs) pollution has become an emergent area of anxiety in the last decade, because their occurrence in hydrosytems often begins in the lakes or canals and expands to rivers and oceans (D'Avignon et al. 2022). In the present experiments, results explored that the pervasiveness of MPs is suggestively augmented in all ecological samples collected from the canals receiving laboratory effluents (Figs. 4 and 5). Outrageously the severity of MPs exceeded the safe level of MPs load index (MPLI) score in all the canal ecological samples (Fig. 5a). For example, in the present investigation, we observed 4–6 times higher MPLI in soil sediment, biota, and water, which is extremely higher than the safe MPLI score (Kasamesiri et al. 2023). Such an inclusion of MPs in addition to heavy metals not only a widespread ecological concern but also a potential threat to human health. For instance, both the metal and MP pollution index for fish samples were above the safe level (Figs. 3 and 5). Additionally, we observed eight different colors of MPs in the laboratory effluent receiving canal ecological samples (Fig. 5c), indicating not only the divergences of MPs availability but also the footing of toxicological consequences in the components of the canal ecosystem (Kasamesiri et al. 2023; Ma et al. 2020).

Generally, the increasing concentration of toxic metals or MPs in the food chain leads to higher concentrations in top predators that trigger associated health risks (Fig. 6) and may be augmented by chemical discharges (Hamidian et al. 2021). Although the present investigation explored the risk index of metals or MPs below the cancer slope factor (SF), the estimated daily intake (EDIs) especially for Cd was found above the critical limit of 0.062 mg kg^{-1} dry weight (Hussein et al. 2023). Again, the repeated ingestion of toxicants even at low concentrations has toxicological effects and may cause organ failures due to the biomagnification process (Schutten et al. 2023). Previous research has explored that the very common anthropogenic discharges are the dominant interacting factor that accelerates pollutant accumulation and associates risk in the terrestrial environment (Häder et al. 2020; Tao et al. 2020). Moreover, the aging process of MPs in the environment is also a potential threat that regulates the mobility of MPs in the ecosystem. For example, Zhou et al. showed that oxidative aging changes the surface morphology, particle size, crystallinity, and chemical composition of polyvinyl chloride microplastic (PVC-MP) results gained strong absorption potential led to the unexpected synergistic toxic effect on aquatic organisms (Zhou et al. 2020). Most surprisingly, such kind of oxidation of MPs facilitated by chemical discharges like sodium hydroxide (NaOH), citric acid, or sodium hypochlorite (NaClO) that are generally used in the laboratory for cleaning or other purposes (Gan et al. 2021). Additionally, the metal complex present in laboratory discharges works as a catalyst agent and promotes the degradation of large plastic to MPs results, thus increasing pollution risk in all the components of the canal ecosystem (Payne et al. 2022).

As an environmental driver, toxic metals or MPs strongly restrict the sensible utilization of canal hydrosystem stirred by environmental discharges of laboratory effluents (de Carvalho et al. 2021; Valavanidis and Vlachogianni 2015). For statistical interpretation, we have performed principal component analysis (PCA) and observed the correlation matrix of toxicity among the components of the canal ecosystem including sediment, water, and biota. Surprisingly, the PCA interfaced data explored that the obtainability of MPs in canal hydrosystem enhanced the metals profundity mostly in soil sediments, snails, crabs, and hydrophytes (Fig. 7). Due to the intrinsic chemical sorptive properties, MP abundance greatly influenced the migration of heavy metals and risk onto the components of the canal ecosystem (Lin et al. 2022). Moreover, the abundance, distribution, structural size, and sorptive affinity to heavy metals of MPs cause the co-occurrence of pollution that leads to the associated environmental risk (Hu et al. 2022; Khalid et al. 2021). For instance, Xiang et al. reported that MPs mediated sorption of heavy metals like Cd, Cr, Ni, Cu, Zn, and As or POPs stimulates their bioavailability in wetland hydrology that adversely affects the growth of soil microbiota, phytoplankton, and plant growth results, reduced the resilience of ecosystem (Xiang et al. 2022).

Conclusions

Globally, the occurrence of chemical effluents in different water bodies and their toxicological effects on components of the canal ecosystem such as sediment, water, and biota have raised strong environmental anxieties. Similarly, in the present experiments. We also observed that laboratory discharges increased the abundance of both the metals and microplastics (MPs) in receiving fluvial canal hydrosystems in Bangladesh. Moreover, laboratory chemical effluents cause the co-occurrence of toxic metals and MPs in receiving canal ecological samples including soil sediments to plankton. Experimental results also unveiled that the rising toxicity was more severe in benthic organisms like snails or crabs due to their omnivorous food habits. Again, although the risk index or hazardous index of pollutants remains below the risk level for fish ingestion, the biomagnification of pollutants due to repeated ingestions of them may have strong toxicological effects leading to organ failures even at low concentrations. Therefore, addressing the enhanced toxicological abundance of heavy metals and MPs in canal hydrosystems resulting from laboratory discharges requires comprehensive chemical waste management practices, regulatory policy, and remediation efforts to minimize environmental and human health risks.

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Authors' contributions Md. Kamrul Hasan: conceptualization, investigation, formal analysis, writing—original draft, writing—review and editing, supervision, funding acquisition. Israt Jahan:

investigation, software, formal analysis. Tonima Islam Suravi: investigation, formal analysis. Md. Abdullah Al HasibImon: investigation. Jannat Shopan: writing—review and editing. Golam Jalal Ahammed: conceptualization, writing—review and editing, supervision, funding acquisition.

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Data availability Data will be made available on reasonable request.

Declarations

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Consent to participate Not applicable.

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Wastewater irrigation impacts on seed germination and seedling growth of rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and mustard (*Brassica napus*) crops†

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Wastewater, particularly industrial water, is being utilised to irrigate agricultural fields owing to the scarcity of freshwater for irrigation. In the present experiment, irrigation wastewater was collected from three different agriculture farms in the Sylhet region of Bangladesh, and their suitability for seed germination and the growth performance of rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and mustard (*Brassica napus*) crops were evaluated. Biochemical analysis revealed that untreated irrigation wastewater (UTW) contained the heavy metals Cd, Cr, Cu, Ni, Pb and Zn at moderate toxicity levels and Ca and Fe at higher toxicity levels. Consequently, wastewater irrigation showed a significant inhibitory effect on seed germination and seedling growth performance (biomass accumulation and root/shoot length ratio) in all crop varieties and regions. Additionally, wastewater irrigation increased seedling mortality by approximately 10–30% and elevated levels of H_2O_2, electrolyte leakage (EL), and malondialdehyde (MDA) in all crop species, indicating enhanced reactive oxygen species (ROS) accumulation. Similarly, industrial wastewater treatment showed inhibitory effects on antioxidant content together with high oxidative stress, reflecting adverse impacts on seed germination and seedling growth.

Water impact

The rapid increase in global population, extreme climate change events and urbanization have increased not only the freshwater scarcity for irrigating agricultural fields, but also the challenges in sustainable agricultural production. This has considerably projected the demand for direct and intended use of sewage effluents and industrial and municipal wastewater (MWW). However, irrigation with untreated wastewater (UTW) could have a drawback related to environmental concerns impacting water, soil, crops, and, ultimately, consumer health. There are numerous studies on the toxicity of heavy metals that pose major environmental and health risks, including Pb, Cu, Cd, Zn, Ni, and Cr. However, to our knowledge, very few studies have reported on toxic heavy metal contamination in irrigated wastewater and their oxidative effect on seed germination in rice, tomato, and mustard crops. Therefore, herein, we aimed to investigate the potential effects of untreated industrial wastewater on seed germination, seedling growth, and development, which is essential for sustainable agricultural development and nutritional security.

1. Introduction

Water availability is a limiting factor on Earth for life and the sustainable development of crop production.[1,2] The rapid rise in human population, climate change, and urbanization has increased the scarcity of freshwater for irrigating agricultural fields, which is challenging to overcome.[3] This has considerably projected the demand for direct and intended use of sewage effluents and industrial and municipal wastewater (MWW).[4] Reclaimed use of this wastewater provides the key to the productive utilization of limited water resources. This alleviates environmental pollution due to the disposal of wastewater effluent and meets the global water demand.[5] However, irrigation with untreated wastewater (UTW) could have a drawback related to environmental concerns, impacting water, soil, crops, and, ultimately, consumer health.[6,7] High concentrations of organic matter, nutrients, toxic heavy metals, and organic chemicals (including herbicides, polychlorinated biphenyl (PCB),

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phenols, and polycyclic aromatic hydrocarbons) are found in wastewater.[8,9] Among the toxic heavy metals, lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), iron (Fe), manganese (Mn), chromium (Cr), and others are present at high concentrations in industrial wastewaters.[10]

There have been numerous studies on the toxicity of heavy metals, including Pb, Cu, Cd, Zn, Ni, and Cr.[11] Heavy metals pose major health risks to humans and the environment because they tend to bioaccumulate in crops.[10,12] They can induce abiotic stress in plants, which reduces leaf expansion and impacts the photosynthetic apparatus and crop yield.[13,14] Additionally, they cause excessive amounts of malondialdehyde (MDA) and reactive oxygen species (ROS) accumulation,[15–17] primarily in the mitochondria and chloroplasts of leaves, roots, and shoots.[18] Although some heavy metals, such as Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, and Ni^{2+}, are essential micronutrients for plants, they become toxic in excessive amounts. The accumulation of nonessential metals, including Cd^{2+} and Pb^{2+}, also causes toxicity in crops.[19,20] It has been reported that the heavy metal lead (Pb) is a significant ecosystem pollutant that has detrimental effects on the environment owing to its high retention period. The major negative effects of Pb on plants are reduced photosynthesis, decreased germination, delayed plant growth, and altered enzymatic activities and root morphology, which is a serious threat to agriculture.[17,21] Similarly, Cr toxicity causes damage to *Brassica napus* L. root ultra-structures and changes plants' natural antioxidant enzymes.[22] Inhibition of seed germination and seedling growth using untreated wastewater (UTW) is extremely toxic in nature.[23] Therefore, irrigation with untreated and treated wastewater containing toxic heavy metals may have a negative impact on plant growth, development, and seed germination.[24,25] However, different plant species exhibit varying levels of wastewater sensitivity and may become more susceptible or tolerant of wastewater contamination.[25] Interestingly, wastewater not only contain heavy metals which are toxic to plants at higher concentrations (>50%) but also contain higher amounts of plant nutrients (*e.g.*, N, P, K, Ca, Mg, Zn, Fe, Cu, and Mn) that can have stimulatory effects on plant growth at lower wastewater concentrations (<25%).[26] Thus, according to a recent worldwide consortium, proper reclamation of wastewater may provide an opportunity to utilize the wastewater as a potential source for irrigation, seed germination, and plant growth in agricultural practice.[27]

We have previously reported the possible ecotoxicological effect of heavy metals by assessing the potential risk from laboratory discharges to canal ecosystem components, including sediments, water, fish, and phytoplankton.[28] However, to our knowledge, very few studies have reported on the toxic heavy metal contamination in irrigated wastewater and their oxidative effects on seed germination in rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and mustard (*Brassica napus*) crops. Here, we selected these three crop species to capture the broad ranges of crop response to untreated irrigated waste water. This study was designed and executed to investigate the potential effects of the untreated industrial wastewater collected from three different locations (Alampur, Majortilla, and Khadimnagar) in the Sylhet region of Bangladesh on germination, seedling growth, and development. To obtain a more complete picture of wastewater effects, the various morphological, physiological, and biochemical properties of seedlings were monitored in detail. Therefore, the study provides a basis for profiling the harmful or safe effects of using UTW in agricultural irrigation and provides some key recommendations for future research regarding its sustainable use.

2. Materials and methods

2.1 Selection of study area, description of geographic conditions, and irrigated wastewater sampling

The present study area was selected based on a preliminary survey ensuring that there were direct discharges of industrial effluents into the agricultural field in the selected experimental regions. Three regions were selected that were irrigated with wastewater, including Alampur, Majortilla, and Khadimnagar in Sylhet city of Bangladesh. These regions were near the industrial areas (such as iron and metal industries, plastic packaging industry, food and spices industry, a rice mill, cosmetics industry, tea industry, ceramics industries and an agricultural machinery manufacturer industry). Notably, there is no documentation regarding current wastewater treatment practices before irrigation in the agricultural field of selected areas. The geographical location of the regions is between 24°36′-25°11′ N and 91°38′-92°30′ E.

We collected irrigated untreated industrial wastewater from the three study locations and analysed their effect on seed germination and growth performance in the laboratory. Separate containers were used to store the water samples from separate regions for heavy metal and essential micronutrient analysis. A one litre conical flask was used to manually collect one litre of the top 20 cm of surface water, covering both the upstream and downstream sections from each region. The samples were brought to the Agricultural Chemistry Laboratory, Department of Agricultural Chemistry, Faculty of Agriculture, Sylhet Agricultural University (SAU), Sylhet, Bangladesh and preserved at 4 °C in a refrigerator for analysing various biochemical properties.[28]

The study was conducted with rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and mustard (*Brassica napus*) as the test crops. Healthy-looking seeds were selected and sterilized with 10% NaOCl solution for five min and then repeatedly washed with distilled water. Afterward, 300 seeds of each treatment and crops were placed in three different Petri-plates (60 mm × 5 mm) containing an untreated (raw) wastewater-soaked double layered filter paper. The experiment was conducted in controlled laboratory conditions in a growth chamber (model ICB-L250B, Bioevopeak, Jinan, China). Seeds were considered germinated when the radical emerged from the seed coats, and germination was monitored for up to seven days after sowing. Similarly, a seedling was considered dead when wilting was observed, which was closely monitored every three days for up to fifteen days after sowing. Prior to the germination test, the industrial wastewater samples were analyzed for the following chemical contents: Pb, Cd, Ni, Cr, Fe, Zn, Ca, Mg, Mn, and Cu.

2.2 Analysis of heavy metals

To assess the ecological risk of trace metals, we examined the abundance of Ca, Mg, Mn, Fe, Zn, Cd, Pb, Cr, and Ni from collected irrigated industrial wastewater samples. The filtered sample was collected in acid-rinsed glass vials and kept in desiccators. Then, 0.5 g of each sample (dry weight) was placed in an acid-washed polytetrafluoroethylene (PTFE) vessel and digested using the di-acid mixture method as previously mentioned.[29] Following digestion, samples were diluted using Milli-Q water to the appropriate volume before being examined using an atomic absorption spectrometer (model: AA7000, Shimadzu, Japan).[28]

2.3 DAB and NBT staining, measurements of H_2O_2, ROS detection, lipid peroxidation and electrolyte leakage in rice, tomato, and mustard seedlings

3,3′-Diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) staining were used to visualise H_2O_2 and $O_2^{\bullet-}$ accumulation in rice, tomato, and mustard seedlings, respectively. Following a 6 hour light incubation period at 25 °C, the seedlings of rice, tomato, and mustard crops were immersed in 1 mg mL^{-1} DAB solution (pH-3.8) to detect H_2O_2 levels. In contrast, $O_2^{\bullet-}$ was detected in the seedlings after incubation in the dark, followed by incubation in a 0.5 mg mL^{-1} (pH-7.8) solution of NBT.[29]

H_2O_2 was measured quantitatively by centrifuging 0.3 g of rice, tomato, and mustard seedlings in 3 mL of ice-cooled 1.0 M $HClO_4$ solution at 6000 × *g* for 5 min in a refrigerator (model: HERMLE Z 326 K, Wehingen, Germany). After that, KOH was used to bring the supernatant's pH down to 6.5, and 0.05 g of charcoal was used to absorb it. Then, the samples were vortexed, centrifuged at 12 000 × *g* for five min, and applied to a pre-packed AG1.8 column. The collected samples were combined with reaction buffer containing 100 mmol potassium acetate (pH-4.4), 1 mmol AzBTS-$(NH_4)_2$ (2,2′-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid), and 0.25 units of HRP (horseradish peroxide) in a 1 : 1 ratio. The samples were assessed at OD412 as previously described.[29]

As previously reported by,[30] we used 2-thiobarbituric acid (TBA) to quantify the malondialdehyde (MDA) equivalents after homogenising 0.5 g samples with 5.0 mL of 10% trichloroacetic acid (TCA) to determine the lipid peroxidation. Following a 10 minute centrifugation at 3000 × *g*, 1 mL of the obtained supernatant was mixed with 4 mL of 20% TCA and 0.65% TBA. The reaction was incubated at 95 °C for 25 min before being halted by submersion in an ice bath. Then, the sample was centrifugation and the absorbance was measured at 440, 532, and 600 nm. To measure the electrolyte leakage (EL), fresh rice, tomato, and mustard seedlings were cut into small pieces and placed in a tube with 10 mL of distilled water, with one set shaken at room temperature for 2 h and another set heated at 120 °C for 20 min.[31]

2.4 Antioxidant assay

The antioxidant activity of extracted samples was measured using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[32] In brief, 3 mL of a 6×10^{-5} methanolic acid solution of DPPH was added to the 2 mL of sample extract, incubated for 30 min in the dark at room temperature, and the OD517 was measured as sample A. In contrast, the OD of the solution without the extract was measured as the blank and the ability to scavenge DPPH radicals was expressed as a percentage of total antioxidant activity using the following equation: antioxidant activity (%) = OD blank − OD sample/OD blank × 100, as previously discussed.[33]

2.5 Measurements of seedling growth parameters

The germination rates (percentage, %) were measured after harvesting three-five seedlings each of rice, mustard, and tomato at 1, 3, 5, and 7 days after sowing (DAS). The mortality rates (%) were measured at 1, 5, 10, and 15 DAS. Next, we measured the plant biomass and the root–shoot length ratio of the rice, mustard, and tomato crops. The total biomass (fresh and dry) was recorded using a digital weighing balance (Model-pH 2601, Ningbo, China) and the values were averaged and represented as grams/seedling. The root–shoot length ratios were measured from ten seedlings of each crop (rice, tomato, and mustard). The roots were separated from the shoots, washed with running water, and air-died in laboratory conditions. Finally, the root and shoot length ratios were measured.

2.6 Data recording and statistical analysis

Statistical analyses were performed with the software package SPSS (version 16.0 Inc., Chicago, IL, USA). The normality and homogeneity of the data of all variables were checked using the Shapiro–Wilk test. The experiment was designed with three replicates for each of the three crops in the three regions. The treatment means were calculated by averaging the values of three replicates. All the experiments were replicated twice to validate the results. Data were analysed using one-way analysis of variance (ANOVA) for comparison among three regions, while the independent-sample Student's test was used to compare three individual samples. Tukey's tests were performed to evaluate the treatment significance (significantly different at $p < 0.05$). Different letters were used to denote the significant differences among the treatment means.

3. Results

3.1 Available heavy metal (essential and toxic) concentrations, metal pollution index (MPI) and essential nutrient index (ENI) in the collected industrial wastewater

Wastewater contains a substantial number of pollutants, such as toxic heavy metals including lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and zinc (Zn).[2] Wastewater application may also lead to increases in essential metals or nutrient levels such as magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe) in the irrigated wastewater.[34] Here, we investigated the abundance of both toxic and essential metals in the irrigated wastewater collected from three regions (Alampur, Khadimnagar, and Majortilla) of Sylhet, Bangladesh (Fig. 1).

The results revealed that the Ca concentration was the highest (5–41 mg L^{-1}) among all essential metals in all the wastewater samples in the three regions, followed by Fe (3–7 mg L^{-1}) and Mn (maximum ~2 mg L^{-1}) (Fig. 1a). The concentration of Pb, Cd, Ni, Cr, Cu, Zn and Mg were found at higher concentration ranges from 0.05–0.24 mg L^{-1}, 0.004–0.07 mg L^{-1}, 0.035–0.14 mg L^{-1}, 0.03–0.2 mg L^{-1}, 0.06–0.1 mg L^{-1}, 0.5–0.64 mg L^{-1} and 0.015–0.02 mg L^{-1}, respectively in an irrigated wastewater of sampled area. However, the traceable amounts of heavy and essential metals (Pb, Cd, Ni, Cr, Cu, Zn, and Mg) in irrigated wastewater were <1 mg L^{-1} lower than the concentration of other essential metal concentrations (Mn, Ca, and Fe). The mean Ca concentrations were significantly higher (41 mg L^{-1}) in wastewater from Khadimnagar, followed by the Alampur region (14 mg L^{-1}), indicating a higher Ca availability and distribution in Khadimnagar. The enrichment of Fe in samples from Khadimnagar was significantly higher (7 mg L^{-1}) than those from the other regions (Fig. 1a). However, the reason for the high level of Ca and Fe in this region could be the geology of the area from which the wastewater was collected.

To determine the loading concentration of different metal pollutants in the industrial wastewater, we calculated the metal pollution index (MPI) based on toxic heavy and essential metal concentrations (Fig. 1b and c). The results revealed that the highest MPI value was scored for Pb accumulation (0.25 mg L^{-1}), followed by Ni (0.13 mg L^{-1}), Cr (0.1 mg L^{-1}), and Cd (0.02 mg L^{-1}) in the Khadimnagar region wastewater sample (Fig. 1b). Among other region samples, the highest MPI values were found for Pb accumulation (0.13 mg L^{-1}) followed by Ni (0.13 mg L^{-1}), Cr (0.1 mg L^{-1}), and Cd (0.01 mg L^{-1}) in the Majortilla region wastewater sample (Fig. 1b). The lowest MPI values for all four heavy metals (below 0.06 mg L^{-1}) were found in the Alampur region (Fig. 1b). Among all the toxic heavy metal elements, Pb accumulation was found to be higher in most of the wastewater samples (Fig. 1b). The above results could be explained by the lower average levels of the other toxic heavy metals in most of the wastewater samples, which might be less toxic due to lower accumulation in rice, tomato, and mustard seedlings.

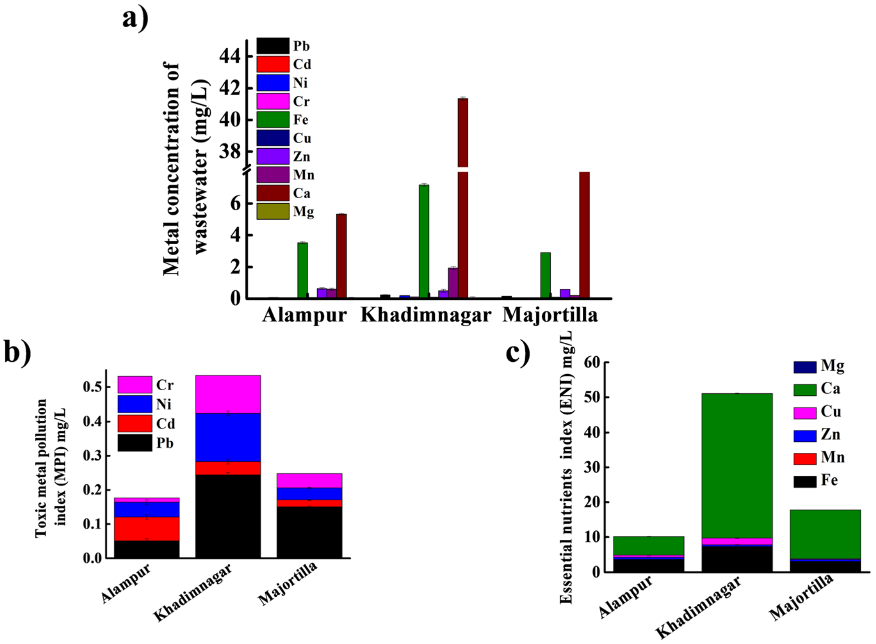

Fig. 1 Metal (Pb, Cd, Ni, Cr, Fe, Mn, Cu, Zn, Ca, and Mg) concentration (mg L^{-1}) and pollution index (mg L^{-1}) in irrigated wastewater collected from three different locations of the Sylhet region of Bangladesh. a) Metal concentration (mg L^{-1}), b) toxic metal pollution index (MPL), and c) essential nutrient index (ENI) in the collected industrial wastewater. The data are presented as the means of three replicates (± SE), and the means denoted by the same letter do not differ significantly at $p < 0.05$, according to Tukey's test.

The results revealed that the industrial wastewater contains a significant load of micronutrients (*e.g.*, Mg, Ca, Cu, Zn, Mn, and Fe), which are necessary for the growth and yield of crops. In context with the essential nutrient index (ENI), the Ca content was the highest (42 mg L^{-1}) in the Khadimnagar region followed by Fe (9 mg L^{-1}) and Cu (1 mg L^{-1}) (Fig. 1c). On the other hand, the highest ENI in Majortilla for Ca and Fe was 10 mg L^{-1} and 5 mg L^{-1}, respectively and a decrease in ENI accumulation in the Alampur region was observed in the following order: Ca (5 mg L^{-1}) > Fe (4.5 mg L^{-1}) > Cu (0.5 mg L^{-1}) (Fig. 1c). Several metal concentration values (Pb, Cd, Ni, Cr, Fe, Cu, Zn, Mn, Ca, and Mg) are plotted on the Smith chart (Fig. 2). In the Smith chart, Pb and Ni display positive angular relation between *j* values of 1.0–0.2 on the centre axis at the point labelled 2.0–5.0. The 2.0–5.0 value start at the point labelled 5.0 on the centre axis and move toward the outer circle, where Ca and Fe show a positive angular relation (Fig. 2).

3.2 Effect of irrigated wastewater on seed germination and mortality of rice, tomato, and mustard seedlings

Rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and mustard (*Brassica campestris*) seeds showed different trends in relation to the physiological parameters recorded (seedling germination and mortality percentage) when treated with irrigation wastewater (Fig. 3a–d and 4a–c). The germination rate increases with a greater number of days after sowing (DAS) between 5–7 days for all crop species (rice, tomato, and mustard) in three regions. The relative maximum germination rates of rice seedlings were between 96–97% at 5–7 DAS, whereas the minimum was 50–63% at 1 DAS in the Alampur and Majortilla regions. The germination rate of rice was significantly lower (<20%) at 1–3 DAS, while the germination rate increases to a maximum of ~80% at 7 DAS in the Khadimnagar region (Fig. 3a). Similar trends were also observed for tomato seedlings. The relative germination rates of tomato were increased by about 45–92% from 1–7 DAS in Alampur and Majortilla regions. The lowest relative germination rates of tomato were 20% at 1 DAS, which increased up to 83% in the Khadimnagar region (Fig. 3b).

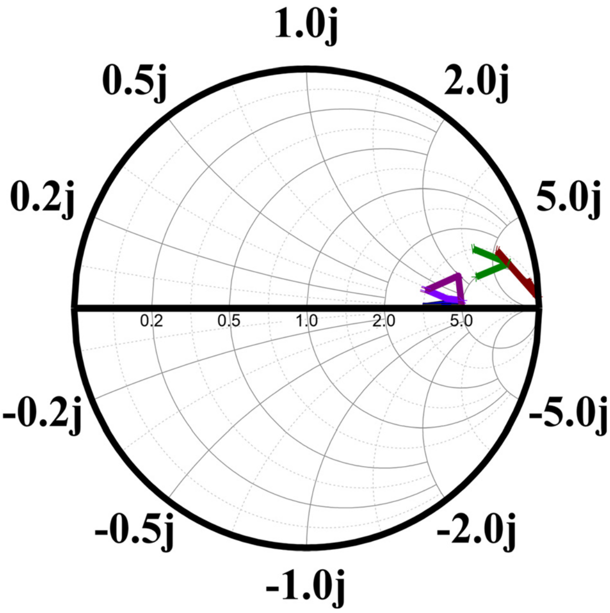

Fig. 2 The impedance Smith chart depicts the angular phase relationships among toxic and essential metal ions detected in irrigated wastewater from different regions of Sylhet, Bangladesh. The horizontal axis represents the metal concentration (0.2–5.0 mg L^{-1}). The positive relation axis starts at the center and goes straight up; the negative relation axis traverses straight down the origin.

Interestingly, the maximum relative germination rates of mustard seedlings were noted between 91–92% in Alampur at 1–7 DAS and in Majortilla at 3–7 DAS, while the minimum was 77% at 1 DAS in the Majortilla region. The lowest germination rate of mustard was 65% at 1 DAS, and the highest was 89% between 3–5 DAS, which did not further increase at 7 DAS in the Khadimnagar region (Fig. 3c). The germination rate was very slow at the beginning for rice and tomato seedlings which slowly increases with a greater number of days after germination in all regions. No significant differences were detected in the germination rates between the Alampur and Majortilla regions for all varieties, indicating that there was no variation in the wastewater treatment effect rather than cultivar or soil characteristics. The highest germination rates were observed in the case of rice seedlings (97%) in the Alampur region. The lowest germination rate was observed in the case of tomato (20%) in the Khadimnagar region. Nevertheless, the germination rate is almost the same for all regions at Alampur and Majortilla, but greater than the Khadimnagar region. Seedling mortality was significantly higher (1–30%) from 1–15 DAS in Khadimnagar for rice, tomato, and mustard seedlings compared with the other two regions when they were treated with irrigation wastewater (Fig. 4a–c). Meanwhile, the seedling mortality rate was significantly lower (0–10%) between 1–15 DAS in the Alampur and Majortilla regions (Fig. 4).

3.3 Effect of irrigated wastewater on biomass (fresh weight) accumulation and root/shoot length ratio of rice, tomato, and mustard seedlings

In the present study, the biomass (fresh weight) accumulation of rice seedlings increased by a maximum of 0.044 g per seedling in the Alampur region (Fig. 5a). Biomass accumulation was between 0.02 and 0.04 g per seedling for all crop varieties in the Majortilla region (Fig. 5a). Meanwhile, biomass accumulation significantly decreased for both rice and mustard seedlings (~0.019 g per seedling) and for tomato seedlings (0.022 g per seedling) in the Khadimnagar region, indicating that the irrigation wastewater could have a harmful effect to facilitate plant growth (Fig. 5a). The root/shoot length ratio significantly differed among the crop

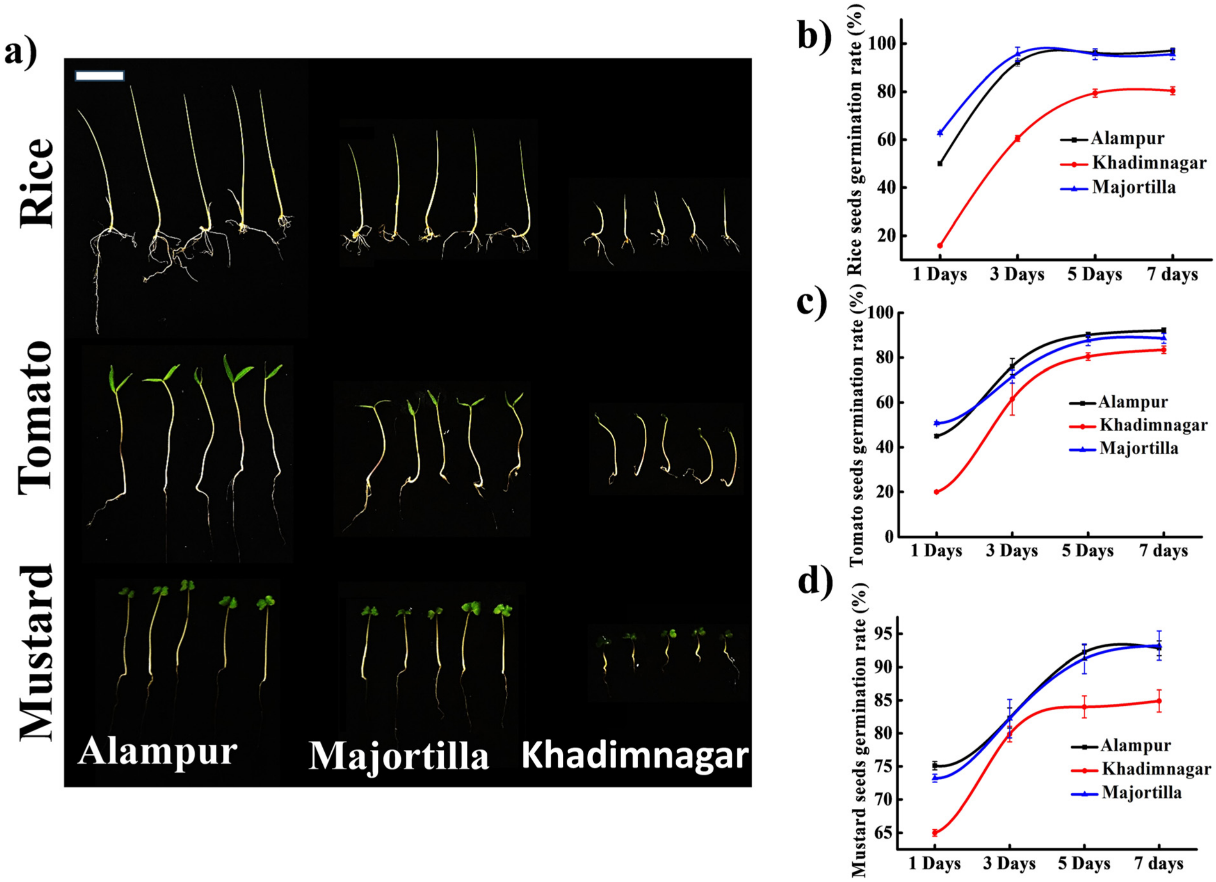

Fig. 3 Effect of irrigated wastewater on seed germination; (a) phenotypes and (b–d) rate of seed germination of rice (b), tomato (c), and mustard seedling (d) irrigated with collected wastewater. The data is presented as the means of three replicates (± SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

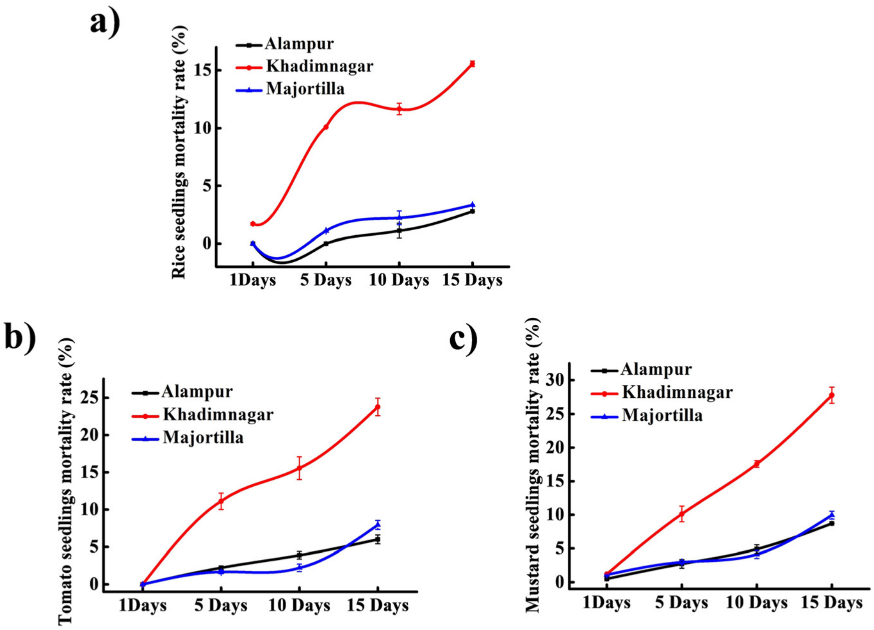

Fig. 4 Effect of irrigated wastewater on the mortality rate of rice (a), tomato (b), and mustard (c) seedlings irrigated with wastewater collected from different locations of the Sylhet region of Bangladesh. The data are presented as the means of three replicates (± SE), and the means denoted by the same letter do not differ significantly at $p < 0.05$, according to Tukey's test.

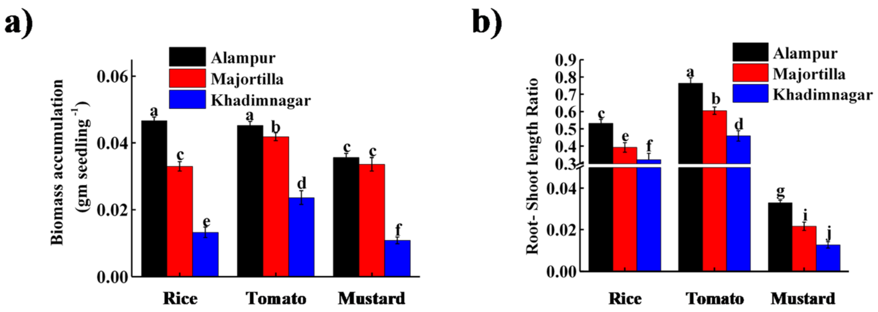

Fig. 5 Effect of wastewater on (a) biomass accumulation; (b) root shoot length ratio of rice, tomato and mustard seedlings irrigated with wastewater collected from different locations of the Sylhet region of Bangladesh. The data are presented as the means of three replicates (± SE), and the means denoted by the same letter do not differ significantly at $p < 0.05$, according to Tukey's test.

varieties in all regions. The highest root/shoot length ratio (0.76) was recorded in tomato seedlings in the Alampur region (Fig. 3a and 5b). A significant reduction in the root/shoot length ratio (0.01–0.03) was found for mustard seedlings in all regions (Fig. 3a and 5b). In rice, the root/shoot length ratio (0.32–0.53) significantly varied compared with the other regions using irrigation wastewater treatments (Fig. 5b). A significant reduction in the root/shoot length ratio (0.01–0.46) was observed in the Khadimnagar region for all crop varieties when seedlings were treated with irrigation wastewater (Fig. 5b). A higher root-to-shoot ratio indicates a healthy plant, whereas a lower ratio indicates that the crops' health has deteriorated.[26] In this study, untreated wastewater (UTW) irrigation gave a better root : shoot ratio in all regions. These findings have been validated by visual examination of seedlings grown in pots and whole seedlings extracted from soil in the laboratory (Fig. 3a).

3.4 Reactive oxygen species (ROS) accumulation and antioxidant activity in rice, tomato, and mustard seedlings upon irrigation wastewater treatment

There were differential biochemical changes in the seedlings of rice, tomato, and mustard following irrigated wastewater treatments for all the cultivars in the three different regions. To understand the effect of irrigated wastewater on the

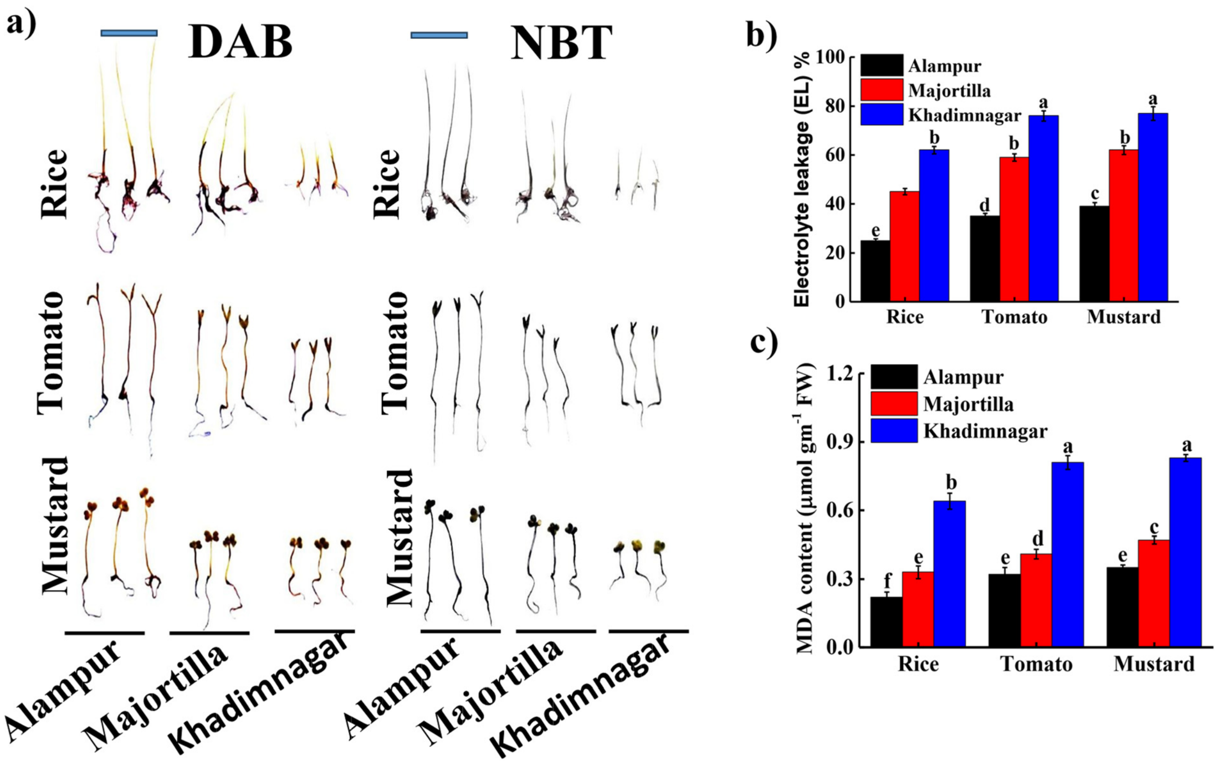

Fig. 6 Effect of wastewater on ROS accumulation determined by (a) DAB and (b) NBT staining; (c) electrolyte leakage (EL) and (d) malondialdehyde (MDA) content of rice, tomato and mustard seedlings treated with industrial wastewater collected from three different locations (Alampur, Majortilla, and Khadimnagar) of the Sylhet region of Bangladesh. The data are presented as the means of three replicates (± SE), and the means denoted by the same letter do not differ significantly at $p < 0.05$, according to Tukey's test.

generation of ROS, we stained rice, tomato, and mustard seedlings with DAB and NBT to visualize H_2O_2 and $O_2^{\bullet -}$ accumulation. Histochemical results showed that ROS accumulation was the lowest in the Alampur region for all crop seedlings, while ROS accumulation was substantially higher in the Khadimnagar region for all crop varieties (Fig. 6a and b). Staining with NBT revealed that an accumulation of $O_2^{\bullet -}$ was comparably lower in rice, tomato, and mustard seedlings for the Alampur and Majortilla regions compared with the Khadimnagar region. In contrast, NBT staining for rice, tomato, and mustard seedlings that were subjected to irrigated wastewater treatment was more evident in the Alampur and Majortilla regions (Fig. 6a and b).

The oxidative damage was evaluated by quantifying the electrolyte leakage (EL) in the rice, tomato, and mustard seedlings. The percentage of EL content increased by a maximum of 80% in the Khadimnagar region for all varieties (Fig. 6c). The EL percentage significantly reduced (20–40%) in all seedlings in the Alampur region (Fig. 6c). The results revealed that there was potential oxidative damage for all seedlings in the Khadimnagar region compared with the Alampur and Majortilla regions. In the Khadimnagar region, the rise in EL content was parallel to elevated ROS accumulation (Fig. 6c). Generally, the malondialdehyde (MDA) concentration is considered to be a reliable indicator of lipid peroxidation through an indirect measurement of membrane integrity under abiotic stress.[35] Therefore, we measured MDA levels in rice, tomato, and mustard seedlings to evaluate ROS with a view of determining the amount of oxidative stress caused by irrigation wastewater treatment compared with that required for seed germination (Fig. 5d). The MDA content from lipid peroxidation was found to be maximum/highest (~0.8 mmol g^{-1} FW) in the Khadimnagar region for tomato and mustard seedlings (Fig. 6d). The MDA content significantly increased in rice, mustard, and tomato cultivars for all regions. The MDA content was between 0.33 and 0.47 mmol mg^{-1} FW for all crop varieties in the Majortilla region. The MDA content was lowest (0.22 mmol mg^{-1} FW) for all crop seedlings in the Alampur region compared with the other regions. However, a minimal trend of MDA accumulation was observed in the Alampur region for all varieties (Fig. 6d).

3.5 Irrigation wastewater pre-treatment induces antioxidant activity in rice, tomato, and mustard seedlings

To further investigate the interaction between irrigated wastewater conditions and its effect on redox homeostasis, we measured the total antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity in wastewater-treated rice, tomato, and mustard seedlings. There was significant improvement in the antioxidant status in rice, tomato, and mustard seedlings with respect to irrigation wastewater treatment. The total antioxidant activity was the highest in the Alampur region (7.65 mg AsA g^{-1} FW) for tomato seedlings, and the lowest was 3.32 mg AsA g^{-1} FW for mustard seedlings (Fig. 7a). In the Majortilla region, the highest antioxidant content was found for tomato seedlings (6.05 mg AsA g^{-1} FW), while there was no significant difference between rice and mustard seedlings (Fig. 7a). Subsequently, there was a decreasing trend in antioxidant activity in the Khadimnagar region for all crop varieties (1–5 mg AsA g^{-1} FW) treated with irrigation wastewater (Fig. 7a). The DPPH activity significantly differed among the regions for all crop varieties. However, seed pre-treatment with irrigation wastewater increased the DPPH activity for all crop seedlings, which was the highest in Alampur regions (2.2–2.5 mmol g^{-1} FW) compared with the other regions (Fig. 7b). The DPPH activity was 1.8–2.5 mmol g^{-1} FW in Majortilla regions, which significantly differed among all crop varieties. Reductions in DPPH activity (1.2–1.8 mmol g^{-1} FW) were found in the Khadimnagar region (Fig. 7b).

4. Discussion

Biochemical analysis of the industrial wastewater revealed that it contained low concentrations (0.007–2.0 mg L−1) of some essential nutrients (Mg, Cu, Zn, and Mn) and various heavy metals (Cd, Pb, Ni, and Cr) (Fig. 1). Meanwhile, the presence of Ca and Fe micronutrient ions was found in high concentrations (3–41 mg L^{-1}). These findings are in accordance with previous observations in urban wastewater,

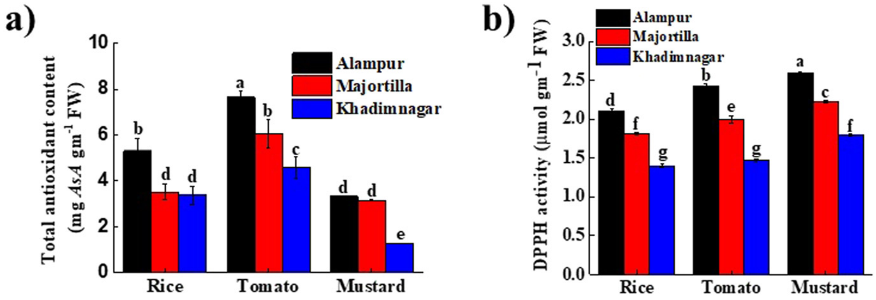

Fig. 7 Effect of irrigated wastewater on antioxidant activity in rice, tomato, and mustard seedlings; (a) total antioxidant content and (b) 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of rice, tomato and mustard seedlings irrigated with wastewater collected from different locations of the Sylhet region of Bangladesh. The data are presented as the means of three replicates (± SE), and the means denoted by the same letter do not differ significantly at $p < 0.05$, according to Tukey's test.

where high concentrations of Ca and Mg ions were observed with decreased levels of Cu (0.1 mg L^{-1}), Zn (1.14 mg L^{-1}), Pb (0.08 mg L^{-1}), and Cd (0.03 mg L^{-1}) concentrations.[23,36] Another report suggested that vegetable crops irrigated with wastewater had higher concentrations of heavy metals, including Pb, Cr, and Cd.[37] Similar results were found by,[38] showing that irrigation with municipal wastewater effluents increased heavy metal concentrations. Several factors affect heavy metal absorption and accumulation in plant tissue, including temperature, moisture, pH, and nutrient availability.[39] These results unequivocally imply that untreated wastewater contains heavy metal concentrations that can be toxic with high inhibition rates to plants at the measured concentrations.[40]

Our results revealed that the germination percentage of rice, tomato, and mustard seedlings increased from 45% to 97% between 1–7 DAS in the Alampur region compared with the other regions. On the other hand, the germination rate was lower (0–89%) for all crops from 1–7 DAS in the Khadimnagar regions (Fig. 3a). These three crop species exhibited a broad range of responses to untreated irrigated wastewater as the rice field needs continuous flooding irrigation, resulting in prolonged exposure to untreated wastewater, whereas tomato and mustard need less frequent irrigation.[41,42] Wastewater irrigation application resulted in significant adverse effects on germination and crop growth performances as reported in a previous study[43] showing that the germination percentage of alfalfa, fescue, and sorghum seeds was considerably lower than the control when irrigated with untreated wastewater (UTW), indicating that UTW exhibits a strong inhibitory effect on germination rates. Wastewater reduced the germination rate and leaf number and accelerated the rate of leaf senescence.[44] There was a significant decline in the percentage of germination, lengths and numbers of roots and leaves when oat (*Avena sativa*) seeds were soaked with untreated wastewater (UTW) effluent for 20 days.[23] It was found that irrigation with municipal wastewater collected from three locations in Quetta, Pakistan had a negative impact on lettuce (*Lactuca sativa* L.) seed germination and seedling growth.[45] It was documented that municipal wastewater pollution had severe impacts on certain vegetables in China, such as sweet peas, carrots, and cabbage.[46] Wastewater effluents from a chemical industry that were extremely saline with high electrical conductivity significantly hindered the growth and yield of soybean cultivars.[47] Thus, several reports observed the inhibition of germination in different species of crop seeds when irrigated with wastewater.

Seed germination can be affected by several biochemical mechanisms, including malondialdehyde (MDA) content, electrolyte leakage (EL), and reactive oxygen species (ROS). In the present study, ROS accumulation was higher for all crops treated with irrigation wastewater in the Khadimnagar region compared with the other two regions, corroborated by high H_2O_2 levels (Fig. 6). Similarly, it was previously observed that irrigation with UTW augmented a significant inhibition of seed germination or delayed germination in many species such as alfalfa, fescue, sorghum, and mustard, which seems to be linked to the generation of high ROS.[31,43] These findings reflected the higher sensitivity of rice, tomato, and mustard crop performances in different regions of wastewater treated samples, with elevated inhibition in the Khadimnagar region due to high oxidative stress.

The total antioxidant content did not significantly differ between the Majortilla and Khadimnagar regions in the rice crop and between the Alampur and Majortilla regions for mustard, while it showed a significant difference among all regions in the tomato crop. The highest antioxidant activity was recorded in tomatoes for all regions, which may partially explain the higher tolerance of tomato plants to oxidative stress generated by irrigation wastewater treatment (Fig. 7). The highest and lowest antioxidant activity was observed in the Alampur and Khadimnagar regions for all crop varieties that were treated with irrigation wastewater, which may be attributed to high oxidative stress (increased MDA levels). These results are consistent with the reports of ref. 48 regarding the reduction of antioxidant activity under stress conditions. In this study, the MDA content was increased in response to wastewater treatment in the Khadimnagar region for all crops, which may be attributed to increased oxidative stress due to the production of ROS.[31,49] In contrast, enhancement of the antioxidant system was observed due to the reduction in ROS production.[50] Our findings align with previous studies indicating that wastewater treatment increases the inhibitory effects on plants. Therefore, it is recommended that farmers irrigate agricultural fields with reclaimed domestic wastewater due to their economic and environmental compatibility, making it suitable for irrigation, offering an effective growth advantage, and mitigating the adverse effects of wastewater in urban gardening.

5. Conclusion

The amount of freshwater availability for agricultural field irrigation is steadily decreasing. The accumulation rate of toxic heavy metals above the threshold level in different plant organs (shoots, seeds, and roots) through wastewater irrigation is alarming. Therefore, treatment with untreated industrial wastewater may interfere with key physiological and biochemical processes. Moreover, considerable seed germination, mortality and growth performance could be observed depending on the crop species used and their resistance to toxicity levels. The results from the current study confirmed that untreated industrial wastewater significantly increased the toxic heavy metals (Cd, Cu, Cr, Zn, Ni, and Pb). On the other hand, wastewater treatment significantly increased the oxidative damage (ROS) effects on rice, mustard, and tomato seedlings by increasing EL and the MDA level while decreasing antioxidant activity which interfered with initial seed germination, biomass accumulation, and root/shoot length ratio of rice, tomato, and mustard seedlings. The results revealed that the percentage of germination and mortality rate of rice, tomato, and mustard crops increased to 97% and reduced to 30% at 7 DAS and 15 DAS, respectively. This finding suggests that reclaimed wastewater could be a profitable option for sustainable agriculture, particularly by eliminating heavy metal accumulation below permissible limits and high plant macro- and micro-nutrient uptake by plants, which is essential for agricultural resilience. Therefore, reclaimed wastewater with certain remedial techniques should be adopted to restrict heavy metal toxicity, which can be beneficial for agricultural irrigation to improve crop growth performances.

Data availability

The data that have been used are confidential.

Author contributions

Md. Kamrul Hasan: conceptualization, writing – original draft, writing – original revised, funding acquisition and supervised the study. Mahfuza Pervin: formal analysis and writing – original draft. Tushar Kanti Das: investigation and formal analysis. Abdur Rakib: investigation and formal analysis. Kamrun Nahar Mousomi: investigation and formal analysis. Jannat Shopan: formal analysis, writing – review and editing.

Conflicts of interest

The authors declare no conflict of interest.

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Article

Silicon-Nanomaterials Enhance Stress Resilience and Early-Maturity of Onions (*Allium cepa* L.) in Acidic Soils

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Abstract

Silicon-nanomaterials (Si-NMs) have emerged as a revolutionary tool in modern agriculture; however, the collaborative role of Si-NMs in onion crop productivity and expansion in acidic soils remains elusive. We conducted a series of sequential experiments, from seed germination to field trials, over two consecutive cultivation years. Intriguingly, the results revealed that among the differential doses, 1.0 mM L^{-1} of Si-NMs significantly ameliorated the acid-stress-induced suppression of onion seed germination and seedling growth. Additionally, a selected dose of Si-NMs reduces seedling mortality and improves plant establishment rate with increased photosynthetic performance, bulb properties, and nutritional quality. These stimulatory effects of Si-NMs on onion crop adaptation to acidic soil were associated with reduced ROS accumulation driven by enhanced antioxidant potential, which further increased upon dolomite supplementation. Furthermore, exogenous Si-NMs spray accelerated the early stages of harvestable onion size, accompanied by increased synthesis of IAA and GA_3 hormones, suggesting the potential of Si-NMs to enhance farm resilience in acidic soils.

Keywords: low soil pH; silicon-nanomaterials; ROS homeostasis; bulbing-ratio; adaptation

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1. Introduction

Onion (*Allium cepa* L.) is a globally significant vegetable and the queen of the kitchen for its distinct flavor, nutrients, and health benefits, primarily due to its high antioxidant and sulfur-containing compound content, including Vitamin C, Vitamin B6, Folate (B9), and Potassium. Onions are also rich in quercetin, which exhibits anti-inflammatory and anti-cancer properties [1]. However, onions require well-drained, nutrient-rich soils with a slightly acidic to neutral pH, typically 5.8 to 6.5, for optimal growth and proper bulb development [2]. Onions have a shallow, fibrous root system that is highly sensitive to low soil pH; roots are stunted when soil pH is below 5.5, limiting nutrient and water uptake.

Poor root growth and nutrient uptake at low soil pH result in smaller, deformed, and lower-quality bulbs [3]. Moreover, poor nutrient availability and uptake result in chlorosis, stunted growth, and delayed maturity. As a result, overall bulb yield and quality are reduced, contributing to economic losses and increasing nutritional insecurity.

Acid soil covers about one-third of the world's land area and only 4.5% of it is under crop cultivation due to its corrosiveness [4]. Acid soils are most dangerous due to their low nutrient availability, caused by fixation or leaching, and the high mobility of toxic elements, which leads to poor crop growth and yields. When soil pH falls below 5.0, the solubility of aluminum, iron, manganese, and/or zinc in the soil solution increases, becoming toxic to most vegetable crops, including onion [5]. Acid soils also hinder microbial activity, affecting nitrogen fixation and organic matter decomposition, which are essential for health and fertility. Again, silicon is the second most abundant element in the Earth's crust, making up about 28% by weight and primarily occurring as silicate minerals (up to 60.6%) and typically in quantities of 10–40 mg L^{-1} in available form as monosilicic acid (H_4SiO_4) in soil solution [6,7]. However, low silicon availability is most pronounced at low soil pH due to enhanced leaching, limited dissolution of silicate minerals, and absorption by Al or Fe oxides, in addition to the phytogenic cycle [6,8]. Consequently, plants become weak in their cellular structures, increasing their susceptibility to biotic and abiotic stress. Hence, addressing soil acidity is essential for achieving sustainable agriculture, as it directly affects soil fertility, nutrient availability, and crop productivity, thereby promoting long-term soil health and contributing to global food security.

In contrast, there is global resilience in the application of plant growth regulators (PGRs) due to their impressive roles in crop growth and development under diverse environmental conditions, such as soil acidity [9–11]. Similarly, Si-NMs have attracted attention as an innovative solution for modern sustainable agricultural development. Silicon is generally not considered essential for plant growth and development, but increasing evidence shows that this metalloid is beneficial to plants, especially under stress conditions across a wider range of agro-ecologies [12]. By addressing key agricultural challenges such as soil degradation, nutrient deficiencies, and environmental stresses, including salt stress, drought, and heavy metals, Si-NMs have demonstrated their significance for sustainable agricultural development and are proving to be a game-changer for global production [13]. Although Si-NMs, as a new step, successfully address numerous intensifying agricultural challenges, their role in onion crop productivity and expansion under low soil pH remains elusive. Moreover, the interaction mechanism and the influence of Si-NMs in agricultural applications are still in their early stages [14]. Therefore, this study aimed to explore the impact of soil acidity on onion crop production and to highlight the role of Si-NMs in mitigating its adverse effects and in ensuring early reaches of harvestable bulb sizes, thereby ensuring sustainable agricultural expansion and productivity.

2. Materials and Methods

2.1. Plant Resources and Seeds Pretreatment with Differential Doses of Silicon Nanomaterial (Si-NMs)

Seeds of onion (*Allium cepa* L. cv. *Taherpuri*) were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Dhaka, Bangladesh, for purity. This is an indigenous Bangladeshi cultivar characterized by excellent plant vigor, large-sized bulbs, and uniformity [15]. After assortment, healthy seeds were sterilized in a 10% NaOCl solution for five minutes and then repeatedly washed with distilled water as previously discussed [12]. To assess the effects of Si-NMs on seed germination and seedling growth, we used differential (0 mM L^{-1}, 0.25 mM L^{-1}, 0.5 mM L^{-1}, 1.0 mM L^{-1}, and 1.5 mM L^{-1}) doses of Si-NMs. Amorphous silicon dioxide (SiO_2) with an average particle size of 30 nm and a purity of 99.9% (metal basis) was purchased from Aladdin Industrial Corporation, Shanghai, China. To obtain a homogenous suspension of differential doses, the measured amount of Si-NMs was dispersed in distilled water and sonicated for 30 min using a Sonic Vibra-Cell (Model-VCX 505, Gütersloh, Germany) as previously discussed [12]. After preparing a homogenous suspension of Si-NMs, onion seeds were soaked in varying concentrations of the suspension for approximately 12 h, and the germination rate and seedling growth were subsequently observed. Afterward, pretreated seeds with varying doses of Si-NMs—300 seeds from each treatment group—were placed in three different Petri plates (60 mm × 5 mm), containing a double layer of acidified water (pH 4.8) soaked filter paper. The acidified water was prepared by adding HNO_3, as previously discussed [12]. The in vitro seed pretreatment experiment was conducted under controlled laboratory conditions in a growth chamber (model ICB-L250B, Bioevopeak, Jinan, China). Each Petri plate was prepared to contain 100 seeds, ensuring uniformity and adequate sample size for the accurate assessment of germination and seedling growth parameters. Seeds were considered germinated when the radicle emerged from the seed coat, and the rate of germination was recorded every 3 days for 20 days. Similarly, root and shoot lengths were measured every three days over a period of twenty days.

2.2. Field Experimental Design and Treatments

Field experiments were conducted in two consecutive cultivation years from 2022/23 to 2023/24 at the research field of the Department of Agricultural Chemistry, Sylhet Agricultural University, Bangladesh, an area located at the 23°57′ to 25°13′ North latitude and 90°56′ to 92°21′ East longitude. The soils of the study area are under the Northern and Eastern Piedmont-plain agro-ecological zone (AEZ-22) of Bangladesh, which comprises soils with strongly acidic pH ranges from 4.5 to 4.8 that are considered to be acid soils (Supplementary Table S1). The soil pH was measured using a glass electrode pH meter (Model: HI-2211, Woonsocket, RI, USA), maintaining a 1:2.5 soil-to-water ratio after collecting soil samples at a depth of 10–15 cm in the study area [12]. The climatic conditions of the selected AEZ Cwa type indicate regions of humid subtropical climate according to the widely used Köppen–Geiger world climate classification system. The average rainfall during the cultivation season was very low (0.4 inches), with average maximum and minimum temperatures of 26 °C and 15 °C, respectively. The relative humidity ranged from 63% to 68%. The experimental structure consisted of six treatments: control, dolomite, Si-NMs soak, Si-NMs spray, Si-NMs soak with dolomite, and Si-NMs spray with dolomite. Healthy seeds were sown into the seed beds. After 25–30 days of sowing, 416 healthy seedlings were transplanted to the one main cultivation bed with a spacing of 20 cm row to row and 12 cm plant to plant [16]. For the soak treatment, we pretreated healthy seedlings with the selected Si-NM dose, whereas for the control seedlings, we pretreated them with normal water for 12 h and then directly sowed them in the main beds. As a world soil correction practice, we have applied dolomite at a rate of 1 ton ha^{-1}. We used finely powdered dolomite one week before transplanting. After the application, the soil was thoroughly plowed to ensure uniform mixing, with a plowing depth of approximately 10–12 cm. We applied a 16 L ha^{-1} Si-NMs solution at a concentration of 1.0 mM L^{-1} as a foliar spray, three times at 25, 45, and 65 days after transplanting. Each treatment had three replications, with a 10 m square (2.5 m × 4.0 m) arranged in a complete randomized block design.

2.3. Assessment of Si-NMs-Mediated Adaptive Potentials of Onion Crops in Acid Soils

Both seedling mortality and seedling establishment rate are key checkpoints in the life cycle of seed plants. Accordingly, to assess seedling mortality and seedling establishment rate, onion seeds were pretreated with or without selected doses (1.0 mM L^{-1}) of Si-NMs and sown in seed beds. The experiment was performed in triplicate with 100 seeds per replication. The cumulative seedling mortality for each treatment was calculated as the percentage of seedlings that died from germination to the transplanting age at 40 days after sowing. Similarly, the survival percentage or seedling establishment rate was calculated as the number of seedlings that successfully reached transplanting age, as previously described [17].

2.4. Measurements of Total Chlorophyll Content and Plant Photosynthetic Performance

To measure total chlorophyll content, fresh onion leaf samples were collected from each treatment at a consistent growth stage after the third spray of Si-NMs. Approximately 0.3 gm of leaf tissue was homogenized with 80% acetone, then centrifuged at 10,000 rpm at 4 °C for 10 min. Then, the collected supernatant was measured spectrophotometrically at 646, 663, and 450 nm for absorbance using a UV-visible spectrophotometer (Model: T80+, PG Instruments Ltd., Wibtoft, Leicestershire, UK), as previously described [18]. The net photosynthesis rate of onion (*Allium cepa* L. cv. *Taherpuri*) was measured by using a portable photosynthesis system (LI-6400; LI-COR Bioscience, Lincoln, NE, USA) under control environmental conditions, following the method previously described [12]. Measurements were taken between 9.0 am to 11.0 am from fully expanded leaves during the third foliar spray of Si-NMs.

2.5. Valuation of Si-NMs-Mediated Growth and Yield Attributes of Onion Under Acid Soil Conditions

Biomass accumulation of onion was assessed after the application of the third foliar spray to evaluate the impact of Si-NMs on overall plant growth. At this stage, both the shoot and root systems were carefully harvested and rinsed with distilled water to remove surface residues, and the weight was measured after gently blotting dry with paper towels. Similarly, at harvest, morphological parameters, including the number of scales per bulb, scale thickness, and individual bulb weight, were assessed to evaluate the effects of Si-NMs on growth and yield attributes of onion under acidic soil conditions [19]. After carefully removing the outer dry layers, each onion bulb was manually dissected and the number of fleshy scales was counted. Similarly, the thickness of the innermost fleshy scales was measured by using a vernier caliper. A total of ten randomly selected bulbs from each treatment were used and the average value was recorded. Each bulb was weighed using a precision electric balance (Model: PA213; Ohaus Corp., Pine Brook, NJ, USA) and recorded in grams to determine the effect of treatment on bulb development and yield.

2.6. Assessments of H_2O_2, Lipid Peroxidation (MDA), and Electrolyte Leakage

To evaluate oxidative stress and membrane damage in onion plants H_2O_2, lipid peroxidation (MDA) and electrolyte leakage were assessed using standard biochemical methods. For H_2O_2, fresh 0.3 gm leaf samples were homogenized in 3 mL ice-cold 1.0 M $HCLO_4$ and centrifuged at 6000× *g* for 5 min at 4 °C. Then, the supernatant was adjusted to pH 6.5 with 4 M KOH, absorbed with activated charcoal, and passed through a pre-packed column and measured at 412 nm wavelength following the methods previously discussed [18]. Lipid peroxidation was measured as malondialdehyde (MDA) content. A total of 0.5 g fresh leaf sample was homogenized in 5 mL of 10% (*w*/*v*) trichloroacetic acid (TCA) and centrifuged. The supernatant was mixed with 0.5% thiobarbituric acid (TBA) in 20% TCA and heated at 95 °C for 30 min. The reaction was stopped in an ice bath, and the absorbance at 532 nm was recorded using a UV-visible spectrophotometer (Model: T80+, PG Instruments Ltd., Wibtoft, UK), as discussed [18]. Membrane stability was assessed by measuring electrolyte leakage with a conductivity meter (Model E1597EU, Shanghai, China). Leaf segments of equal size were incubated in 10 mL of deionized water at room temperature for 24 h, and the initial electrical conductivity was measured. The samples were then autoclaved at 121 for 20 min, cooled, and the final conductivity was recorded. Finally, the EL value was calculated as a percentage [20].

2.7. Total Polyphenol Content (TPC), Enzymes, and Hormonal Assay

To evaluate the biochemical response of onions to Si-NMs under acidic soils, total polyphenol (TPC), antioxidant, DPPH, AsA, and GSH were measured. TPC was determined spectrophotometrically (Model: T80+, PG Instruments Ltd., Wibtoft, UK) using the Folin–Ciocalteu method as previously described [20]. The total antioxidant potential of onion extract was determined using a phosphomolelybdenum assay. In brief, 0.1 mL of the methanolic extract was mixed with 1 mL of the mixture solution (containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and then the absorbance at 695 nm was measured after incubation in hot water bath [10]. The AsA content was measured spectrophotometrically by monitoring the reduction of Fe^{3+} to Fe^{2+} by AsA in acidic solution, with the resulting Fe^{2+} forming a color complex. Glutathione (GSH) was quantified using 5,5′-dithiobis (2-nitrobenzoicacid) (DTNB) and the glutathione reductase recycling method, as discussed [12]. Endogenous levels of indole-3-aceic acid (IAA) and gibberellic acid (GA_3) in onion bulb tissues were quantified using high performance liquid chromatography (HPLC; Shimadzu Prominence-i Plus) following a previously described method with modifications to assess the Si-NMs-mediated hormonal response in bulb development [21]. The method was validated by the Rice Analytical Laboratory (RAL) of the Bangladesh Rice Research Institute [22]. In brief, fresh tissue samples (1 g) were finely ground in liquid nitrogen using a Mixer Mill MM 400 (RETSCH, Haan, Germany), followed by extraction in 80% cold methanol. The extracts were agitated for 30 min using an orbital shaker (Stuart SSL1, Staffordshire, UK) and subsequently centrifuged (Sigma 3-30KS, Osterode am Harz, Germany) at 12,000 RPM for 5 min at 4 °C. The supernatant was filtered (0.22 μm syringe filter) before analysis by HPLC using a C18 analytical column (4.6 mm × 250 mm, 5 μm). Detection wavelengths of 265 nm (IAA) and 208 nm (GA_3) were employed, utilizing a mobile phase of 40% methanol and 0.1% acetic acid. Column temperature was maintained at 25 °C, with a flow rate of 1 mL min^{-1} and an injection volume of 20 μL. Data processing was conducted using Lab Solutions software, version 6.9, comparing analyte elution profiles with standard IAA and GA_3 solutions.

2.8. Assessment of Bulbing Ratio and Maturity Index of Onion and Nutrients

The bulbing ratio of the onion was calculated as the ratio of the equatorial diameter to the neck diameter of the bulb. This index serves as an indicator of bulb development and is a crucial determinant of harvesting maturity of the onion bulb. A bulb ratio of 2.0 or greater is generally considered a mature, well-formed bulb [23]. The maturity index was assessed based on the morphological indicator neck fall or top-down drying of foliage, as previously discussed [16]. This was visually monitored and recorded weekly throughout the crop cycle to determine the point of physiological maturity. The nutritional properties of the onion were analyzed after harvesting the mature bulbs. Both macro and micronutrients were determined following the standard protocol as previously described [10]. Briefly, 0.1 gm of dry onion bulb tissue was digested with 5 mL of a di-acid mixture ($HClO_4$: HNO_3, v/v = 1:3). The digested samples were then filtered and diluted to volume. Subsequently, the macro- and micronutrients were determined using a flame atomic absorption spectrophotometer (Shimadzu AA-6300, Kyoto, Japan) following the method previously described [24].

2.9. Statistical Analysis

All experimental data were subjected to statistical analysis using analysis of variance (ANOVA) to determine the significance of treatment effects. The data were processed using

SPSS (version 16.0 Inc., Chicago, IL, USA). The mean values were compared using Tukey's test at a 5% level of significance ($p < 0.05$). The experiment was conducted over two consecutive cultivation years (2022/23 and 2023/24) to validate the results following a completely randomized block design (CRBD). Results are expressed as the mean ± standard error and the graphs were generated using OriginLab software (version: 9.1, Origin Corporation, Northampton, MA, USA) to visualize treatment effects and variability.

3. Results

3.1. Role of Differential Doses of Si-NMs on Onion Seeds Germination, Seedlings Growth, and Establishment

To understand the effective role of Si-NMs on onion seed germination, seedling growth, and establishment at the field level, we initially trialed a range of Si-NM doses (0, 0.25, 0.5, 1.0-, and 1.5 mM L^{-1}). Results displayed that all doses of Si-NMs had stimulatory effects on onion seed germination and seedling growth. However, among the differential doses, 1.0 mM L^{-1} Si-NMs was found to be most responsive in seed germination and seedling growth. Compared to the control, the 1.0 mM L^{-1} dose of Si-NMs showed about 38.8% additional germination rate, followed by 0.5, 1.5- and 0.5 mM L^{-1} doses of Si-NMs, respectively (Figure 1a,b). Similarly, enhanced seedling growth was also observed upon Si-NMs pretreatment. Again, among the doses, 1.0 mM L^{-1} Si-NMs showed the best performance. For example, the root length and shoot length increased about 2.6-fold and 1.9-fold at 1.0 mM L^{-1} of Si-NMs compared to their control (Figure 1c,d). For further confirmation of dose selection, we also performed a field experiment both at field soil (pH-6.5) and acid soil (pH-4.8) conditions and observed the seedling growth and development.

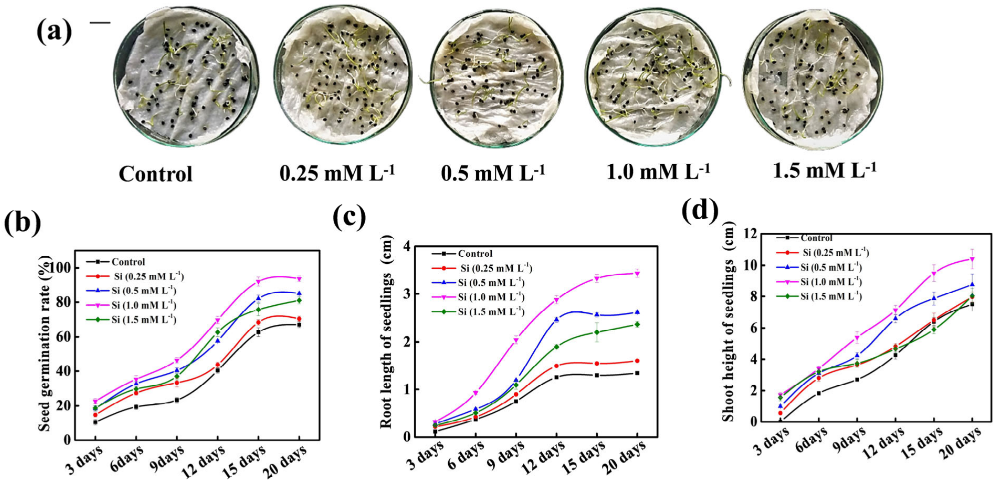

Figure 1. Effect of differential doses (0–1.5 mM L^{-1}) of silicon-nanomaterials (Si-NMs) on onion seed germination and seedling growth: (**a**) photographic images of seed germination (bar = 1 cm); (**b**) seeds germination, (**c**) root growth, and (**d**) shoot growth (cm) at 20 days of sowing under acidic (pH-4.8) conditions. The data are presented as the means of three replicates with standard error of the mean (±SEM).

Interestingly, the selected dose (1.0 mM L^{-1}) of Si-NMs showed a pronounced effect on seedling growth under both soil conditions, as shown in Supplementary Figure S1. Based on both in vitro and in vivo experiments on seed germination and seedling growth under field conditions, we have selected a 1.0 mM L^{-1} dose of Si-NMs for further experiments to observe the efficacy of Si-NMs on onion crop adaptation and farming resilience to acid soil conditions.

3.2. Si-NMs Improve Net Photosynthetic Performance and Reduce Seedling Mortality Under Acidic Soil Conditions

To understand the influential effects of a selected dose of Si-NMs on plant adaptation under stressful acidic soil conditions, we applied Si-NMs (seed pretreatment and spray) in addition to dolomite, a widely used soil amendment, and observed the net photosynthetic rate during seedling establishment over two consecutive years. Interestingly, results showed that both methods of Si-NM application significantly improved total chlorophyll, net photosynthesis, and thereby biomass accumulation, which were significantly inhibited by soil acidity (Figure 2a). However, among the techniques, exogenous spray of Si-NMs showed the most significant response. For example, exogenous spray of Si-NMs showed about 18.0–22.7% increased chlorophyll content and 7.8–9.2% increased net photosynthetic rate in onion crops grown in acidic soil compared to control crops; consequently, total biomass accumulation increased by about 27.9–41.4% over two consecutive years of cultivation (Figure 2d–f). Interestingly, application of dolomite on Si-NMs, as a world soil correction practice for acid soils, further augmented total chlorophyll content, net photosynthesis, and biomass accumulation, which were suppressed by soil acidity.

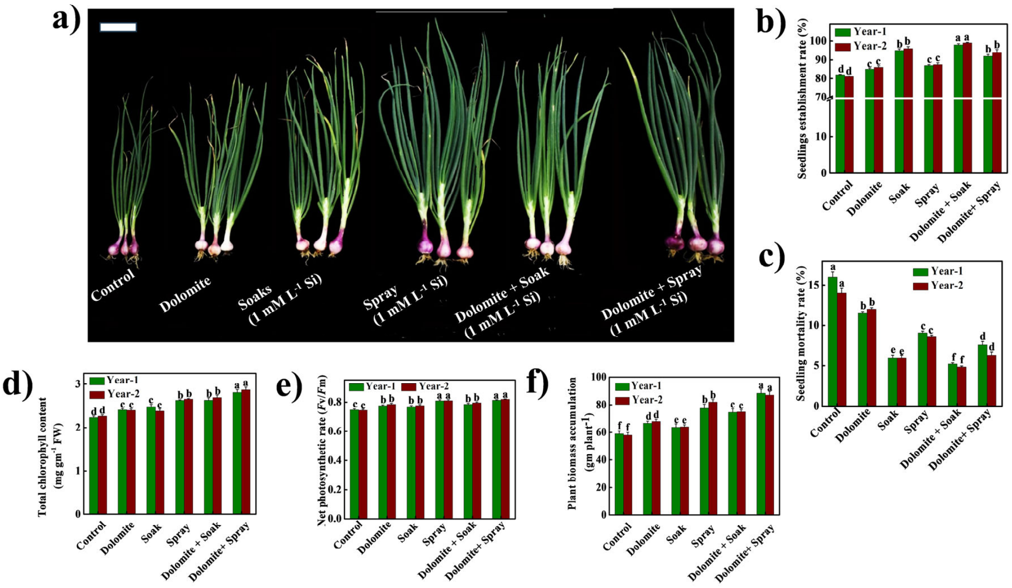

Figure 2. Effect of differential application techniques of selected nano-Si dose (1.0 mM L^{-1}) on onion seedlings growth: (**a**) phenotypic traits, (**b**) establishment rate, (**c**) mortality rate, (**d**) total chlorophyll content, (**e**) net photosynthesis, and (**f**) plant biomass accumulation. Bar = 12 cm. The data shown here are the averages of three replications, with the standard deviations indicated by vertical bars. Different letters above the bars indicate significant differences according to Tukey's test ($p < 0.05$).

Again, seedling mortality is a crucial factor affecting plant establishment and agricultural productivity. Interestingly, experimental results showed that the death of young plants before maturity was also largely influenced by the seed's pretreatment with Si-NMs, thereby improving the plant establishment rate under stressful conditions such as soil acidity (Figure 2b,c). For example, results showed that seedling mortality in acid soils varied by about 14–16% across two consecutive years of cultivation. In contrast, Si-NMs in both application methods significantly reduced seedling mortality; however, among the soak and spray techniques, seed pretreatments showed the best results, with rates of 2.3- to 2.7-fold lower than the control. Accordingly, the establishment rate increased by about 15.8–18.5% compared to untreated control seedings in two consecutive cultivation seasons (Figure 2b,c). Remarkably, dolomite supplementation as soil correction further augmented the seedling establishment rate upon Si-NM applications by reducing seedling mortality stimulated by soil acidity, indicating the influential role of Si-NMs in onion plant adaptation to stressful conditions such as soil acidity.

3.3. Si-NMs Enhance Onion Bulb Properties and Yield Under Acidic Soil Conditions

Enhancing onion bulb properties is crucial for increasing marketable yield and prolonging postharvest shelf life. To understand the role of Si-NMs in enhancing onion bulb properties, we have observed onion bulb phenotypes, scale numbers, scale thickness, bulb dry matter content, individual bulb weight, and yield of onions over two consecutive years. The phenotypic results showed that soil acidity strongly affects the fleshy structure and overall bulb size. However, the applications of Si-NMs, specifically spray techniques, improve overall bulb size and fleshiness, further enhancing the effectiveness of dolomite supplementation as an acid soil correction practice (Figure 3a). However, only dolomite supplementation failed to compensate for the acid-stress-induced suppression of bulb growth. Similarly to the phototypes, the experimental results showed that the individual bulb weight, number of bulb scales, thickness of bulb scales, and dry matter content of bulbs significantly improved with Si-NM application, which suppresses soil acidity. For example, the individual bulb weight, number of bulb scales, and thickness of bulb scales increased about 39.8–39.3%, 65.2–79.1%, and 48.5–58.1%, respectively, compared to their controls in two consecutive cultivation years, which were followed by soak and dolomite application (Figure 3b–d). Accordingly, the dry matter content and total bulb yield also increased by about 65.5–78.8% and 59.6–65.5%, respectively, under Si-NMs spray treatment compared to their acid soil control in the consecutive cultivation year (Figure 3e,f). Interestingly, applying dolomite after Si-NM application further improved the studied properties. For example, the total bulb yield of Si-NMs spray treatment with dolomite supplementation increased by about 11.5–13.8% compared to Si-NMs spray treatment alone, indicating the suitability of Si-NMs from seedling establishment through harvest for onions under acidic soil conditions.

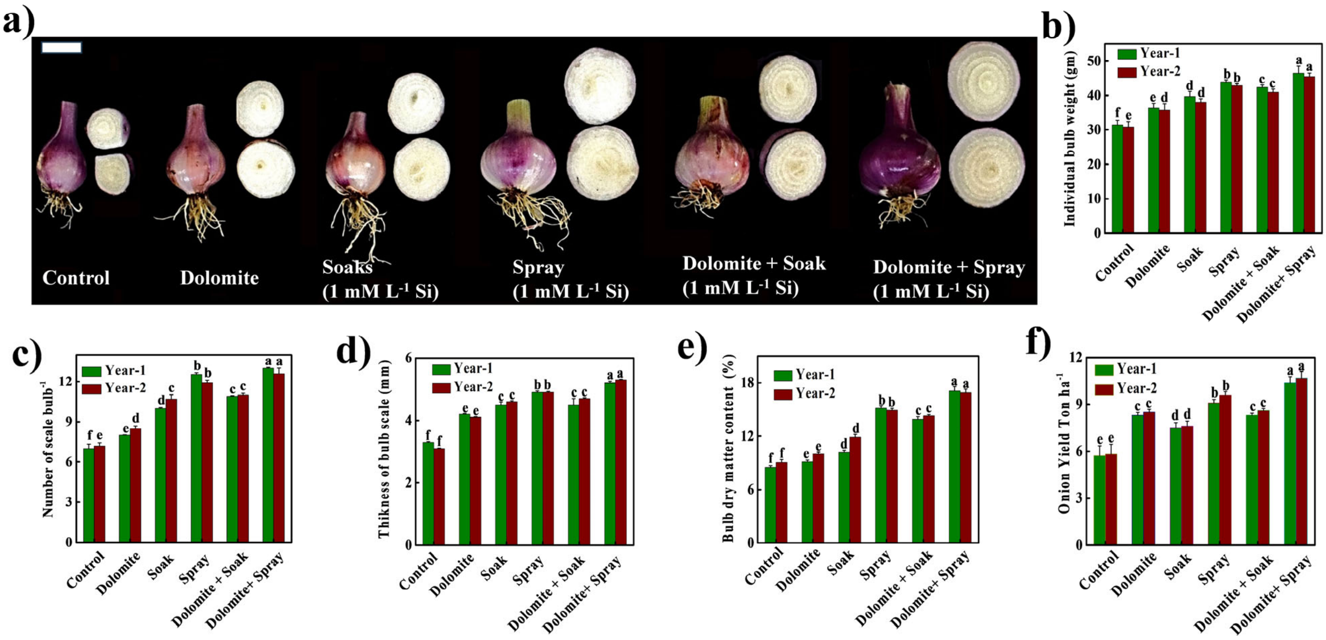

Figure 3. Role of Si-NMs on onion bulb attributes: (**a**) bulb phenotypes, (**b**) individual bulb weight, (**c**) number of bulb scales, (**d**) thickness of bulb scales, (**e**) dry matter content, and (**f**) yield of onions in two consecutive years. Bar = 4 cm. The data shown here are the averages of three replications, with the standard deviations indicated by vertical bars. Different letters above the bars indicate significant differences according to Tukey's test ($p < 0.05$).

3.4. Si-NMs Play a Critical Role in Soil Acidity-Induced ROS Homeostasis in Onion Crops

Overproduction of reactive oxygen species (ROS) affects plant growth by inducing protein, lipid, and DNA damage. To understand the role of Si-NMs in ROS homeostasis, we initially measured H_2O_2 accumulation, electrolyte leakage, and lipid peroxidation as MDA content. Remarkably, results revealed that soil acidity causes an excessive amount of 0.29 µM gm^{-1} FW of H_2O_2 accumulation in onion crops, thereby showing an increased level of electrolyte leakage (EL) and MDA content (Figure 4a–c). Interestingly, SI-NM application in both ways reduced ROS accumulation and EL and MDA content. However, among the application techniques, exogenous Si-NMs spray showed the best performance in reducing H_2O_2, EL, and MDA content. However, the Si-NMs spray technique showed reductions of H_2O_2, EL, and MDA content of about 1.5 fold, 2.3 fold, and 1.5 fold, respectively, compared to their controls (Figure 4a–c). Again, the dolomite alone or dolomite and Si-NM seed pretreatment failed to compensate for ROS accumulation. But the Si-NMs spray upon dolomite supplementation caused a further reduction in H_2O_2, EL, and MDA content of about 26.3%, 48.2%, and 37.9%, respectively, compared to their Si-NMs spray treatments.

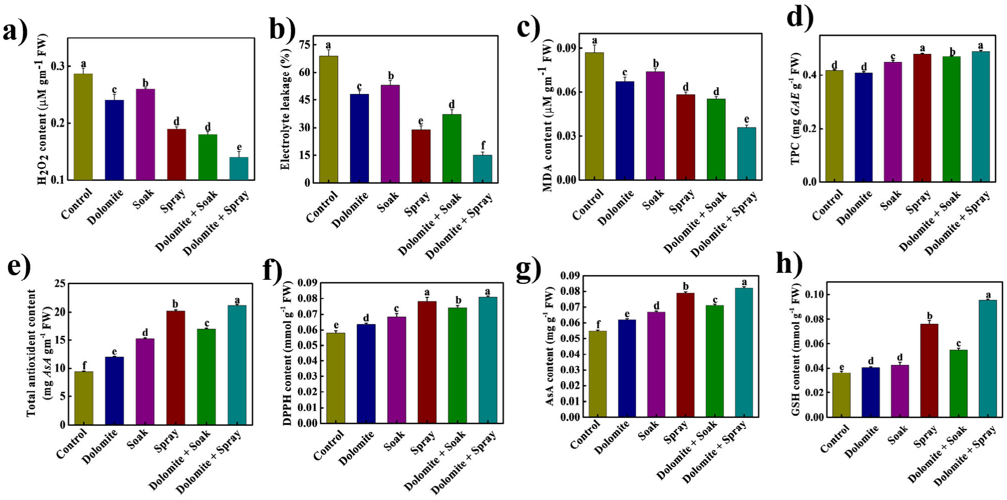

Figure 4. Role of Si-NMs in ROS homeostasis in onions under acidic soil conditions. (**a**) H_2O_2 accumulation, (**b**) Electrolyte leakage, (**c**) MDA content, (**d**) Total polyphenol (TPC) content, (**e**) total antioxidant, (**f**) DPPH, (**g**) ascorbic acid (AsA), and (**h**) GSH content in onions under acid stress conditions. The data shown here are the averages of three replications, with the standard deviations indicated by vertical bars. Different letters above the bars indicate significant differences according to Tukey's test ($p < 0.05$).

To further understand how Si-NMs amplify ROS homeostasis, we measured total polyphenol (TPC), total antioxidant capacity, DPPH, ascorbic acid (AsA), and GSH content in onions under acid stress conditions. Interestingly, results showed that prolonged soil acidity strongly affects the antioxidant defense system of onion crops, and only dolomite supplementation failed to fully optimize the enzymatic systems (Figure 4d–h). For example, dolomite supplementation as a world soil correction practice only increases total antioxidant, DPPH, ascorbic acid (AsA), and GSH content by 26.1%, 8.6%, 12.7%, and 11.1%, respectively, compared to their controls, and no significant differences were observed for TPC (Figure 4d–h). In contrast, the exogenous application of Si-NMs showed about 14.3%, 113.6%, 34.5%, 43.6%, and 111.2% increases in TPC, total antioxidant capacity, DPPH, ascorbic acid (AsA), and GSH content, respectively, compared to their controls, which were further augmented upon dolomite supplementation (Figure 4d–h). These results indicated the intricate mechanisms underlying onion crop adaptation in acid soils by reducing ROS stress.

3.5. Si-NMs Improve the Nutritional Properties of Onions Grown Under Acidic Soil Conditions

Enriching the nutritional quality of onions, which is equally important for harvest quality and human health, has been a major research focus. To understand the potential roles of Si-NMs in nutritional enrichment, we analyzed the macro-(N, P, Ca, Mg, Na, K, and S) and micronutrient (Zn, Fe, Cu, and Mn) content of onions. Results showed that all nutrients varied significantly among treatments, except Cu content (Table 1). Interestingly, all the studied macronutrient contents significantly improved with Si-NM applications, while spray showed the best performance in nutrient enrichment, which was were suppressed by acidic soil conditions. For example, the Ca, Mg, K, P, N, Na, and S increased about 64.3%, 24.6%, 37.1%, 21.9%, 26.2%, 48.1%, and 36.4%, respectively, in comparison to the onions grown in acid soils, which further enriched when dolomite was applied as part of soil correction practices (Table 1). Similarly, Zn increased about 2.0-fold, and Fe and Mn decreased about 1.6-fold and 1.5-fold, respectively, in Si-NMs-sprayed onions compared with the control. Interestingly, dolomite supplementation in Si-NMs-sprayed onion crops showed about 7.1% further enrichment of Zn compared to the Si-NMs-sprayed treatments (Table 1). However, dolomite alone shows little or no effect on nutritional enrichment and homeostasis in onions, suggesting the potential roles of Si-NMs, which have broader implications for harvested quality and human health benefits.

Table 1. Effect of different application forms of nano-Si on the nutritional properties of onion. The average nutritional properties of an onion bulb over two years are presented here. Values are mean (*n* = 3). Values containing the same letters are statistically similar. ** = Significant at 1% level ($p < 0.01$); * = Significant at 5% level ($p < 0.05$); ns = non-significant.

Treatments	Ca^{2+} mg/100 g	Mg^{2+} mg/100 g	K^{+} mg/100 g	P mg/100 g	N mg/100 g	Na^{+} mg/100 g	S content mg/100 g	Zn mg/100 g	Fe mg/100 g	Cu mg/100 g	Mn mg/100 gm
Control	23.83 ± 0.43 e	16.25 ± 0.13 d	12.75 ± 0.43 d	33.32 ± 0.79 d	39.52 ± 0.5 d	2.06 ± 0.03 e	28.3 ± 0.1 f	0.21 ± 0.01 e	0.33 ± 0.02 a	0.16 ± 0.01 b	0.51 ± 0.01 a
Dolomite	37.12 ± 0.32 c	15.23 ± 0.11 e	15.05 ± 0.1 c	35.51 ± 0.25 c	38.31 ± 0.23 d	2.24 ± 0.04 d	34.5 ± 0.45 d	0.27 ± 0.02 c	0.29 ± 0.01 a	0.19 ± 0.02 a	0.43 ± 0.01 b
Soak (1 mM/L nano-Si)	28.67 ± 0.72 d	17.15 ± 0.30 d	15.19 ± 0.7 c	33.89 ± 0.53 d	41.56 ± 0.15 e	2.31 ± 0.06 d	30.3 ± 0.2 e	0.24 ± 0.01 d	0.23 ± 0.01 b	0.17 ± 0.01 a	0.34 ± 0.01 d
Spray (1 mM/L nano-Si)	39.12 ± 0.5 bc	20.21 ± 0.29 b	17.48 ± 0.13 a	40.61 ± 0.40 a	49.85 ± 0.24 a	3.06 ± 0.26 b	38.6 ± 0.41 c	0.42 ± 0.02 a	0.18 ± 0.01 d	0.16 ± 0.01 b	0.29 ± 0.02 e
Dolomite + Soak	42.81 ± 1.2 b	19.11 ± 0.1 c	16.11 ± 0.1 b	37.75 ± 0.1 b	39.89 ± 0.12 d	2.86 ± 0.06 c	40.7 ± 0.3 b	0.31 ± 0.03 b	0.20 ± 0.01 d	0.18 ± 0.01 a	0.37 ± 0.02 c
Dolomite + Spray	46.82 ± 0.49 a	23.28 ± 0.24 a	19.42 ± 0.49 a	41.94 ± 0.54 a	44.56 ± 0.26 b	4.32 ± 0.21 a	47.2 ± 0.54 a	0.45 ± 0.02 a	0.17 ± 0.01 b	0.18 ± 0.02 a	0.28 ± 0.01 e
***p* value**	**	**	**	**	**	*	**	**	*	Ns	**

3.6. Si-NMs Affect the Bulbing Ratio and Early Maturity of Onion Crops

The bulb diameter-to-neck diameter ratio is an important factor in onion maturity and quality. To understand the role of Si-NMs on early reaches of harvestable sizes we determined the bulbing ratio over time (Figure 5). The phenotypical images show that at twenty days of sowing, the bulbs treated with Si-NMs have no visual differences in blubbing sizes, while at forty days of sowing, the changes are obvious, showing that the differences among the treatments becomes more prominent over time (Figure 5a). Interestingly, among the treatments, the Si-NMs spray displayed more enlarged bulbing sizes, which further augmented upon dolomite supplementations. In accordance with the visual observations, the numerical values also showed that acid soils strongly inhibited the bulbing ratio, which remained mostly insignificant with Si-NMs soak treatments, whereas exogenous Si-NMs spray showed a distinct ability to enhance the bulbing ratio. For examples, exogenous Si-NMs spray exhibited about 22.6% higher bulbing ratio at 80 days of sowing compared to the control crops, further augmented by about 7.6% upon dolomite sedimentations (Figure 5b). Surprising, the Si-NMs soak or seeds pretreatment showed about 15.4% reduced bulbing ratio, even with dolomite supplementations, compared to exogenous Si-NMs spray, indicating the appropriateness of Si-NMs spray in the adaptation of onion crops to acidic soil conditions.

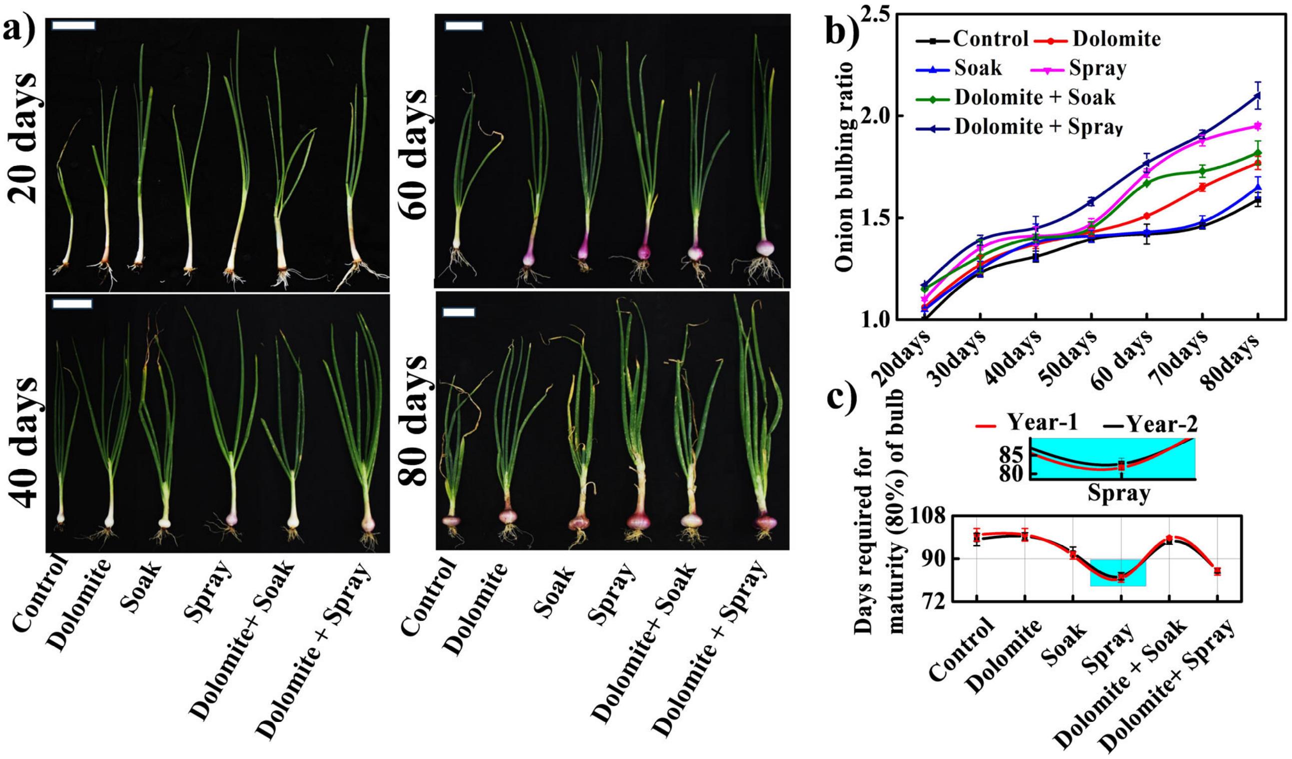

Figure 5. Role of Si-NMs in bulbing ratio and maturity index of onions grown under acidic soil conditions: (**a**) phenotypes, (**b**) bulbing ratio, and (**c**) the days required for 80% maturity of the onions upon Si-NMs applications under acid stress conditions. The data shown here are the averages of three replications, with the standard deviations indicated by vertical bars.

Furthermore, we examined the periods required to reach harvestable onion sizes. Remarkably, results showed that control plants and only the dolomite-complemented plants needed about 98–100 days to achieve 80% maturity. However, exogenous Si-NMs showed the shortest time period, only 81–82 days in two consecutive cultivation years, to reach the harvestable sizes of onion bulbs, followed by other treatments (Figure 5b). Most surprisingly, dolomite supplementation as soil correction again shows no or little impact on the onions reaching harvestable sizes, indicating that only dolomite has no executive potential to regulate the maturity index without Si-NMs. Again, to understand the mechanisms by which Si-NMs stimulate the bulbing ratio and early maturity, we measured the two key hormones, gibberellins (GA_3) and auxin (indole-3-acetic acid, IAA), that regulate bulbing. Interestingly, the results indicated that both the IAA and GA_3 content showed the lowest values, 5.1 and 29.2 ng g^{-1} FW, in onions grown in acidic soils, values which remained similar to those in onions grown using dolomite supplementation as a soil correction practice (Figure 6). Surprisingly, both treatments (soak and spray) of Si-NMs significantly affected IAA and GA_3 synthesis in onion crops, and among these two treatments, exogenous spray of Si-NMs had the most significant effect. For example, Si-NMs increased IAA and GA3 content by about 2.9- and 7.0-fold, respectively, compared to their control crops grown in acid soils. Interestingly, again, dolomite supplementation has little effect on Si-NMs spray and further augments IAA and GA_3 content by about 13.3% and 3.1%, respectively, compared to Si-NMs spray alone. These results illuminate the intricate mechanisms by which Si-NMs in onion crops enhance bulbing ratio and early maturity.

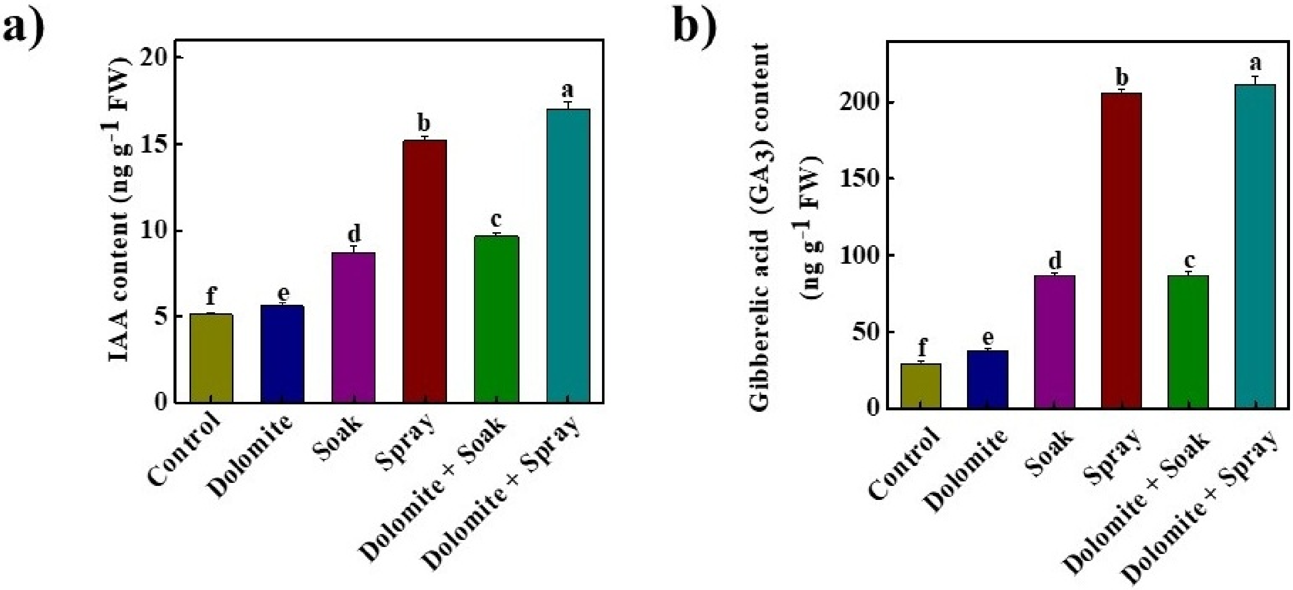

Figure 6. Involvement of Si-NMs in the hormonal regulation associated with onion bulbing: (**a**) indoleacetic acid (IAA) content and (**b**) gibberellic acid (GA_3) content in onions collected at 45 days of sowing. The data shown here are the averages of three replications, with the standard deviations indicated by vertical bars. Different letters above the bars indicate significant differences according to Tukey's test ($p < 0.05$).

4. Discussion

Soil acidity is a major limiting factor that globally restricts crop growth and agricultural expansion in several ways, including reduced root growth, reduced microbial activity, reduced nutrient availability, and increased metal toxicity [20,24–26]. In contrast, in recent years, silicon nanomaterials (Si-NMs) have attracted significant attention for agricultural applications due to their ability to enhance crop growth and stress tolerance [12]. Here, we performed a series of sequential experiments from seed germination to field trials in two consecutive cultivation years. Remarkably, results showed that among the differential doses, 1.0 mM L^{-1} of Si-NMs had the most stimulatory effects on onion seed germination and seedling growth, which were suppressed by soil acidity (Figure 1 and Supplementary Figure S1). The persuasive effects of a selected dose of Si-NMs in plant adaptation to stressful acidic soil conditions were associated with the minimization of amplified ROS accumulation, electrolyte leakage, and lipid peroxidation through promoting total polyphenol (TPC), total antioxidant, DPPH, ascorbic acid (AsA), and GSH content in onions. Consequently, we observed reduced seedling mortality and improved plant photosynthetic performance, overall bulb properties, and the nutritional quality of onions under stressful acidic soil conditions (Figures 2–4 and Table 1). Furthermore, results also showed that Si-NMs spray enhanced the early reaches of harvestable-sized onions, as determined by bulbing ratio over time. Interestingly, these enhanced bulbing ratios and early maturity in response to Si-NMs were associated with increased synthesis of IAA and GA_3 hormones (Figures 5 and 6). Additionally, Si-NMs spray upon dolomite supplementation as a soil correction practice completely reverses the acid-soil-induced suppression of onion crop growth, yield, and quality, with early reaches of the harvestable sizes of onions signifying the farming resilience of Si-NMs, essential for sustainable agricultural expansion in problematic soils like acidity and food security [4,10,12,27,28].

Agricultural harvested yield is crucially affected by seed germination and seedling establishment, or by reduced seedling mortality [29,30]. However, it has been observed that seed germinability and post-germination seedling establishment are largely affected by ecological constraints such as drought, salinity, heavy metals, or soil acidity [12,31]. Despite traditional breeding efforts to improve germination and establishment through harvesting, nano-priming enhanced not only germination percentage but also reduced mortality rates, promoting seedling vigor under environmental stress [31,32]. Similarly, in the present experiments, we observed the competency of Si-NMs in onion seed germination, post-germination seedling growth, and mortality in differential dose trials (0, 0.25, 0.5, 1.0, and 1.5 mM L^{-1}). Interestingly, experimental results showed that all Si-NM doses had positive stimulatory effects on germination, seedling growth, and post-germination seedling establishment rate. However, 1.0 mM L^{-1} of Si-NMs showed the most distinct effects on seedling growth and establishment rate at both the normal (pH-6.5) and acid soil (pH-4.8) conditions. For example, 1.0 mM L^{-1} of Si-NMs showed about 38.8% additional germination rate compared to the non-treated control, which might be due to the increased activity of amylase (Figure 1). As previously observed, Si-NMs significantly improve the alpha amylase and β-amylase activity by about 111–186% in seeds during germination at 1500 mg L^{-1} doses [33]. It has also been observed that, at optimal doses, priming of seeds with Si-NMs enhanced seed coat permeability to water, resulting in improved germination and seedling growth [34,35]. In contrast, at higher Si-NM doses (1.5 mM L^{-1}), excess ions in tissues may promote the overaccumulation of ROS in cells, thereby damaging cellular macromolecules and affecting the overall success of seed germination and seedling growth [36–38].

Seedling mortality is a crucial aspect that directly affects final agricultural productivity. In accordance with this, the present experiment elucidated that among the different techniques of Si-NMs application, exogenous spray with a 1.0 mM L^{-1} dose of Si-NMs showed the lowest mortality rate by about 2.7-fold. The mortality rate was augmented by soil acidity stress by about 14–16% compared to normal acid soil conditions observed in two consecutive cultivation years (Figure 2). This might be due to improved nutrient homeostasis under stressful conditions like acidity, which enhanced plant total chlorophyll content and overall photosynthetic performance [34]. Correspondingly, we also observed that exogenous Si-NMs enhanced the homeostasis of essential macro- and micronutrients. For example, exogenous Si-N spray increased the macronutrients Ca, Mg, K, P, N, Na, and S by up to 64.3% compared to their acid controls. Similarly, the micronutrient Zn increased by about 2.0-fold, and Fe and Mn decreased about 1.6-fold and 1.5-fold, respectively, compared to the control, and this was further amplified upon dolomite supplementation (Table 1). This optimal nutrient homeostasis crucially regulates the synthesis of photosynthetic chlorophyll pigments in plants, thereby promoting increased photosynthesis and successful crop growth [34,39]. Chlorophyll is indispensable for photosynthesis and is crucially responsible for light absorption [40]. In accordance, we also observed that exogenous Si-NMs spray increased chlorophyll content by about 18.0–22.7%, thereby increasing net photosynthetic rate and total bulb yield by about 8–9.2% and 59.6–65.5%, respectively, in the consecutive cultivation year, compared to their acid soil control (Figures 2 and 3).

Furthermore, this enhanced nutrient homeostasis in onion crops may stimulate the antioxidant potential of onion plants, which in turn induces plant systemic resistance to acid stress, as shown in Figure 4 [12,41]. Generally, at low soil pH or under acid stress conditions, essential nutrients like P, Ca, and Mg become less available to plants, but Al, Mn, and Fe become excessively available, leading to the overaccumulation of ROS and triggering oxidative damage to proteins, membranes, and DNA, or chromosomal aberrations [20,42]. ROS usually attack membrane lipids, causing lipid peroxidation that weakens cell membranes, leading to ion leakage, reduced water retention potential, and increased cell death [42,43]. Similarly, we observed that acid stress stimulates H_2O_2 accumulation, thereby increasing electrolyte leakage (EL) and MDA content. In response, plants generally activate the antioxidant system; however, this leads to prolonged stress due to the rate-limiting potential of defense mechanisms [44]. Remarkably, the current experiments revealed that exogenous spraying of Si-NMs achieved the highest reduction in H_2O_2, EL, and MDA content. For example, the Si-NMs reduced H_2O_2, EL, and MDA content by about 52.6%, 137.9%, and 58.2%, respectively, in onion crops under acid stress conditions compared to their controls (Figure 4). Si-NMs mediated this enhanced ROS homeostasis in onion crops were potentially associated with the triggers of enzymatic activity and biosynthesis of secondary metabolites like total phenol and ascorbic acid content [45]. Comparably, the present experiment also revealed that the exogenous spraying of Si-NMs increased the TPC, total antioxidant, DPPH, ascorbic acid (AsA), and GSH content by about 14.3%, 113.6%, 34.5%, 43.6%, and 111.2%, respectively, compared to controls, which was further augmented upon dolomite supplementation. These results explain the intricate mechanisms of agricultural resilience and productivity under a stressful environment like soil acidity [12,45].

Additionally, the early stages of harvestable size are crucial for the maturity and market value of onions [1]. Interestingly, we observed here that in two consecutive cultivation years, exogenous Si-NMs-sprayed onion crops needed only 81–82 days to reach harvestable sizes, whereas control plants needs about 98–100 days to reaches harvestable maturity (Figure 5). Si-NMs mediating this enhanced maturity of onion crops might be associated with enhanced resistance to stressful acid soil conditions, which generally delay the maturity and quality of crops by hampering nutrient homeostasis and steady growth [46,47]. Again, Si-NMs were also found to enhance water retention, uniform bulb growth, and dry matter accumulation by refining photosynthesis and carbohydrate transport, which eventually led to a higher bulbing ratio and reaching harvestable onion sizes more quickly [8,48,49]. Moreover, an Si-NMs-attributed enhanced bulbing ratio may be associated with the transcriptional activation of flowering locus T (FT) genes. As many researchers have shown, transcriptional activation of *AcFT*1 and suppression of *AcFT*4 transcriptional abundance, regardless of the order, play a key role in the induction of bulbing or maturity in cultivated onions [50,51].

In fact, bulbing is a complex physiological process regulated by many factors, including hormonal signals, photoperiod (day length), and temperature. However, among the regulatory factors, hormonal signals are considered to be the most important, and endogenous modular systems play a crucial role in initiating and regulating the process [52]. Most surprisingly, we also observed that Si-NM treatment profoundly affects the synthesis of the two key hormones, gibberellins (GA_3) and auxin (indole-3-acetic acid or IAA), which are responsible for bulbing. For example, the exogenous spraying of Si-NMs increased IAA and GA3 content by about 2.9- and 7.0-fold, respectively, compared to their control crops grown in acid soils, and this effect was further augmented by dolomite supplementation (Figure 6). As an imperative phytohormone, gibberellins regulate many developmental processes in plants, including bulbing [52]. It has been observed that GA_3 can promote bulbing by enhancing cell expansion and increasing bulb size, even in the absence of optimal photoperiod or deficit irrigation conditions [9,52]. GA_3 treatment at an optimal dose was found to sharply increase the number of cloves and whorls per bulb of garlic, with enhanced nutritional quality [53]. Similarly, IAA has been found to induce bulbing in non-inductive photoperiods, but the mechanism remains unclear [9]. Recent studies have shown that IAA interacts with ethylene biosynthesis and is positively involved in onion bulb development [52,54]. Additionally, GA_3 and IAA were found to improve environmental stress tolerance by fine-tuning antioxidant enzymatic activity [54–56]. Taken together, these findings illuminate the intricate mechanism of Si-NM-mediated enlargement of bulbing, as well as the relevance of advancing the sustainable expansion of onion farming, especially in areas affected by soil acidity.

5. Conclusions

Soil acidity, or low soil pH, is a major challenge that affects crop growth and limits agricultural expansion worldwide. Here, we observed that among the differential doses, 1.0 mM L^{-1} of Si-NMs had the most stimulatory effect on onion seed germination and seedling growth, which are suppressed by soil acidity. Si-NMs mitigate soil acidity by reducing excessive ROS and improving nutrient homeostasis, thereby enhancing antioxidant defenses, including TPC, DPPH, AsA, and GSH, in onion. This strengthens plant resilience to acidic stress, highlighting Si-NMs as a promising tool for sustainable agricultural improvement (Figure 7). Additionally, as an innovative approach, exogenous Si-NMs advance the early stages of harvestable onion bulb sizes, accompanied by the enhanced regulation of GA_3 and IAA hormones. Interestingly, Si-NMs sprayed on dolomite-supplemented soil as a soil correction practice further improve adaptive potential, resulting in increased yield and quality of onions. Therefore, the role of Si-NMs in nutrient homeostasis, stress resilience, and hormonal regulation of bulbing and early maturity highlights their potential as a promising tool for sustainable agricultural expansion, particularly in problematic soils such as acidic soils. However, further studies are essential to elucidate the underlying molecular mechanisms and to perform a detailed analysis of soil health.

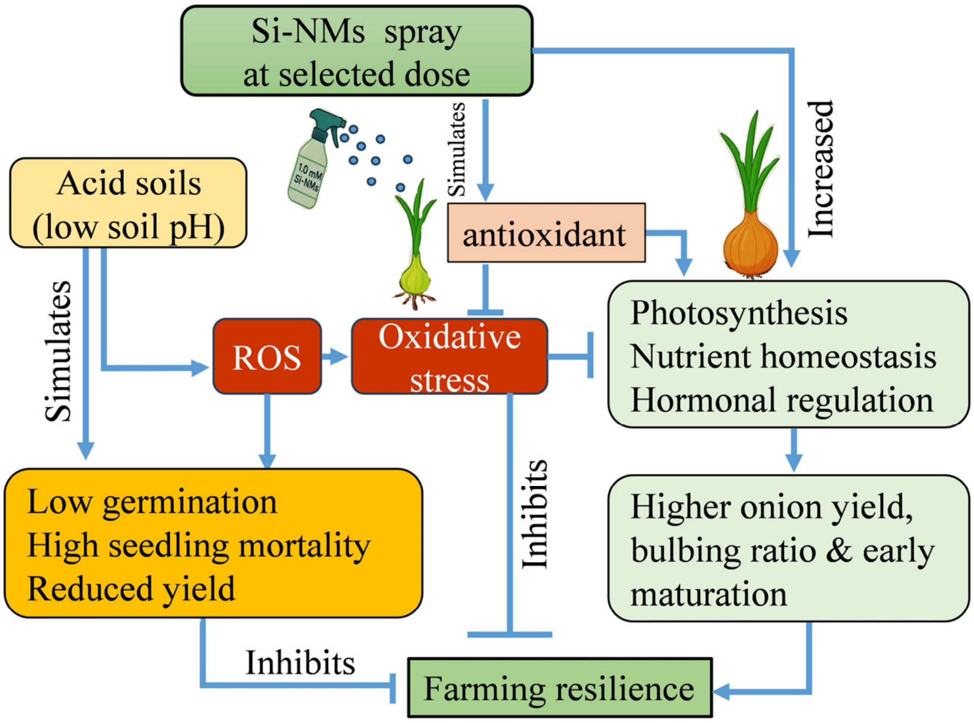

Figure 7. Proposed model of Si-NMs involvement in reducing onion crop sensitivity to low soil pH. The exogenous application of Si-NMs at 1.0 mM L^{-1} mitigates the adverse effects of soil acidity on onion crops by promoting ROS homeostasis by upregulating enzymatic activity. Additionally, Si-NMs influence hormonal signaling pathways, accelerating the early stages of onion development toward harvestable sizes, thereby enhancing farming resilience.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11111407/s1, Figure S1: Effect of selected dose (1.0 mM L^{-1}) of amorphous-Si on onion seedling growth at 27 days of sowing in both the field soil (pH-6.2) and acidic soil (pH-4.5) condition. Table S1: Pre- and post-harvest soil physicochemical properties.

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Data Availability Statement: The data are available from the corresponding author upon reasonable request due to copyright restrictions.

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Silicon Nanomaterials Enhance Seedling Growth and Plant Adaptation to Acidic Soil by Promoting Photosynthesis and Antioxidant Activity in Mustard (*Brassica campestris* L.)

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Abstract: Soil acidity is a divesting factor that restricts crop growth and productivity. Conversely, silicon nanomaterials (Si-NMs) have been praised as a blessing of modern agricultural intensification by overcoming the ecological barrier. Here, we performed a sequential study from seed germination to the yield performance of mustard (*Brassica campestris*) crops under acid-stressed conditions. The results showed that Si-NMs significantly improved seed germination and seedling growth under acid stress situations. These might be associated with increased antioxidant activity and the preserve ratio of GSH/GSSG and AsA/DHA, which is restricted by soil acidity. Moreover, Si-NMs in field regimes significantly diminished the acid-stress-induced growth inhibitions, as evidenced by increased net photosynthesis and biomass accumulations. Again, Si-NMs triggered all the critical metrics of crop productivity, including the seed oil content. Additionally, Si-NMs, upon dolomite supplementation, further triggered all the metrics of yields related to farming resilience. Therefore, the present study highlighted the crucial roles of Si-NMs in sustainable agricultural expansion and cropping intensification, especially in areas affected by soil acidity.

Keywords: soil acidity; silicon nanomaterials; redox homeostasis; yield; farming resilience

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1. Introduction

Low soil pH is an important factor in soil chemistry that largely influences soil nutrient cycling by affecting the soil microbial community and diminishing soil health and fertility. The unavailability of essential nutrients, like calcium, magnesium, phosphorus, sodium and potassium, by means of leaching or fixation leads to deficiency, poor plant growth and low crop productivity [1,2]. The key interconnected phenomenon of low soil pH-induced nutrient deficiency is the generation of excess reactive oxygen species (ROS) in plants that impact plant heath and productivity. Usually, excess ROS accumulation in plants oxidizes the vital cell ultrastructure organelles and inactivates the general cellular functions; as a result, plants not only become vulnerable to adaptation in acidic soils but also show reduced productivity. For example, it has been observed that the grain filling in crops is strongly affected by low pH through contributing to enhanced lipid peroxidation or membrane permeability [3]. However, the possessed antioxidant system may be activated to quench the excess ROS in plants during stress. Shockingly, such an activation of the defense system may not be sufficient to protect the oxidative damage to the plant over the long term [3,4]. Moreover, low soil pH could impact the effectiveness of plant antioxidant defense systems indirectly through nutrient misbalancing in cellular biosynthetic pathways [5].

Acid soils occupy approximately 30–40% of the world's land surfaces and about 50% of arable land, which affects germination niche, plant adaptation and the overall crop growth and productivity [6,7]. It has been observed that acid soil causes a burden of about USD 6.0 billion, which is 6% of the value of the total current production [8]. An extensive number of studies have explored how acid stress affects crop productivity via the inhibition of root elongation and branching and the reduced membrane permeability of roots for water and nutrient uptake [7]. Moreover, low soil pH augmented the replacement of essential base cations with toxic elements like aluminum (Al) by promoting their solubility, creating widespread toxicity. It has been observed that high levels of aluminum- or manganese-induced redox imbalances are the key vulnerability behind damage to plant root systems in acidic soils that reduce not only the water and nutrient absorption but also lead to stunted growth and productivity [7,9]. Therefore, understanding the interplay between soil acidity and ROS homeostasis is essential for developing strategies to improve plant health and productivity in acidic soils.

In contrast, there is an increasing demand for the expansion of agriculture to unfavorable soils, like those of low soil pH, to meet the global demand for food security [10]. Hence, sustainable management practices are crucial to maintain acid soils and stimulate agricultural productivity. Importantly, the efforts to increase and/or sustain the agricultural productivity are often driven by the innovation of technologies and effective management practices that could mitigate the adverse effects of soil acidity and oxidative stress. Expectedly, in recent years, the application of biostimulants like hormones or engineered nanoparticles resembling silicon nanomaterials (Si-NMs) has been widely used in agriculture as an eco-friendly approach to enhance the crop productivity and tolerance to multiple stresses through the addition of limestone [11]. Primarily, silicon is taken up by plants as monosilicic acid (H_4SiO_4) through specific transporters and deposited in tissues as amorphous silica ($SiO_2 \bullet nH_2O$), which regulates plant growth under adverse conditions like soil acidity [12]. However, as a complex abiotic factor, the overwhelming role of low soil pH or soil acidity on plant survival potential has been poorly explored, and very few studies have focused on the involvement of Si-NMs in plant adaptation to harsh acidic soil [13]. Therefore, we hypothesized that the application of Si-NMs might provide an additional strength to reduce the vulnerability of plants to soil acidity. Our study provides a comprehensive understanding of Si-NM-mediated effects on seed germination, seedling growth and their association with antioxidant activity and redox homeostasis. Moreover, we also studied the influential role of Si-NMs with or without dolomite as a global soil correction practice on photosynthesis, yield attributes and seed yield of mustard with an oil percentage essential for sustainable agricultural development and to address the global food demand.

2. Results

2.1. Effect of Differential Doses of Si-Nanomaterials (Si-NMs) on Seed Germination and Seedling Growth of Mustard under Acidic Conditions

To understand the heightened effect of Si nanomaterials on mustard germination and seedling growth, we first performed an in vitro experiment with different doses (0, 0.25, 0.5, 1.0 and 1.5 mmol L^{-1}) of Si-NMs and observed the competency at 7 days after sowing. The results showed that all the differential doses of Si nanoparticles had stimulatory effects on seed germination and seedling growth under normal conditions, as visualized in Figure 1. However, among the doses, 0.5 mmol L^{-1} showed the finest results, followed by others. Like the visual display, our numerical data also revealed that the 0.5 mmol L^{-1} dose of nano-Si enhanced, by about 8.7% and 7.8%, additional germination in BARI mustard-14 and BARI mustard-17 compared to their respective control. Similarly, the seedling height and biomass accumulation were also augmented by seed pretreatment with Nano-Si. For example, the results showed that, among the doses, 0.5 mmol L^{-1} Nano-Si enhanced the root and shoot height by about 140% and 54.8% in BARI mustard-14 and 189.3% and 47.2% BARI mustard-17, respectively, compared to their control, which was followed by 1.0 mmol L^{-1} (Figure 1d,e). In contrast, higher doses (1.5 mmol L^{-1}) of Nano-Si displayed 15.9% and 14.5% reduced biomass accumulation and 6.2% and 11.2% reduced germination in BARI mustard-14 and BARI mustard-17, respectively, compared to 0.5 mmol L^{-1} doses, indicating that Si-NM-mediated mustard seed germination and seedling growth are dose-dependent.

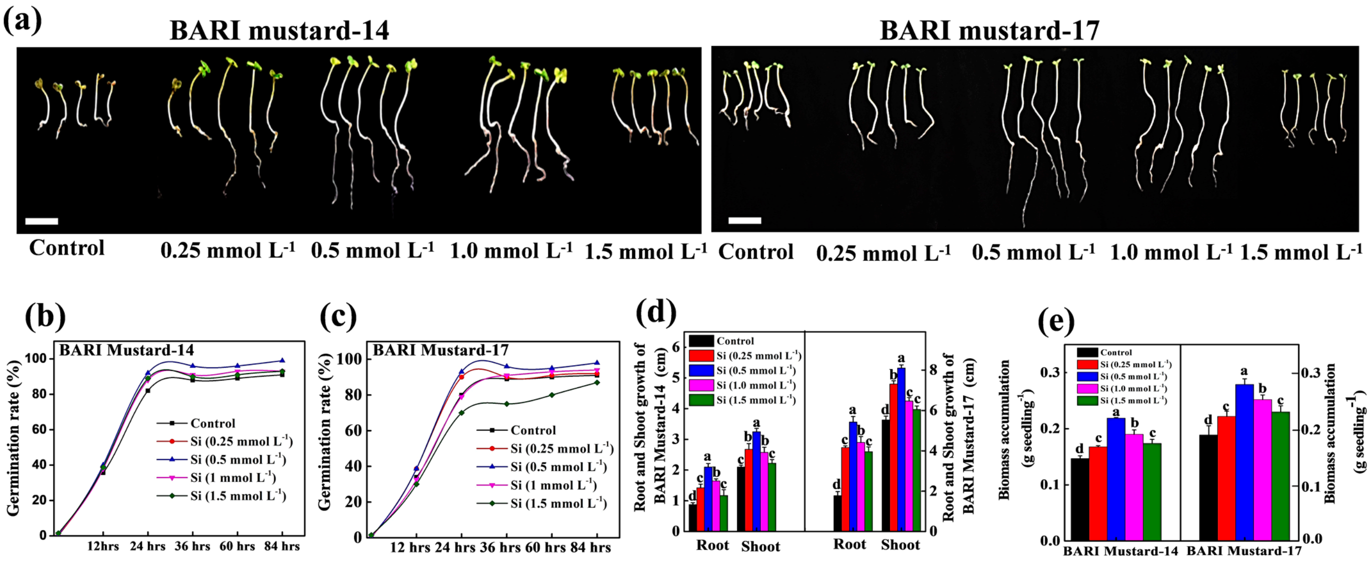

Figure 1. Selection of Si dose based on seed germination and seedling growth. (**a**–**c**) Effect of different doses (0–1.5 mmol L^{-1}) of Si nanomaterials (Si-NMs) on seed germination, bars = 1 cm and (**d**,**e**) root shoot growth of seedling of two mustard varieties, BARI-14 and BARI-17, at 7 days of germination. The data presented as the means of three replicates (±SE) and the means denoted same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

After preliminary selection of the Nano-Si dose (0.5 mmol L^{-1}), we performed another experiment under acidic conditions at pH 4.5 to observe the efficacy of Nano-Si in stressed conditions (Figure 2), as soil acidity is one of the major concerns that strongly reduces mustard seed germination, seedling growth and adaptation. Interestingly, the results showed that Nano-Si significantly improved the germination and seedling biomass accumulation in both BARI mustard-14 and BARI mustard-17, which were firmly suppressed by the acidic medium. The results showed that under acidic conditions, the germination and biomass accumulation of BARI mustard-14 and BARI mustard-17 was reduced by 55.3% and 61.8% and 21.7% and 31.8%, respectively, compared to their control, indicating that BARI-14 is more sensitive to acid stress compared to BARI-17 (Figure 2a–e). However, seed pretreatment with Nano-Si improved germination and biomass accumulation in both varieties. For example, in BARI-14, the germination and biomass accumulation were improved by 1.4-fold and 3.4-fold, respectively, compared to their acid-only treatment, similar to the response of BARI-17. These results indicated that Nano-Si has a positive stimulatory effect on mustard seed germination, seedling growth and biomass accumulation under stressed acidic conditions, while this response varied with the varietal consistencies.

2.2. Si-NM-Mediated Mustard Seedling Growth and Establishment Are Associated with ROS Homeostasis

To understand the interaction between acidic growth conditions and the generation of ROS, we stained the mustard seedlings with DAB and NBT to visualize the H_2O_2 and $O_2{}^{*-}$. The histochemical results showed that ROS accumulation remained almost constant in the control condition, while ROS were substantially accumulated in acidic conditions (Figure 3a,b). However, seed pretreatment with Si-NMs strongly minimized the acidic-growth-condition-induced ROS accumulation in both mustard varieties. Similar to the visualized symptoms, our biochemical values also revealed that the acidic growth condition augmented a more than 2.0-fold higher H_2O_2 accumulation in both BARI mustard-14 and BARI mustard-17, compared to their respective control. However, seed pretreatment with

Si-NMs reduced about 22.3% and 30.7% less H_2O_2 accumulation in both BARI mustard-14 and BARI mustard-17 seedlings compared to their acid-only treatment (Figure 3c). Furthermore, the malondialdehyde (MDA) content from lipid peroxidation and the percent of electrolyte (%EL) were also found to be increased in both mustard seedlings under acidic growth conditions, while seed pretreatment with Si-NMs minimized the excessive MDA and electrolyte leakage content. For example, seed pretreatment with Si-NMs reduced the EL by about 38.6–45.8% MDA and 30.8–36.9% in both tested varieties (Figure 3d,e), indicating the potential role of Si-NMs in ROS homeostasis.

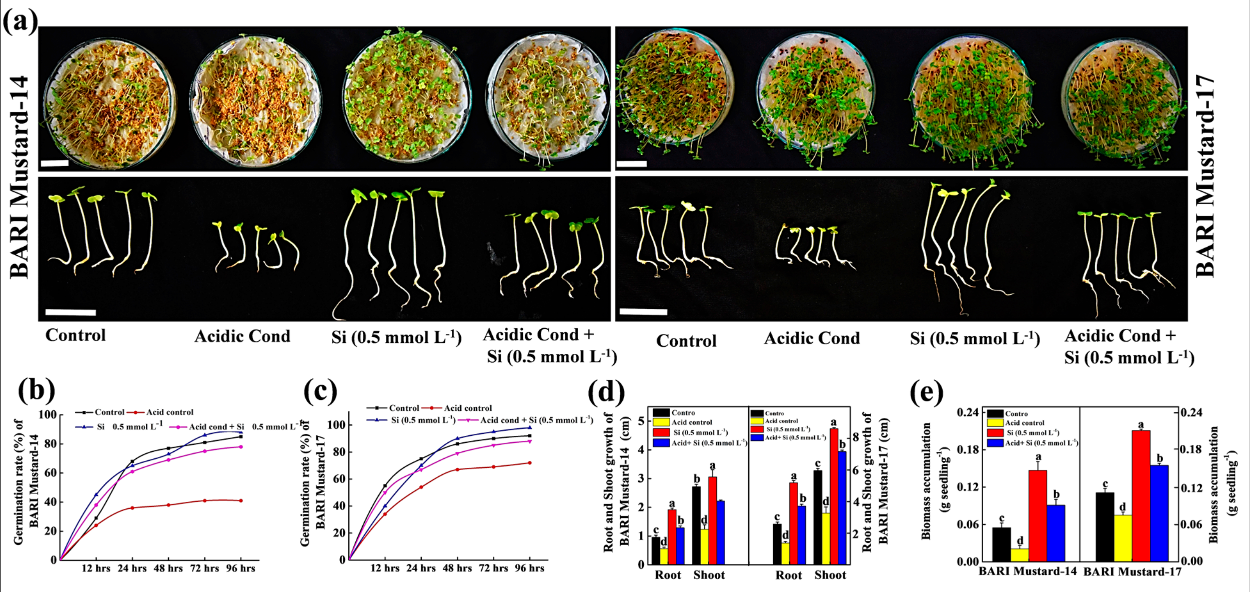

Figure 2. Effect of selected dose (0.5 mmol L^{-1}) of Si-NMs on germination and seedling growth under acidic conditions (at pH 4.5); (**a**–**c**) effect of Si-NMs at 0.5 mmol L^{-1} doses on seed germination, bars = 2 cm upper panel and bars = 1 cm lower panel and (**d**,**e**) root shoot growth and biomass accumulation of seedlings of two mustard cultivars, BARI-14 and BARI-17, at 7 days of germination under acidic conditions. The data presented as the means of three replicates (±SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

To further investigate the physiological interplay of Si-NMs on redox homeostasis under acidic conditions, we measured the total antioxidant, DPPH and the ratio of GSH/GSSG and AsA/DHA. Interestingly, the results displayed that the total antioxidant and DPPH activity was strongly inhibited by acidic growth conditions in both varieties. For example, the total antioxidant and DPPH activity was reduced by 30.7% and 8.9% and 22.8% and 19.9%, respectively, in BARI mustard-14 and BARI mustard-17 compared to their control conditions (Figure 4a,b). In contrast, although seed pretreatment with Si-NMs under normal conditions showed no effect, Si-NMs significantly hindered the reduced activity of DPPH and the total antioxidant content under acidic growth conditions, showing about 47.8% and 65.4% and 91.8% and 96.0% higher activity in BARI mustard-14 and BARI mustard-17 compared to their acid control (Figure 4). Similarly, the ratio of reduced glutathione to oxidized forms of glutathione (GSH/GSSG) and the ratio of ascorbate to dehydroascorbate (AsA/DHA) in both mustard seedling varieties were strongly reduced under acidic growth conditions compared to their respective control (Figure 4c,d). The reduction in the GSH/GSSG and AsA/DHA ratios ranges from 73.4 to 103.7% and 84.1 to 112.3%, respectively, under acidic conditions compared to their control conditions. In contrast, although the seed pretreatment with Si-NMs revealed no significant change in the ratio of GSH/GSSG and AsA/DHA under normal growth conditions, but under acidic growth conditions, Si-NMs prominently uphold the ratio of GSH/GSSG and AsA/DHA in the seedlings of both tested mustard varieties (Figure 4c,d). For example, the ratios of

GSH/GSSG and AsA/DHA in seedlings of BARI mustard-14 were increased by about 68.5% and 76.8%, respectively, under acidic growth conditions compared to non-treated seedlings. These results indicate that seed pretreatment with Si-NMs plays a critical role in redox homeostasis under acidic conditions by stimulating the antioxidant and DPPH activity and upholding the ratio of GSH/GSSG and AsA/DHA.

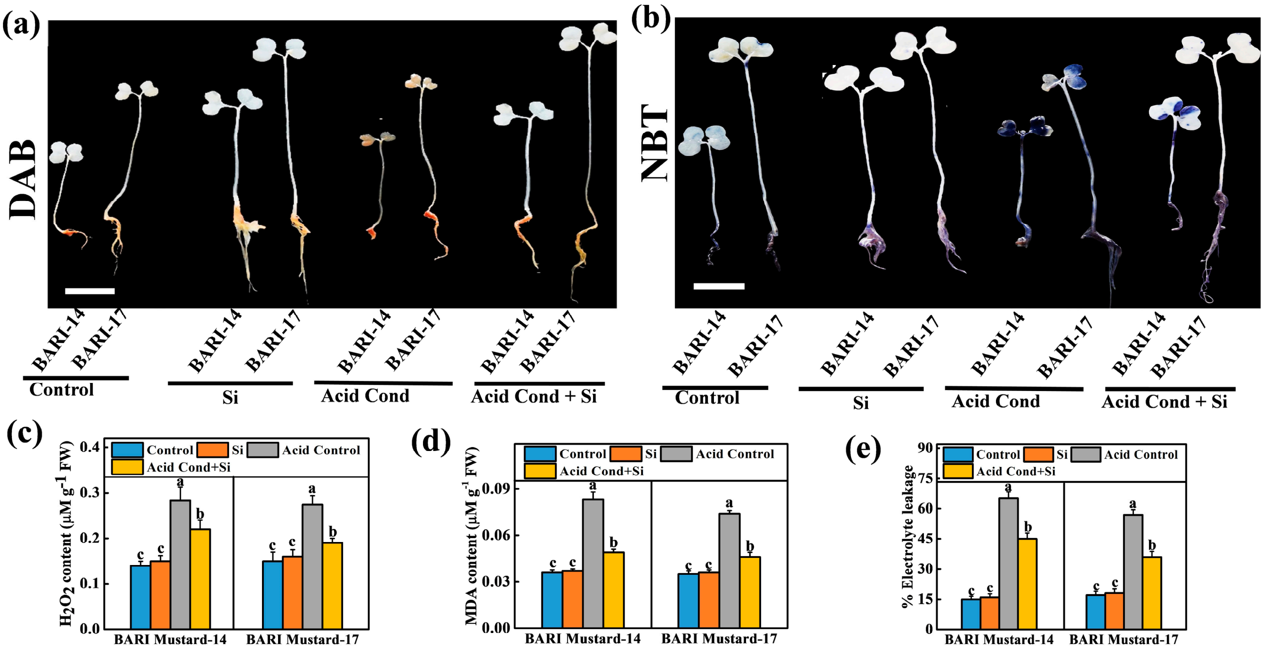

Figure 3. Si-NMs at selected dose (0.5 mmol L^{-1}) reduce acid stress (at pH 4.5) induced over accumulation of ROS in mustard seedlings; (**a**,**b**) histochemical staining of mustard seedlings with DAB and NBT, respectively, bars = 1 cm; (**c**) H_2O_2 accumulation, (**d**) lipid peroxidation (MDA) content and (**e**) electrolyte leakage (EL) of two mustard varieties, BARI-14 and BARI-17, at 7 days of germination under acidic conditions. The data presented as the means of three replicates (±SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

2.3. Exogenous Si-NMs Enhanced Plant Growth and Biomass Accumulation under Acidic Field Soil Condition

Soil acidity is a limiting factor of crop growth and productivity. To understand the promising opportunities of Si-NMs to manage the adverse soil acidity effect and improve crop productivity, we extended our study at the field level under acidic soil conditions. The results displayed that soil acidity (pH 4.5–4.8) in field conditions strongly suppressed the normal growth of both mustard varieties compared to the crops grown at the normal pH 6.2–6.3 (field soil) level (Figure 5a). However, the exogenous application of Si-NMs at the selected dose (0.5 mmol L^{-1}) significantly improved the acidic-soil-condition-induced suppression of mustard crop growth. For example, the total biomass accumulation of BARI mustard-14 and BARI mustard-17 was reduced by 4.1-fold and 1.2-fold, respectively, compared to the plants grown in normal acidic soil conditions (Figure 5d). However, the exogenous application of Si-NMs improved the total biomass accumulation by about 3.7-fold and 1.9-fold compared to the non-treated plants grown in acidic soil conditions. Similarly, the total chlorophyll content, net photosynthesis and root shoot ratio of acid-stressed plants were also improved via the exogenous application of Si-NMs in both tested varieties. For instance, the total chlorophyll content and net photosynthesis were improved by 35.1% and 76.4% and 157.2% and 200.4% for BARI mustard-14 and BARI mustard-17, respectively, compared to the acid-stressed plants. In contrast, the exogenous application of Si-NMs caused a further increase in the total chlorophyll content, net photosynthesis and growth biomass in both mustard varieties grown in normal field soil conditions. The results showed that in normal field soil conditions, Si-NMs stimulated further plant biomass accumulation by about 48.9% and 80.7% in BARI mustard-14 and BARI mustard-17, respectively, compared to their respective control (Figure 5d).

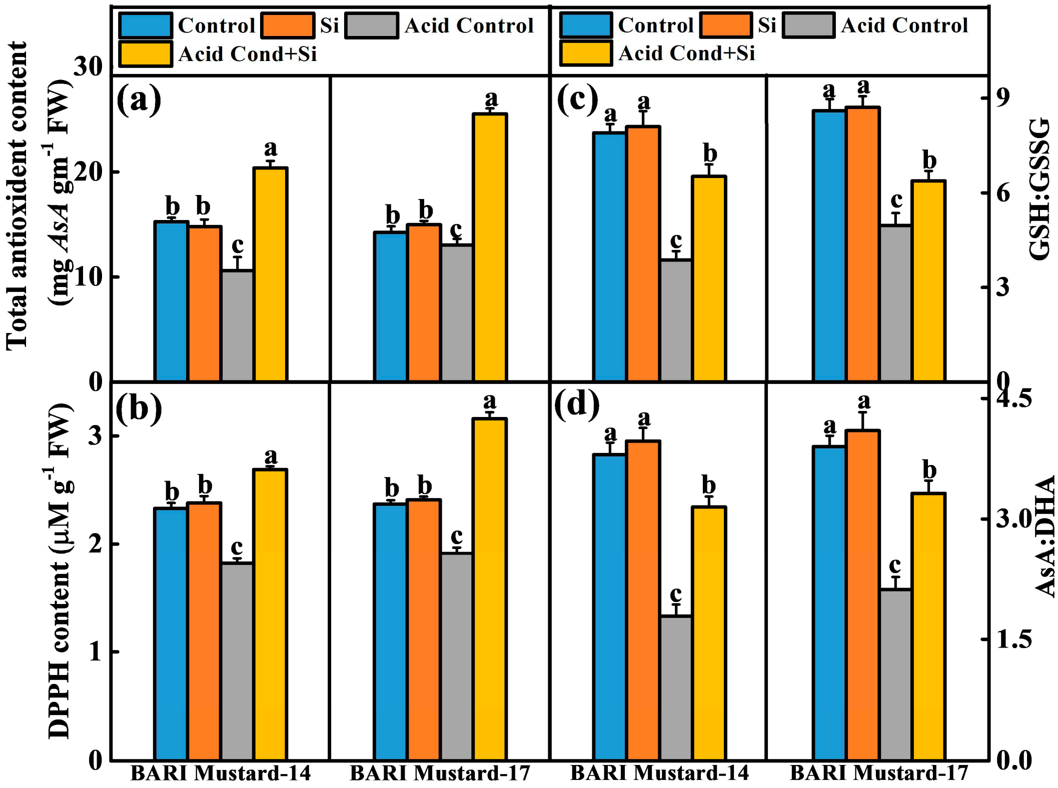

Figure 4. Si-NMs at selected dose (0.5 mmol L^{-1}) enhance antioxidant acitivity and cellular redox state in mustard seedlings under acid stress conditions; (**a**) total antoxidant activity, (**b**) DPPH content, (**c**) GSH/GSSG ratio and (**d**) AsA:DHA rato of two mustard varieties, BARI-14 and BARI-17, at 7 days of germination under acidic conditions. The data presented as the means of three replicates (±SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

Although the crop growth significantly improved upon Si-NM application under both the acidic and normal field conditions, under the acid-stressed condition, the total chlorophyll content, net photosynthesis and biomass accumulation growth contribution remained behind the optimized level of crops cultivated in normal field soil conditions. Hence, as a soil management strategy, we also applied the dolomite at a rate of one ton ha^{-1} and observed the potential of Si-NMs on the growth attributes of mustard crops. Interestingly, the results showed that only dolomite supplementation failed to diminish the acid-stress-induced delay in mustard crop growth and biomass accumulation (Figure 5). For example, the dolomite-only treatment showed 20.2% and 17.7% and 18.9% and 7.2% reduced net photosynthesis and biomass accumulation, which was statistically insignificant, with the Si-NM-only treatment in BARI mustard-14 and BARI mustard-17, respectively, compared to their respective field soil control (Figure 5b,d).These results indicate that neither dolomite nor Si-NMs are independently capable of optimizing the acid-stress-induced growth suppression of mustard crops. However, the application of Si-NMs upon dolomite supplementation as a soil amendment completely reversed the acid-stressed-induced suppression of mustard crop growth, as evident by the chlorophyll content, net photosynthesis and biomass accumulation in both tested varieties (Figure 5). For example, regarding the application of Si-NMs upon dolomite supplementation, the net photosynthesis and biomass accumulation increased about 3.9% and 10.5% and 15.8% and 39.7% in BARI mustard-14 and BARI mustard-17, respectively, compared to the crops grown in normal field conditions.

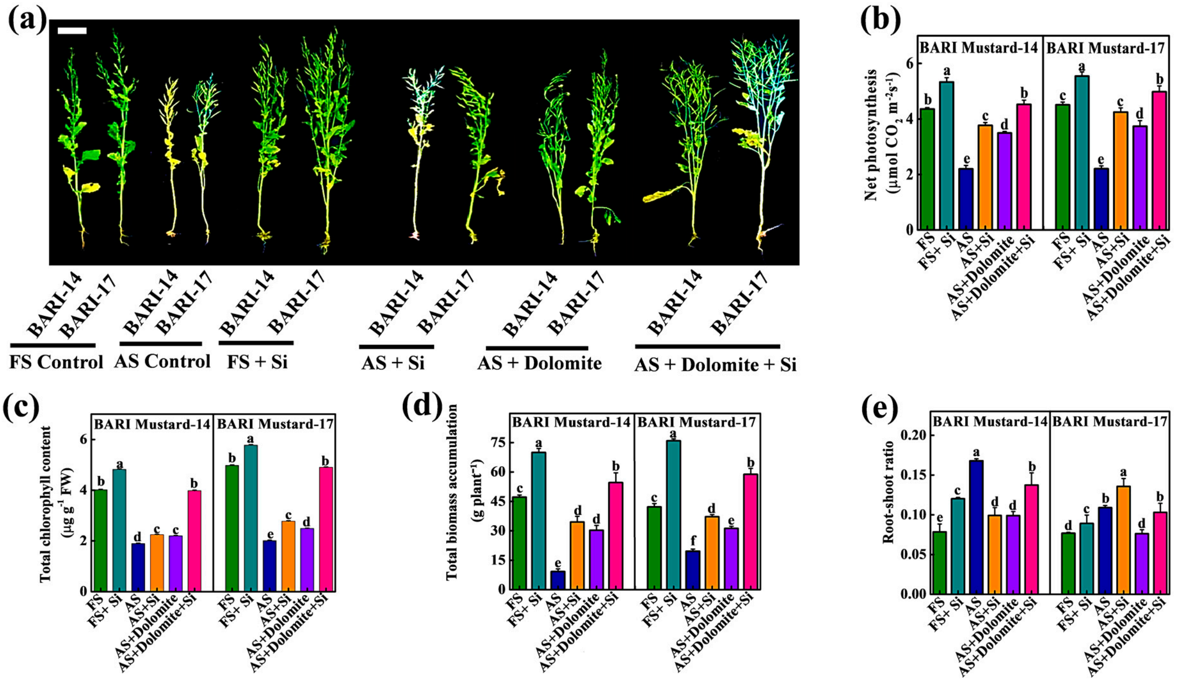

Figure 5. Effect of exogenous application of selected dose (0.5 mmol L^{-1}) of Si-NMs on photosynthetic activity and biomass accumulation under field soil (pH 6.2–6.3) and strongly acidic soil (pH 4.5–4.8) conditions; (**a**) photographic image of mustard plants, bar = 24 cm; (**b**) net photosynthetic rate, (**c**) total chlorophyll content, (**d**) biomass accumulation and (**e**) root–shoot ratio of two mustard varieties, BARI-14 and BARI-17, at 60 days of sowing. The data presented as the means of three replicates (±SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

2.4. Effects of Si-NMs on Yield Attributes of Mustard Crop under Acidic Field Soil Condition

Yield attributes are critical metrics responsible for crop productivity. To observe the efficiency of Si-NMs on the yield attributes of mustard, we recorded the total numbers of siliqua per plants, number of seeds per siliqua and hundred seeds weight under both acid-stressed and field soil conditions, with or without Si-NMs and/or dolomite. The results displayed that siliqua size largely varied among the treatments; specifically, the tiniest sized siliqua was observed in both tested varieties under acid soil conditions (Figure 6a). However, the siliqua sizes were significantly enlarged upon the independent application of both the Si-NMs and dolomite as a soil supplementation. Conversely, their combined application caused a further increase in the siliqua and even the leaf size under acid soil conditions. In accordance with the physiological display, the numerical values also showed that the total number of siliqua plants^{-1} significantly increased in both BARI mustard-14 and BARI mustard-17 under acid soil conditions upon Si-NM or dolomite application. The results showed that, compared to the field soil control, the total number of siliqua plants^{-1} reduced by 173.9% and 90.1% in BARI mustard-14 and BARI mustard-17 under acid soil conditions, showing only 23.4% reduced siliqua in BARI mustard-14 but 84.1% increased siliqua in BARI mustard-17 upon Si-NM application, indicating varietal differences of adaptive response. On the other hand, only dolomite application as a soil supplementation had a much lower effect and showed about 52.1% and 24.8% reduced siliqua settings plant^{-1} under acid soil compared to field soil control. However, their combined application showed about 71.5% and 161.4% increased numbers of siliqua in BARI mustard-14 and BARI mustard-17 under acid soil conditions, which were statistically insignificant or little tuned with Si-NM treatment in field soil (FS) conditions (Figure 6b). Similarly, the total number of seeds per siliqua and the hundred seeds weight were also reduced by 14.8% and 50.1% and 38.1% and 48.6%, respectively, in BARI mustard-14 and BARI mustard-17 under acid soil conditions compared to the plants grown in field soil with a normal pH. The application of exogenous Si-NMs significantly improved the traits but failed to optimize; however, it caused a further increase in the total number of seeds per siliqua and the hundred seeds weight in plants grown in field soil conditions. In contrast, the counting remained statistically similar or showed a slight change with the counting in plant soils treated with dolomites (Figure 6c,d). However, their combined application completely reversed the acid-stress-induced suppression of total seed counting per siliqua and the hundred seeds weight. These results indicated that the combined application of Si-NMs and dolomite, instead of their solo use, plays a potential role in promoting the yield attributes of mustard crops suppressed by acid soil conditions.

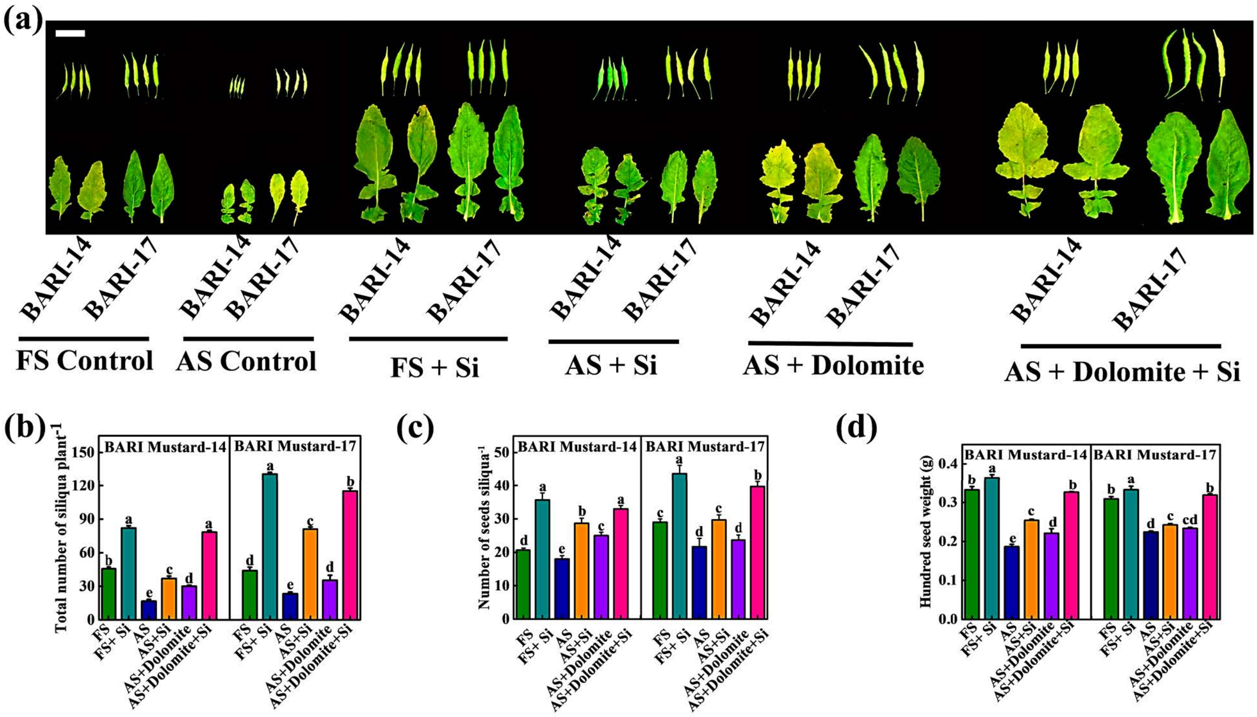

Figure 6. Exogenous Si-NMs at 0.5 mmol L^{-1} dose enhanced yield attribute characteristics of mustard crop under acidic field soil conditions. (**a**) Photograph of siliqua and leaves of mustard crops, bar = 2 cm, (**b**) total number of siliqua per plants, (**c**) numbers of seeds per siliqua and (**d**) hundred seeds weight after harvest of two mustard varieties, BARI-14 and BARI-17, cultivated at two different field soil conditions, having soil pH 4.5–4.8 and pH 6.2–6.3, respectively. The data presented as the means of three replicates (±SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

2.5. Si-NMs Enhanced Seed Yield and Oil Percentage of Mustard Reduced by Acidic Field Soil Condition

To understand the role of Si-NMs on the sustainable intensification of mustard crops under stressful conditions, we examined the mustard seed yield and analyzed the oil content for economic resilience. The results showed that the mustard seed yield and oil content in seeds were highly susceptible to low soil pH and showed 2.9-fold and 2.7-fold reduced seed yield and 25.7% and 22.2% reduced oil content in BARI mustard-14 and BARI mustard-17 under acid soil conditions compared to the plants grown in normal field soil conditions (Figure 7). However, the exogenous supplementation of Si-NMs significantly improved both the seed yield and oil content in both tested varieties. Upon Si-NM application, the seed yield increased by about 155.3% and 126.5% and the oil content increased by about 16.5% and 10.3% in BARI mustard-14 and BARI mustard-17, respectively, in acid soil conditions. Interestingly, these results were mostly statistically insignificant with the soils cultivated upon dolomite supplementation as a soil amendment and completely failed to optimize the acid-stress-induced suppression. However, their combined application caused a further increase in both the seed yield and oil content and remained statistically insignificant with the field soil controls (Figure 7). In contrast, the exogenous supplementation of Si-NMs to the crops cultivated at normal soil pH showed further augmentation in the seed yield and oil content in both tested varieties. Compared to the field soil control, the seed yield and the oil content were increased by about 24.1% and 12.8% and 6.2% and 8.1%, respectively, in BARI mustard-14 and BARI mustard-17 in Si-NM-treated field soil crops, indicating the economic resilience of Si-NMs, essential for sustainable agricultural developments.

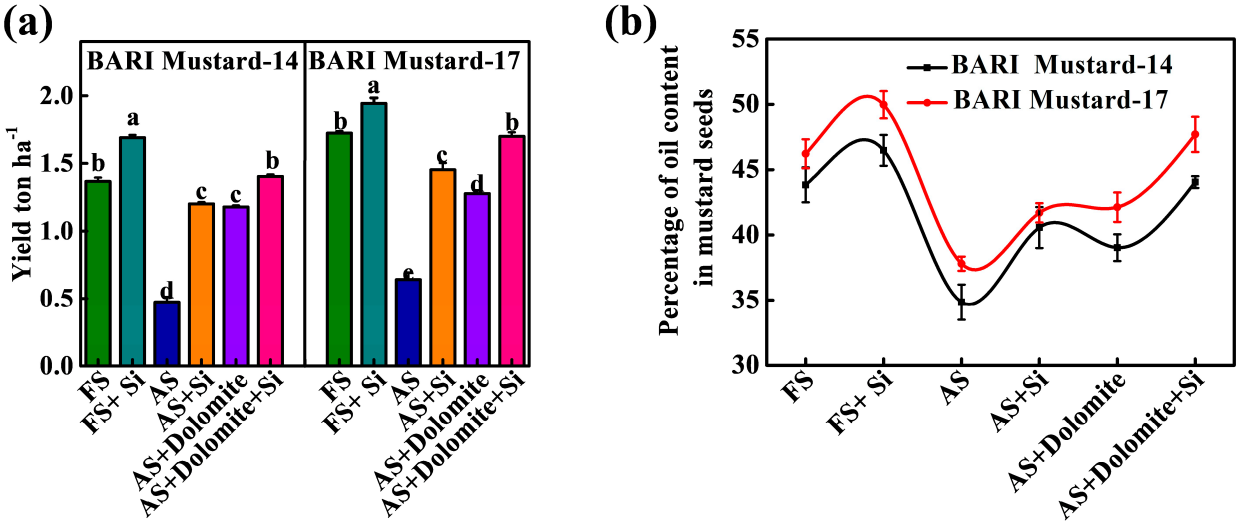

Figure 7. Si-NMs promote seed yield and oil content restricted by soil acidity; (**a**) total seed yield and (**b**) percent of oil content of seeds of two mustard varieties, BARI-14 and BARI-17, cultivated at two different field soil conditions, having soil pH 4.5–4.8 and pH 6.2–6.3, respectively. The data presented as the means of three replicates (±SE) and the means denoted by the same letter do not differ significantly at $p < 0.05$ according to Tukey's test.

3. Discussion

The growing concern for global food security has expanded to the further expansion and intensification of agriculture, particularly the region of soils affected by acidity [10]. However, soil acidity or low soil pH is thought to be one of the key stress factors restricting the total acreage and crop productivity, subsequently threatening global food security [14]. However, the most recent year's nano-fertilizers like silicon nanomaterial (Si-NMs) raised a healthier option for sustainable agricultural development and is observed to improve growth, physiological development and biochemical response in plants under diverse stress conditions, like drought, salinity or metal stress [15,16]. Regarding the benefits of Si-NMs as an ecologically sustainable and economically stable food production strategy, we designed the present experiments to understand its suitability for mustard crop production under acidic soil conditions, which remains elusive. To understand the influential effect of Si-NMs, we performed a sequential experiment, from seed germination to field trial, of mustard crops. The results showed that, among the deferential doses, 0.5 mmol L^{-1} of Si-NMs showed the finest stimulatory effects on seed germination and seedling growth that was firmly suppressed by the acidic medium (Figures 1 and 2). The promotion of growth associated with the management of the adverse effects of acid stress dominantly minimized the generated ROS, lipid peroxidation (MDA) and electrolyte leakage (EL) by stimulating the total antioxidant activity, DPPH and the ratios of GSH/GSSG and AsA/DHA (Figure 4). Furthermore, our experimental results also showed that Si-NMs are promising to manage the adverse effects of soil acidity and improve crop productivity at the field level, as evident by the improved chlorophyll content, net photosynthesis, biomass accumulation and the metrics of yield attributes upon Si-NM application in both tested varieties, BARI mustard-14 and BARI mustard-17 (Figures 5–7). However, the application of Si-NMs upon dolomite supplementation as a soil amendment completely reversed the acid-stress-induced suppression of mustard crop growth, seed yield and oil content, indicating the economic resilience of Si-NMs, essential for the sustainable intensification of agricultural growth [17,18].

Germination is a critical aspect of the lifecycle and plays a vital role in successive regeneration, ecological balance and food production [19]. To identify the threshold-type response of Si-NMs to low pH, initially, we observed the seed germination competency with different doses (0, 0.5. 1.0 and 1.5 mmol L^{-1}) of Si-NMs in BARI mustard-14 and BARI mustard-17 (Figure 1). The experimental results showed that Si-NMs mediated mustard seed germination and seedling growth extremely dose-dependently, and 0.5 mmol L^{-1} showed the finest results. However, higher doses (1.5 mmol L^{-1}) of Nano-Si displayed 6.2% and 11.2% reduced rates of germination and 15.9% and 14.5% decreased biomass accumulation in BARI mustard-14 and BARI mustard-17, respectively, compared to 0.5 mmol L^{-1} doses. The toxicity of nanoparticles in plants at higher doses is limited but thought to be regulated by a complex phenomenon of multiple intrinsic factors [20]. One common hypothesis by scientists suggests that at higher doses, Si-NMs might produce excess metal ions in plant tissues that promote the generation of reactive oxygen species (ROS) in cells, leading to cellular structure and macromolecular damage [21,22]. Moreover, Si-NMs at higher doses might interfere with the hormonal balances that restrict the overall germination process. For example, it has been reported that Ag-NPs alter the hormonal balance at higher doses in many species, including Arabidopsis, pepper, cucumber and wheat and shakes the germination and growth [23]. Therefore, we selected the finest dose (0.5 mmol L^{-1}) of Si-NMs for further experimentation under acidic or low pH 4.5 conditions, a major limiting factor restricting mustard seed germination, seedling growth and adaptation [24,25]. Interestingly, the experimental findings showed that Si-NMs dramatically upgraded the germination, seedling growth and biomass accumulation in both tested varieties that were firmly suppressed by the acidic medium (Figure 2); however, these positive stimulatory responses change from variety to variety [26]. Though the mechanism remains unclear, Si-NM-mediated enhanced mustard seed germination under stressed conditions may be associated with increased water absorption through enhanced aquaporin gene expression that stimulates the metabolic activity in seed results, thus improving germination [20]. As optimum water or moisture content in the seed is the key factor of metabolic degradation carbohydrates to sugar and the increased activity of amylase and protease allow for quicker cell division in the embryo, this consequently enhanced the normal germination that was halted at <0.1 g H_2O content in per gram seeds weight [20,27]. Previously, it has also been observed that seed priming with Si-NMs enhanced not only the germination but also improved the seedling growth and biomass accumulation by improving stress tolerance, similar to our current experimental findings [28].

Generally, the active mitochondria in germinating seeds are likely one of the principal sites of ROS generation that initially produce superoxide anions ($O_2{}^{\bullet-}$) and then hydrogen peroxide (H_2O_2). However, under normal growth conditions, the generated ROS were scavenged by the antioxidant enzymatic activity, such as SOD, POD, CAT, APX, AsA and GSH [29]. In accordance, our present experimental findings also displayed that under acid stress conditions, mustard seedlings showed higher $O_2{}^{\bullet-}$ and accumulation of H_2O_2, resulting in increased malondialdehyde (MDA) content and the percent of electrolyte leakage in both tested varieties (Figure 4). These might be due to the rate-limiting antioxidant potential of seedlings [4,30]. However, seed priming with Si-NMs showed an enhanced activity of total antioxidant activity, DPPH and the ratios of GSH/GSSG and AsA/DHA in both mustard varieties, BARI mustard-14 and BARI mustard-17, under acidic conditions, indicating the critical interplaying role of Si-NMs in improving redox homeostasis (Figures 3 and 4) [31]. Accordingly, a number of studies recently explored that, as an innovative technique, Si-NMs could be an ecologically sound alternative to mitigate the oxidative stress generated in plants under stressed conditions [32]. Si-NMs improve the DPPH and total antioxidant activity up to 65.4% and 96.0% in mustard seedlings under acid stress conditions compared to their respective control, in addition to upholding the ratios of GSH/GSSG and AsA/DHA (Figure 4). Moreover, very recently, Si-NMs were found to control the LOX and redox-related gene expression that decreased ROS-induced cellular damage under stressful conditions, consequently enhancing the plant adaptation potential to adverse conditions like soil acidity [29].

As a critical factor, low soil pH not only regulated the seed germination but also negatively impacted the plant adaptation and crop productivity results, affecting the livelihoods of farmers across the world [1]. Considering soil acidity as the most divesting factor, we also extended our experiments at the field level, specifically in soil consisting of low pH (4.5–4.8), and evaluated the opportunities of Si-NMs for managing the adverse effects of soil acidity. Increased H^+ ions in soil are a critical indicator of the evaluation of environmental pollution [6]. Our field experimental results showed that soil acidity critically regulated the normal growth of both tested varieties, as evident by the reduced chlorophyll content, net photosynthesis and root shoot ratio and biomass accumulation (Figure 5). Many recent studies unveiled that soil acidity affects every aspect of plant growth and productivity by inhibiting nutrient availability, root development and soil microbial activity [13,33,34]. Interestingly, our results showed that the exogenous application of Si-NMs improved net photosynthesis by about 157.2% and 200.4% and the total biomass accumulation by about 3.7-fold and 1.9-fold in BARI mustard-14 and BARI mustard-17, respectively, compared to the non-treated plants, flattening their use for sustainable agricultural expansion to the acid stress region [35].

Again, the yield attributes, such as the total numbers of siliqua per plants, number of seeds per siliqua and hundred seeds weight of mustard, are thought to be the critical metrics responsible for crop productivity [36]. Surprisingly, the results displayed that all the yield attribute metrics significantly improved upon feeding with Si-NMs in both tested varieties, thereby increasing the total seed yield that was strongly suppressed by soil acidity (Figures 6 and 7). For example, upon Si-NM application, the seed yield increased by about 155.3% and 126.5% in BARI mustard-14 and BARI mustard-17, respectively, in acid soil conditions (Figure 7a). In accordance, a number of studies explored that Si-NMs enhanced crop growth and productivity in numerous crop species, such as cucumber, pea, wheat, oat, maize, grape, bean, rice, etc., under diverse types of stress, like salinity, water deficiency or heavy metal stress [16,37]. Generally, Si is primarily taken up by plants as monosilicic acid (H_4SiO_4) through specific transporters in roots and then various tissues, where they are deposited as amorphous silica ($SiO_2 \bullet nH_2O$), regulating the plant growth and adaptation to adverse conditions like soil acidity [12]. This means that the stimulatory potential of Si-NMs might be associated with enhanced cellular protection by promoting antioxidant activity and redox homeostasis. Moreover, Si-NMs have been found to improve lateral root or even main root growth, which eventually increases the nutrient uptake potential of plants, leading to crop yields [38]. Despite the mustard seed yield Si-NMs being found to increase the oil content in seeds (Figure 7a), it has been observed that under low soil pH conditions, the oil content in BARI mustard-14 and BARI mustard-17 increased by about 16.5% and 10.3%, respectively, compared to non-treated plants, signifying the economic resilience of farmers [1].

However, Si-NM application to optimize the crop growth and yield performed poorly in extremely low soil pH conditions [39]. Similarly, we also found that Si-NMs failed to completely compensate the yield and yield attributes of mustard crops when compared with crops grown in field soil (slightly acidic pH 6.2–6.3) conditions (Figures 5–7). In contrast, in field soil or slightly acidic conditions, Si-NM application caused further augmentation of all the metrics associated with farming resilience. Hence, as a soil management strategy, we also applied the dolomite at a rate of one ton ha^{-1} as a global acid soil correction practice, which consequently reduced the crop sensitivity to low soil pH [33]. For example, Si-NM application upon dolomite supplementation increased the seed yield and the oil content by about 24.1% and 12.8% and 6.2% and 8.1%, respectively, in BARI mustard-14 and BARI mustard-17 compared to the crops grown in field soil conditions, indicating the wider feasibility of Si-NM application across diverse soil pH ranges [39].

4. Materials and Methods

4.1. Plant Materials and Seed Pretreatment with Silicon Nanomaterials (Si-NMs)

Seeds of two mustard (*Brassica campestris*) varieties, BRAI Mustard-14 and BRAI Mustard-17, were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Dhaka, Bangladesh. After assortment, healthy seeds were sterilized with 10% sodium hypochloride solution for five minutes and then repeatedly rinsed with distilled water. Once washed, the seeds were soaked with graded level of silicon nanomaterials (Si-NMs) at rate of 0, 0.25, 0.5, 1.0 and 1.5 mmol L^{-1} for about twelve hours to observe the competency. The Si-NMs with average primary particle sizes 30 nm and 99.9% pure at metal basis were purchased from the Aladdin Industries Corporation, Shanghai, China. The measured amount of Si-NMs with distilled water was sonicated for thirty minutes to prepare the homogenous mixture using sonic's vibra-cell (Model-VCX 505, Gütersloh, Germany), as previously discussed [28].

After seed pretreatment with differential doses of Si-NMs, three hundred seeds of each treatment were transferred into three Petri dishes (60 mm × 15 mm), comprising a double layer of filter paper. The filter papers were soaked with distilled water to ensure seed moisture during germination. In case of acid treatment, the pretreated seeds with or without Si-NMs placed into the Petri plate containing acidified water (pH-4.5) soaked the double-layered filter paper. The acidified water prepared by adding the HNO_3 drop by drop to the tap water and adjusted at 4.5 pH level using a benchtop pH meter (Model-pH 2601, Ningbo, China), as previously described [40]. All the in vitro experiments were performed in a growth chamber (model-ICB-L250B, Bioevopeak, Jinan, China) at laboratorial conditions. The number of seeds germinated were counted every 12 h and continued to 96 h. The seeds were considered germinated when their radicle emerged from seed coat. Finally, the potential of Si-NM-mediated seed germination under acid treatment or control condition was assessed in terms of percentage of seed germination. Root–shoot growth and biomass accumulation of seedlings of two mustard cultivars, BARI mustard-14 and BARI mustard-17, were calculated at 7 days of seed germination.

4.2. Description of Field Experiments

The field experiment was conducted in two different agro-ecological zones (AEZ), AEZ-20 (Eastern Surma Kushiara Flood-plan) and AEZ-22 (Northern and Eastern Pedmont-plain), of Bangladesh due to the variation in soil acidity. The soils of AEZ-20 located at (24°54′30.4″ N 91°47′17.5″ E) are slightly acidic (pH ranges from 6.2–6.3) and considered as field soil. In contrast, the soils of AEZ-22 located at (24°54′32.4″ N 91°56′33.5″ E) are strongly acidic (pH ranges from pH 4.5–4.8) and considered as acid soil (Supplementary Table S1). The soil acidity status was measured using a glass electrode (Model: HI-2211, Washington Hwy, Smithfield, VA, USA) and a soil to water ratio of 1:2.5 after collecting the three subsamples from each experimental site at a depth of 15 cm, as previously described [41]. The climate of the selected AEZ is Cwa type, humid subtropical with hot summer and cool winter according to Köppen-Geiger climate classification [42]. In strongly acidic soil, we also applied dolomite limestone at a rate of 1 ton ha^{-1} as a world soil correction practice, in addition to Si-NMs sprayed 15 L ha^{-1} at a rate of 0.5 mmol L^{-1} [33]. We spared Si-NMs three times, at seedling stage, vegetative stage and reproductive stage. The field experimental design was a complete randomized block design with six treatments and three replicates. The area of each treatment plot was 10 m square (2.5 m × 4.0 m) in size.

4.3. Measurements of Plant Growth and Yield Parameters

The plant biomass and the root–shoot ratio were measured after harvesting of ten plants of two mustard varieties, BARI mustard-14 and BARI mustard-17, from each experimental site at 65 days of sowing. Just after harvesting, the roots were separated from the shoots, washed and air-died at laboratory conditions and weighed in grams using a digital balance (Model-pH 2601, Ningbo, China). The number of pods for each plant, number of seeds per pod and hundred seed weight were estimated after the harvested period of mustard varieties. The counted pods of ten plants of each treatment were averaged and expressed as numbers of pods plant^{-1}. Similarly, ten pods per plant and ten replications of each treatment were shelled to extract the seeds and counted after harvest at 90 days of sowing to average the number of seeds per pod. Finally, the hundred seeds of each treatment with three replicates were weighed using an analytical balance and expressed as grams.

4.4. Determination of Chlorophyll Content and Net Photosynthetic Rate

To determine the photosynthetic pigment contents, 100 mg of homogenous leaf sample was grounded by using a mortar and pestle with 10 mL of 80% of acetone. Afterward, the homogenate was filtered and we measured the Chl*a* and Chl*b* spectrophotometrically (Model:T80+, PG Instruments Ltd., Wibtft Leicestershir, UK) at a wavelength of 645 nm and 663 nm for the total chlorophyll determination [43]. The net photosynthetic rates (*P*n) were determined on the third fully expanded leaves from the top of plants using an infrared gas analyzer (IRGA) portable photosynthesis system (Model: Li-CHOR 6400, Lincoln, NE, USA) and maintained the conditions, as previously described [4].

4.5. Histochemical Staining of H_2O_2 and $O_2^{\bullet-}$ and Measurements of H_2O_2, Lipid Peroxidation and Electrolyte Leakage in Mustard Seedlings

The H_2O_2 and $O_2^{\bullet-}$ accumulation in mustard seedlings was visualized by staining with 3, 3-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) staining, respectively. The H_2O_2 seedlings of both mustard varieties were submerged in 1 mg mL^{-1} solution (pH-3.8) after 6 hrs light incubation at 25 °C. In contrast, $O_2^{\bullet-}$ visualized in seedling by incubating in 0.5 mg mL^{-1} (pH-7.8) solution of NBT after dark adaptation [4]. Quantitative measurements of H_2O_2 0.3 gm of mustard seedlings were homogenized in 3 mL of ice-cooled 1.0 M $HClO_4$ solution and centrifuged at 6000× *g* for 5 min in refrigerated conditions (Model: HERMLE Z 326 K, Wehingen, Germany). Afterward, the pH of supernatant was adjusted at 6.5 with KOH and absorbed with 0.05 gm charcoal. Then, the samples were vortexed and centrifuged at 12,000× *g* for 5 min and passed through an AG1.8 pre-packed column. The collected samples were mixed with reaction buffer containing 1 mmol 2,2-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) and 100 mmol potassium acetate (pH-4.4) and 0.25 units of horseradish peroxide at a ration of 1:1 and measured at OD_{412}, as previously discussed [4].

To determine the lipid peroxidation, 0.5 g samples were homogenized with 5.0 mL 10% trichloroacetic acid (TCA) and we quantified the MDA equivalents using 2-thiobarbituric acid (TBA), as previously described [44]. After centrifugation at 3000× *g* for 10 min, 4 mL of 20% TCA with 0.65% TBA was added to the 1 mL of the collected supernatant, incubated at 95 °C for 25 min, and we stopped the reaction by placing in an ice bath. We then centrifuged and measured absorbance at 440, 532 and 600 nm. To determine the electrolyte leakage (EL), fresh mustard seedlings were cut into small pieces and placed in a tube with 10 mL of distilled water, with one set shacked at room temperature for 2 h and another set heated at 120 °C for 20 min. We then measured the conductivity EC_1 and EC_2 using an EC meter (Model: DDS307, Changsha, China) and calculated as percentage [45].

4.6. Antioxidant Assay

The antioxidant activity of extracted samples was measured by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenge assay [46]. In brief, 3 mL of 6×10^{-5} methanolic acid solution of DPPH was added to the 2 mL of sample extract and left for 30 min in dark at room temperature, and we measured the OD_{517} as sample A. In contrast, the solution without the extract measured the OD as blank and the ability of scavenge DPPH radicals expressed as percentage of total antioxidant activity by following an equation of antioxidant activity (%) = $OD_{blank} - OD_{sample}/OD_{blank} \times 100$. The total glutathione (GSH) and oxidized glutathione (GSSG) were measured by using 5,5′-dithio-bis (2-nitrobenzoicacid)-GSSG reductase recycling method and we calculated GSH by deducting GSSG from the total glutathione. The ascorbate (AsA) and dehydroascorbate (DHA) content were also measured spectrophotometrically by following previously described method based on the reduction of Fe^{3+} to Fe^{2+} by AsA in acidic solution [31].

4.7. Measurement of Oil Percentage of Mustard Seeds

For the determination of oil percentage, two varieties of mustard seeds were initially cleaned by passing the sieve to remove the sand and stones and air/oven-dried at 60 °C for 10 min. Afterward, seeds were grounded to powder, and the oils of seed flours were extracted with n-hexene in a Soxhlet apparatus for 6 h following the operating conditions specified in IUPAC [47]. The oil was recovered by evaporating the solvent n-hexene by using a rotary evaporator and the extracted oil percentage assay following the equation $w_1 - w_2/w_1 \times 100$, where w_1 is the initial sample weight placed in the thimble and w_2 is sample weight after evaporation.

4.8. Statistical Analysis

Statistical analyses were performed using SPSS (version 16.0 Inc., Chicago, IL, USA). The normality and homogeneity of data of all variables were checked by using Shaprio–Wilk test. The field experimental design was a completely randomized block design with three replications. The treatment means calculated by averaging the values of three replications and Tukey's test ($p < 0.05$) were performed to evaluate the treatment significance. All the experiments were replicated twice to validate the results. Different letters were used to denote the significant differences among the treatment means.

5. Conclusions

Acidic soils dominantly functioned as a key stress factor, often influencing the soil physical chemical and biological processes, leading to the generation of ROS in plants and, thereby, reducing the crop growth and productivity. However, the utilization of engineered nanomaterials like silicon nanomaterials (Si-NMs) has been hailed as a blessing of modern agricultural intensification by overcoming the ecological barrier. Here, we found that the seed pretreatment with Si-NMs (at 0.5 mmol L^{-1} doses) plays a critical role in redox homeostasis under acidic conditions by stimulating the antioxidant activity and upholding the ratio of GSH/GSSG and AsA/DHA, thus increasing the mustard seed germination, seedling growth and biomass accumulation. Moreover, the exogenous application of selected doses of Si-NMs at the field level showed improved plant growth and biomass accumulation in low soil pH conditions that might be associated with increased chlorophyll content and net photosynthesis. The results also showed that exogenous Si-NMs improves all the critical attributes of crop productivity and economic resilience, such as the total numbers of siliqua per plants, number of seeds per siliqua and hundred seeds weight, seed yield and oil content percentage under stressed conditions in both tested varieties, BARI mustard-14 and BARI mustard-17. These caused further augmentation in plants grown in normal or slightly acidic field conditions. The results also revealed that Si-NMs failed to completely compensate for the yield and yield attributes of mustard crops at extremely low soil pH (pH 4.5–4.8) conditions than the crops grown in field soil (slightly acidic pH 6.2–6.3) conditions. However, dolomite supplementation as a global acid soil correction practice upon Si-NM application not only reduced the crop sensitivity to extremely low soil pH but also improved all the metrics of mustard yields associated with farming resilience (Figure 8). These studies highlighted the potential of Si-NMs to sustainably expand the agricultural intensification of crops in areas affected by low soil pH, essential for global food security and agricultural resilience [37]. However, further study is essential to unveil the cardinal molecular basis salted by Si-NMs before reaching the farm-gate.

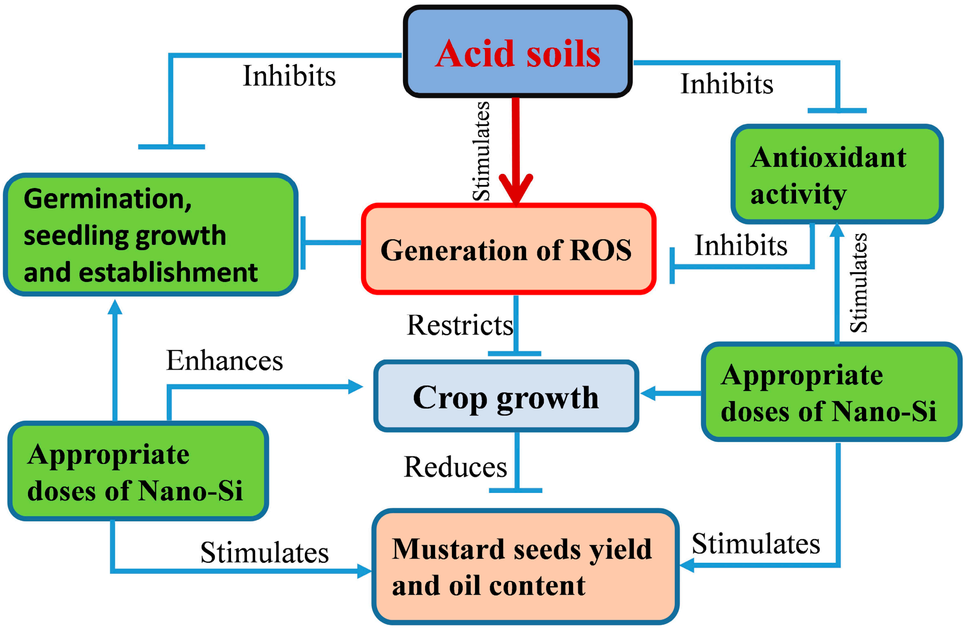

Figure 8. A proposed model depicting the involvement of Si-NMs in reduction in crop sensitivity to extremely low soil pH. Exogenous Si-NM application at 0.5 mmol L^{-1} doses enhanced ROS homeostasis by promoting enzymatic activity and thereby improves all the metrics of mustard yields associated with farming resilience.

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An update on post-harvest losses of onion and employed strategies for remedy

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ABSTRACT

Onions (*Allium cepa* L.) are widely cultivated staple vegetables in daily cooking while, harvested onions as living entities that undergo various physicochemical changes including respiration, weight loss, rotting, and sprouting at storage. Due to high moisture content fresh onions are prone to rapid spoilage which is aggravated by inappropriate handling. Storage is a complicated issue regulated by numerous pre- and post-harvest features encompasses irrigation, fertilization, bulb maturity, harvesting period, curing techniques, storage environment, packaging materials, and chemical treatments; causing about 30–40 % losses of total yield. Again, the attritional changes at physiological, fructant and hormonal levels of stored onions enhance sprouting and microbial spoilage. Among the onion diseases, only *Fusarium* causes 50 % of rotting at storage, which is augmented by elevated temperatures and humidity. Such an alteration of onions during storage has a robust effect on both their quality and marketability. Although the storage life of onions is an inherent trait, it could be improved through efficient pre and post-harvest practices such as application of growth regulators, ensuring balanced nutrition, nanofertilizers application, and chemical treatments in addition to curing. Curing largely helps to prevent spoilage at storage and increases the availability of onions around the year. Moreover, issues such as sprouting, drying, and microbial spoilage could be minimized by employing advanced techniques like perfect handling, modified ventilation and controlled storage atmospheres with elevated CO_2. Here, we present an overview of the strategies used to minimize post-harvest losses and discuss recent advances in preserving quality and extending the shelf-life of onions at storage.

1. Introduction

Onion (*Allium cepa* L.) is a vital cooking ingredient of almost every culture as a staple vegetable, which belongs to the *Allium* family. Throughout the world, onions are utilized as raw ingredients in salads and/or in the creation of many cuisines for their flavor, aroma, and taste. In addition to savory flavor onions add very low calories (only 40 kilocalories per 100 g) to the recipes. Onions contain mostly water (89 %) with carbohydrates (9 %), fiber (1.7 %), protein (1.1 %), sugar (4.2 %) and fat (0.1 %) (Bahram-Parvar and Lim, 2018). Onions have a unique combination of fructans, flavonoids and organosulfur compounds, which exhibit strong salutary effects on human health. Moreover, fructans as a source of short-chain fatty acids like butyrate potentially contribute to colon health and reduce the risk of colon cancer (Loredana et al., 2019). The high concentrations of flavonoids and quercetin suggestively improve lipid profiles and antioxidant levels, thereby greatly reducing the risk of capillary fragility and cardiovascular arrest (Liguori et al., 2017). Therefore, throughout the world onions are cultivated as an important vegetable crop for generating currency addition of recognized therapeutic uses (S Bisen and Emerald, 2016).

Global onion production reached around 106 million metric tons, which made the onion the second-highest vegetable crop after tomatoes,

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contributing 9 % of total vegetable production worldwide as mentioned in Food and Agriculture Organization Statistics (FAOSTAT. Onion Production, 2021). Nowadays, India is the world's top producer of onions, followed by China, Egypt, the United States and Turkey. For small, medium, and large-scale production many other developing countries also contribute a lot to meet the local demand in addition to generating foreign currency. Despite tremendous progress in onion production across the world, many countries face a scarcity to meet the demand (Etana et al., 2019). The main reason for this lower productivity and adoption of onion farming is most probably due to a lack of improved cultivars, inappropriate agronomic practices, adoption susceptibility to diverse soil ecology, domestic farmer's inefficiencies in utilizing the accessible agricultural resources and/or lack of attention given to the storage system (Premi and Premi, 2017). Generally, winter seasons considered as perfect time for onion cultivation and harvested onion-bulb stored for year-round use as shown in Fig. 1.

Understanding market demand and aligning production accordingly could maximize farmer profitability. However, factors like soil, water, and farming practices affect the quality and marketable yield of harvested and/or stored onion (Premi and Premi, 2017; Salari et al., 2020). Although onions have good nutritional properties, a high-quality check of their nutritional stability largely depends on the appropriate storage facilities like perfect ventilation. As a semi-perishable crop, onions are prone to deterioration during the storage period, resulting in a significant monetary loss ascending year after year. The storage loss of onions is primarily attributed to physiological weight loss, rotting, sprouting and rooting (Petropoulos et al., 2017). Spoilage during storage is influenced by various factors, including cultivars, maturity of the bulb, moisture content, and storage environment. Post-harvest diseases, predominantly induced by bacterial and fungal pathogens, pose a significant threat to onions. Once they are harvested, acting as potential carriers for disease transmission and ultimately lead to a decline in their shelf life at storage. Microbial contamination and mechanical distortion during transportation additionally result in 20–30 % post-harvest loss (Kumar et al., 2015). Although bactericides and fungicides are commonly utilized to prevent onion post-harvest disease, there is a critical requirement for more environmentally friendly techniques to prevent transition by altering prevailing practices (Zhao et al., 2021a). Though substantial progress has been made in identifying superior cultivars and adopting new yield-increasing strategies, improved post-harvest management practices remain sparse. In this review, we aim to provide a better insight into different ways of post-harvest loss of onion and discuss the advances of pre and post-harvest technologies to improve the storage quality and marketability of onions.

2. Global scenario of postharvest losses of onion

Postharvest loss encompasses food loss along the entire food supply chain, from the point of crop harvesting to consumption (Kumar and Kalita, 2017). The losses fall into several categories, which include weight loss from spoiling, loss of quality, nutritive loss, sustainability of seed loss, and economic loss. It has been estimated that 30–40 % of the production never reaches consumer levels (Tripathi and Lawande, 2019). In fact, the quality of harvested onion mainly deteriorated at storage through physiological weight loss, rotting and sprouting (Gorrepati et al., 2018). In general, only physiological weight loss contributed 20–30 % losses in addition to sprouting and decay losses that caused another 10–12 % and 4.5 % respectfully. The degree of post-harvest losses in the food distribution system varies substantially across crops, regions, and economies. For example, among the total losses, 29.78 % of post-harvest losses occur at the farmer, wholesale, retail and consumer levels, of which the highest proportion (35.5 %) of losses were observed only at the farmer's level (Yeshiwas et al., 2023). Another 35–40 % losses occur during the movement at the postharvest stage as shown in Fig. 2 (Shankar et al., 2023). Recently, witnessed that the main contributor to postharvest losses of onion is diseases like rot, which aggravated poor storage facilities and transport (Falola et al., 2023). Lack of knowledge, inappropriate technology, and/or inadequate

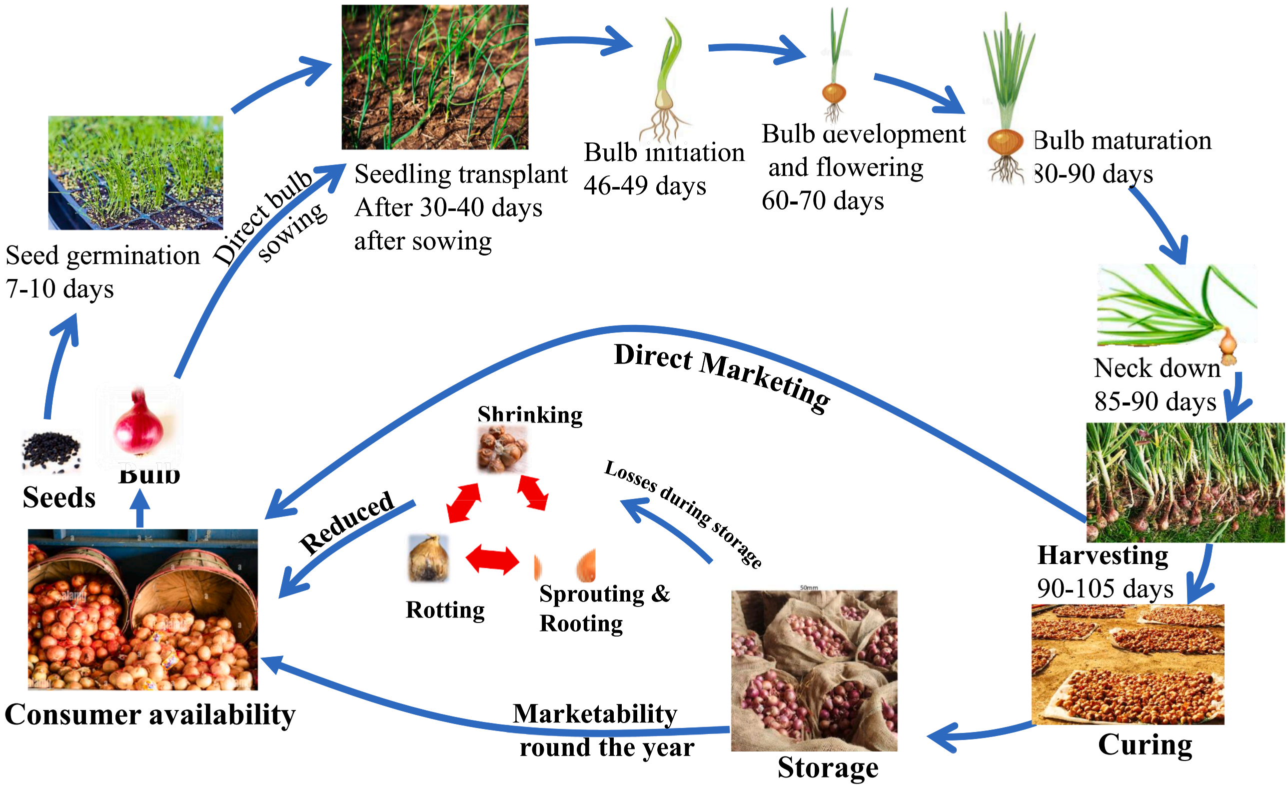


Fig. 1. An overview of the onion crop life cycle from field fabrication to kitchen.

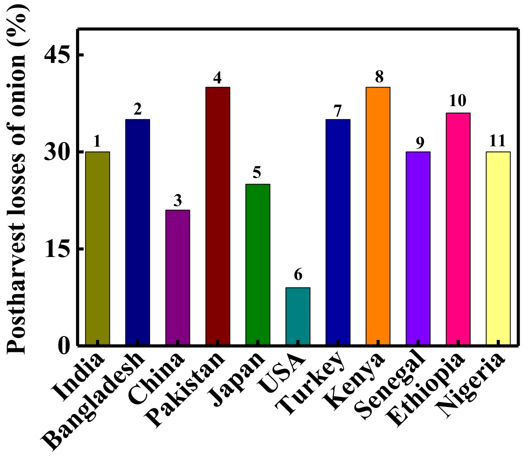


Fig. 2. Postharvest losses of onion in different areas of the world. 1. (Gorrepati et al., 2018), 2. (Biswas et al., 2010), 3. (Zheng et al., 2000), 4. (Soomro et al., 2016), 5. (Tsuchiya et al., 2021), 6. (Currah et al., 2012), 7. (Kaynas, 2016), 8. (Gathambiri et al., 2021), 9. (Beye and Komarek, 2020), 10. (Emana et al., 2017), 11. (Abubakar et al., 2019).

storage facilities specifically in underdeveloped (such as Kenya, Ethiopia, and Nigeria) and developing countries (like India, and Turkey) causes a significant quantity of deterioration at the last stage of the supply chain. In contrast, such a loss in developed countries like America, Japan and China are generally low might be due to better technological advancement and infrastructures (Petropoulos et al., 2017). However, the extend of losses varied year to year based on weather conditions and other variables including immature or over-mature harvesting, physical damage, unfavorable storage conditions, inadequate curing, fungal and bacterial contamination and others. In tropical countries, such losses may be higher as their ambient temperature is comparatively high (Tripathi and Lawande, 2019). Surprisingly, it is important to note that the precise information on post-harvest losses of onions may not comprehensive that make challenges to take initiatives in many regions.

Postharvest losses could be interpreted from two perspectives: qualitative and quantitative. The quantitative approach assesses postharvest loss as a decrease in the physical count of food products over time and geographical area while, qualitative losses involve those that influence the product's nutrient or caloric composition, acceptability, and edibility (Falola et al., 2023). Such a harvested loss of onions not only has notable financial forfeiture but also has food security consequences, as onion is a major vegetable and/or spicy crop that plays a crucial role throughout the world in culinary preparations and food preservation.

3. Ways of postharvest losses of onion

Existence evidence unveiled that several factors contributed to the losses of onions from harvesting to storage and transport. Among the multiple reasons, physiological weight loss, rotting loss and sprouting loss because of poor storage facilities are thought to be the principle contributors to postharvest losses of onion as shown in Fig. 2 (Falola et al., 2023). Such losses of onion at the postharvest stage affect not only the economic return of the farmers but also augment the nutritional insecurity (Falola et al., 2023; Gathambiri et al., 2021). Therefore, here we have discussed the specific ways and reasons for postharvest losses of onion in order to facilitate the development of efficient management technologies for future food or nutritional security.

3.1. Physiological weight loss

Physiological weight loss refers to the natural and unavoidable loss of water and other substances that occurs during the normal postharvest stage of onions. Although there are several biological processes of physiological weight loss the key contributing factors include water loss, respiration, transpiration and drying of outer layers of bulbs (Kakade et al., 2023; Sohany et al., 2016). Generally, onions are cultivated during the cool-dry season and stored for longer periods, but the absence of cold-storage facilities forced farmers to rely on traditional shelter-based storage methods that contributed the highest 22 % of physiological weight losses of onions at the postharvest stage (Sohany et al., 2016). Onions comprise a significant amount of water that evaporates over time from the cells through respiration and transpiration, resulting in physiological weight loss. A number of variables, including storage temperature, relative humidity, air circulation, and bulb moisture content, affect the physiological weight loss of onions during preservation. However, the maximum physiological weight loss takes place during the dry months, when mean temperatures are above 27 °C and with low (70–75 %) humidity levels (Islam et al., 2019a; Sohany et al., 2016).

Again, although the drying process acts as a barrier to pathogen attack and moisture loss during prolonged storage it evacuated the mass loss of onion and reduced profitability (Kakade et al., 2023). Generally, onions lose maximum weight by respiration or transpiration through the outer surface at 30–40 days of storage. It has been observed that in tropical environments, the weight loss may range from 5 to 6 % every month of storage and which contributes a total of 20–30 % of losses as shown in Fig. 3 (Tripathi and Lawande, 2019). A number of experiments have explored that physical damage has a significant impact on metabolic activity like respiration and transpiration that drives-up to 10 % of the total physiological weight loss of onion bulbs (Islam et al., 2019a). Therefore, minimizing the mass losses of onion bulbs from fresh harvesting to consume is crucial for reducing the uncertainty of marketability and economic loss.

3.2. Rotting loss

Rotting is a primary factor of postharvest losses of onion during prolonged storage and contributes to substantial revenue losses in the onion industry. Rotting basically occurs due to the proliferation of microbes, primarily fungi and bacteria, that break down the onion's tissues and cause approximately 35–40 % of the post-harvest loss and lead up to 15 % loses of total production (Ahmed et al., 2022; Tripathi and Lawande, 2019). However, the severity of loss significantly increased throughout the pre-harvest and post-harvest periods due to the existence of excessive humidity as shown in Fig. 4. Losses by numerous diseases like fungal, bacterial and viral diseases augmented at high moisture levels (Islam et al., 2019b). The most severe diseases of onion at the postharvest stage are rot, mold, sour and slippery skin in addition to viral soft rooting as shown in Table 1. There are presently 26 different pathogens identified that cause storage bulb rot, of which 14 are fungi and 12 are bacteria (Vahling-Armstrong et al., 2016). However, fungi are the most predominant pathogen causing significant economic loss of bulbs at storage (Ewekeye et al., 2021). Among the fungi, the occurrence of fusarium infection has risen as an emerging concern due to their predominant rotting of onion bulbs at the postharvest stage and even surpassing the botrytis. It has been also observed that among the fusarium species *F. proliferatum* is more aggressive on onions rotting causes >50 % of disease in onions at storage (Haapalainen et al., 2016). Moreover, onion at storage is affected by various Aspergillus pathogens-mediated mold on the surface of bulbs with clear signs of spoilage. Additionally, many Aspergillus species like *Aspergillus niger* able to produce mycotoxins, leading to a decline in the quality and quantity of food products, and posing a notable hepatocarcinogenic threat to both humans and animals (Haapalainen et al., 2016; Saranya et al., 2017). Interestingly, most of these causal pathogens are prevalent in soils and sustain themselves on organic matter and onion debris, consequently posing a risk to multiple host plants including onions and fatal, particularly after the harvesting (Borkar et al., 2020).

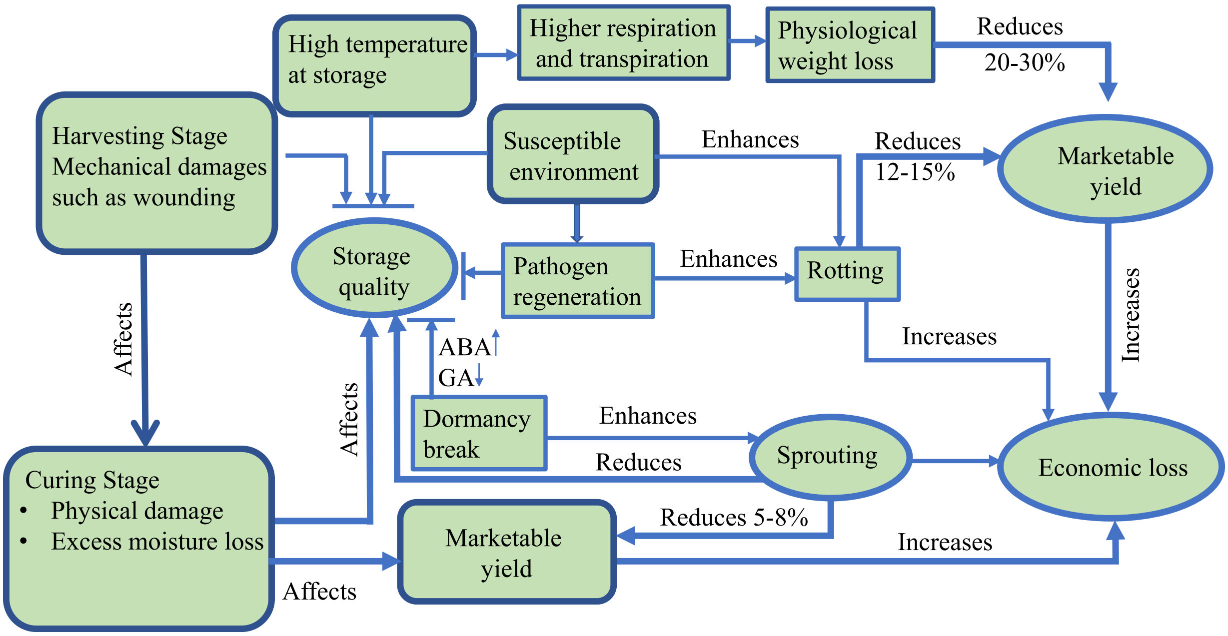


Fig. 3. Represents multiple ways of post-harvest quality losses of onion and their effects on ultimate economic losses. ABA indicates abscisic acid, GA indicates gibberellin. The thick lines and arrows indicate the major contributing factors and the "T" shape indicates inhibitory factors affecting post-harvest quality and marketable yield of onion bulbs.

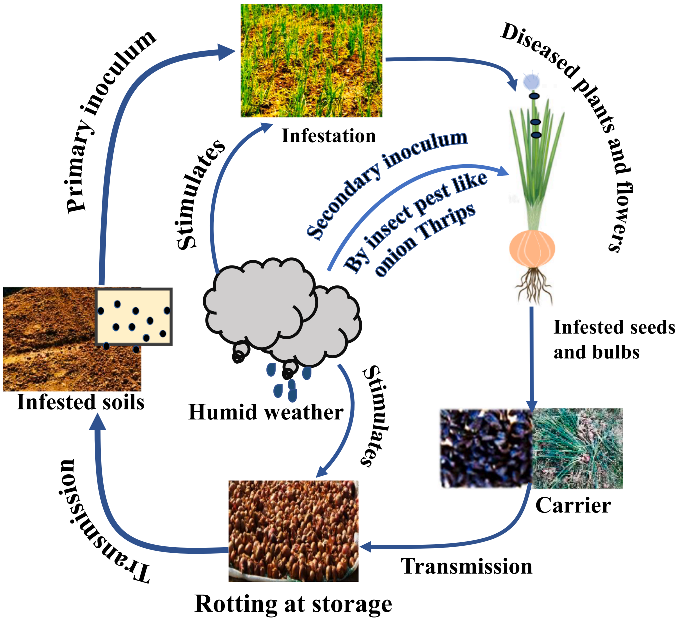


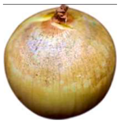
Fig. 4. A simple schematic diagram of the disease cycle and the rotting loss of onions caused by *Fusarium proliferatum* modified and redrawn after Gálvez and Palmero (2022).

3.3. Sprouting loss

The sprouting of onions during storage is an undesirable frequent issue triggered by multiple factors including storage environment (temperature, humidity, light and storage container), variety and improper curing (Petropoulos et al., 2017; Romo-Pérez et al., 2020). The suspension of dormancy in postharvest onion not only negatively impacts the bulb quality but also reduces the prolonged shelf life and arrests the economic returns (Forney et al., 2022). As living organs onion bulbs remain active and undergo various physiological processes while in storage. For example, Romo-Pérez *et al.* reported that among the 189 studied metabolites, 128 undergoes notable changes during storage (Romo-Pérez et al., 2020). In fact, the metabolic activity in stored onion largely depends on the source (scale) to sink (base plate) transition that kept the bulbs metabolically active (Sharma et al., 2016a). However, the bulbs become vulnerable to growth at the end of dormancy due to the absence of sprouting inhibiting factors, which manifests as either the formation of shoots or the elongation of roots (Petropoulos et al., 2017). As an external factor storage temperature above the critical level (27 °C) strongly inhibits the shoot growth while wounding the bulbs during or after harvest promotes shoot growth (Sahu et al., 2021). Hence, the observed sprouting percentages towards the end of the storage period may be attributed to either a decrease in temperature or the loss of dormancy of bulbs.

Although the eco and paradormancy suspension regulates the signals originating from external environmental conditions the endodormancy breaks are largely regulated by the endogenous signals generated by the hormonal alteration (Forney et al., 2022). Generally, after harvesting onion bulbs face three major rest, dormancy and sprouting periods. Once harvested, onions go through a resting period of about 30–40 days before entering the dormancy period where bulbs show reduced responsiveness to environmental changes controlled by internal hormones and genetic factors. Phytohormones, including auxins, cytokinins, gibberellins, abscisic acid, ethylene, and salicylic acid, are essential endogenous signaling molecules that play diverse and crucial roles in regulating plant growth, organ development, and adaptation to environmental stimuli (He et al. 2024; Liu et al. 2024; Shi et al. 2024; Zhang et al. 2024). For example, the endogenous accumulation of cytokinin enhances sprouting by stimulating cell division as proven by benzyladenin application. Similarly, the reduction of abscisic acid (ABA) and stimulation of gibberellins (GAs) synthesis could suspend dormancy through the breakdown of starch to simple sugar essential for growth or development of root or shoot of bulbs (Sharma et al., 2016a). Therefore, paying attention to the regulating factors could help us to minimize the sprouting loss and prolonged storage of onion bulbs essential for nutritional security.

Table 1
Commonly observed onion storage diseases and their causal organisms with common signs and symptoms.

Disease Name	Causal Organisms	Phenotype	Symptoms	References
Fungal Diseases				
1. Neck Rot	*Botrytis allii,* *Botrytis aclada* *Botrytis byssoidea*		Scales on the neck region initially get wet, then dry out and turn brown, with visible gray mycelium between them.	(Steentjes et al., 2021)
2. Blue mold	*Penicillium* spp.		Pale blemishes, yellow lesions and soft areas are the initial signs. On lesions, a blue-green mold grows. In advanced stages, bulbs may begin to completely rot.	(Duduk et al., 2014)
3. Black mold	*Aspergillus niger*		Masses of black spores are seen on the outer surface and between the scales. Whole surface of the bulb becomes black and shrivels.	(Saranya et al., 2017)
4. Purple Blotch	*Alternaria porri*		Water-soaked lesions turn brown to purplish color as the spot enlarges. After harvest bulbs begin to rot, and scale tissue changes color from yellow to wine red.	(Mohsin et al., 2016)
5. Smudge	*Colletotrichum circinans*		Smudge first appears as dark green spots that eventually turn black on the dry outer scales and lower sections of the bulb.	(Chen et al., 2022; Maude, 2018)
6. Fusarium Basal Plate Rot	*Fusarium oxysporum, F. proliferatum, F. solani*		Affected tissues become brown, dry and shriveled. Black or brown fungal spore masses form on the diseased bulbs.	(Mandal and Cramer, 2020)
7. Pythium Soft Rot	*Pythium* spp.		Plants first show yellow and stunted appearances. Lower stems and onion bulbs and roots eventually start to develop a watery rot that eventually becomes black.	(Le et al., 2016)
8. White Rot or sclerotium Rot or White mold	*Sclerotium cepivorum*		Bulbs in storage may decay due to small sclerotia forming in and on the surface of the affected bulb.	(Hussain et al., 2017)
9. Rhizopus Soft Rot	*Rhizopus* spp.		It leads to rapid softening and decay of the bulbs, often accompanied by a fluffy gray mycelium and black sporangia formation.	(Kolhe et al., 2021)
10. Cladosporium Rot	*Cladosporium* spp.		A dark-colored, fuzzy mold appeared on the bulbs accompanied by soft rot. Infected tissues become discolored and show a slimy texture.	(Shahnaz et al., 2018; Wang et al., 2021)

Bacterial Diseases				
1. Bacterial Streak and Bulb Rot	*Pseudomonas viridiflava*		Infected inner scales of the bulb start as lemon yellow, quickly changing to reddish brown or dark brown, leading to rot and discoloration.	(Tsuji and Fuji, 2021)
2. Bacterial center Rot	*Pantoea ananatis* (syn. *Erwinia ananatis*), *P. agglomerans* (syn. *E. herbicola*)		Whitish, bleached and necrotic lesions appear on interior leaves. Interior bulb tissue is affected, resulting in rot and foul odors.	(Achbani et al., 2016)
3.Enterobacter Bulb Decay	*Enterobacter cloacea*		Symptoms are not present in the field but are seen in the storage period. While outside bulbs seem healthy, inside scales exhibit light to dark brown discoloration and rot.	(Asselin et al., 2016)
4.Soft Rot	*Dickeya (ERwinia) chrysanthemi, Bacillus cereus*		The decay and decomposition of bulbs and the emergence of a pungent odor.	(Sawada et al., 2021)
5.Slippery skin	*Burkholderia gladioli* pv. *allicola*		The central scales of a bulb decay and can be dislodged by squeezing the neck during severe infections.	(Félix-Gastélum et al., 2017)
6.Sour skin	*Burkholderia (Pseudomonas) cepacia*		Infection of scales within the bulb with discoloration and rotting with a foul odor. A distinctive tan colored slimy ring seen in cross section.	(Konduru et al., 2015)
7. Bacterial Blight	*Pantoea ananatis*		Affects onions during storage, resulting in water-soaked lesions on bulbs. The lesions may progress to rotting and browning.	(Choi et al., 2021)
Viral Diseases				
Causal organisms	Vector	Phenotype	Symptoms	References
1. Irish Yellow Spot Virus (IYSV)	Onion thrips		Causes light green or tan necrotic lesions on the leaves and bulbs. The storage life of bulbs reduces due to soft rotting.	(Hamza et al., 2023)
2. Onion Yellow Dwarf Virus	Aphids		Leaves may be crinkled or flattened with yellow streaks. Bulbs are undersized and enhanced vulnerability to storage diseases.	(Verma et al., 2015)
3. Shallot Latent Virus	Infected planting materials		Reduce the height and yield of onion plants. Infected bulbs may not show visible symptoms but act as a source of virus for future planting.	(Harti et al., 2020)
4. Leaf Yellow Stripe Virus	Onion thrips		Induces chlorotic stripe symptoms on leaves, resulting in yield reduction and reduced shelf life by enhancing the susceptibility to storage diseases.	(Parthasarathy et al., 2016)

4. Factors regulating the post-harvest excellence of onion

Prolong preservation of onions is a complicated process as it involves multiple factors both before and after harvest including the conditions they grow in, how they are handled, temperature control, humidity, disease management, and post-harvest treatments (Falola et al., 2023; Petropoulos et al., 2017). This indicates onions go through the phases that start in the field and end with the consumer. Therefore, the quality of stored onions actually depends on a combination of genetical, physiological, biochemical, nutritional and cultural factors, with post-harvest management practices (Fig. 5 and 6). These factors collectively affect the texture, flavor, and overall desirability of the onions in storage. The storage duration of onions is also impacted by the timing and method of harvest, curing process, storage environment and the choice of packing materials (Yeshiwas et al., 2023). Each of these situations has the potential to cause injuries such as bruising, cuts, ruptures, and scratching, which can have a substantial influence on onion quality and postharvest life. Inappropriate management of these processes may have serious consequences like significant drops in nutritional content and outbreaks of foodborne illnesses that enlarge the financial losses for everyone involved in the supply chain from growers to consumers.

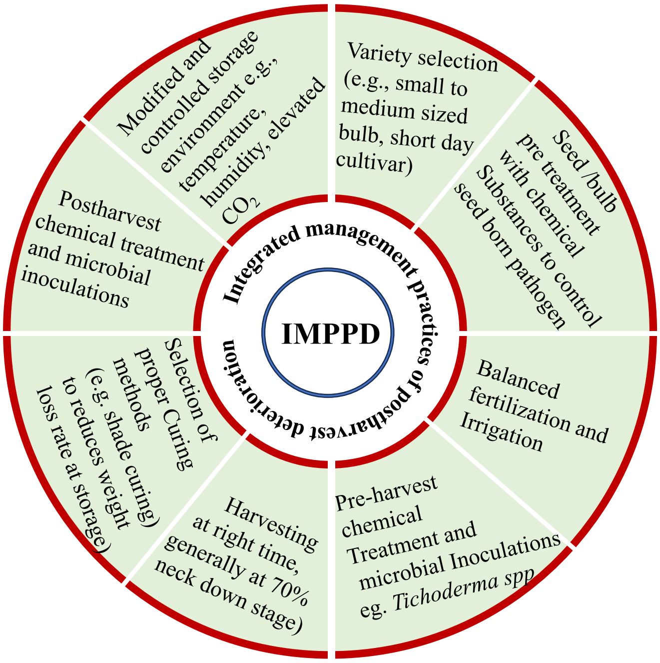


Fig. 6. Integrated management practices of post-harvest deterioration (IMPPD).

4.1. Genetic factors

The factors that determine the inherent characteristics of onion such as bulb size, shape, firmness, color, dry matter accumulation, growth cycle or maturity index, photoperiod, disease resistance flavor and storability or shelf-life after harvest called genetic factors that we broadly discussed in two physicochemical and nutritional categories. Generally, the duration onion remains viable after harvest, known as shelf-life that intricately influenced by the complex interplay of all the above mention key genetic factors (Petropoulos et al., 2017). It has been observed that onions with high dry matter content with total soluble solids (TSS) are well-suited for long-term storage due to their inherent ability to resist deterioration over extended periods (Tekeste et al., 2017). For example, onions with an amplified (11.9 %) total soluble solids (TSS) showed not only an extended shelf life of about twelve months but also noticed excellent storage quality (Tripathy et al., 2016). In addition to dry matter accumulation pectin and cellulose were found to be correlated with the firmness of the bulb and increased self-life after harvest (Coolong, 2007). Similarly, onion storage life is influenced by the color of their outer scales and the scales with red varieties have the best storage potential followed by the yellow and white types (Abubakar et al., 2019). Advanced genomic tools like RNA sequencing or genetic mapping reveal the important genes and pathways that influenced onion bulb or scale color. For example, very recently, it has been reviewed that onion bulb or scale color is inherently regulated by five major loci, such as, loci I, loci C, loci G, loci R and loci L (Sharma et al., 2024). Among these loci, locus C- a basic color factor predominantly required for red or yellow color development in homozygous (CC) or heterozygous (Cc) genotypes. Again, two complementary loci, R and L, also regulate red scale color by interacting with flavonoid biosynthesis (for details, please review Sharma et al., 2024). Expression profiling explored that the *CHI*

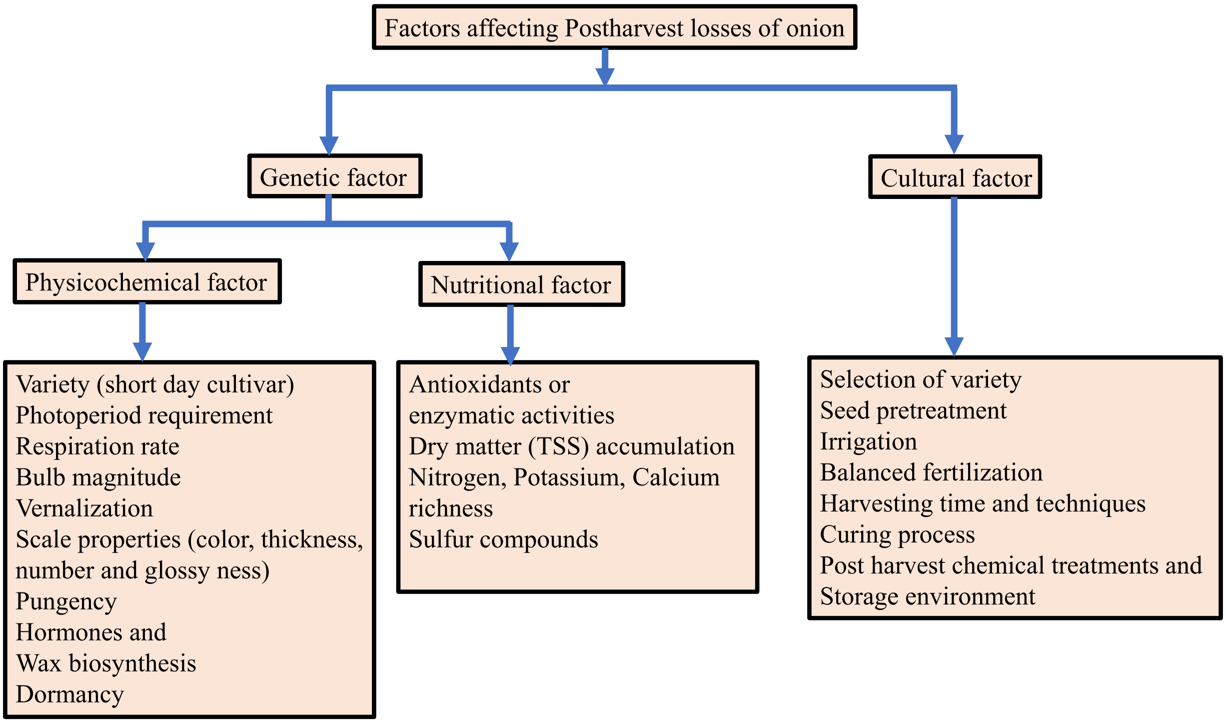


Fig. 5. Factors affecting the post-harvest storage quality of onion.

gene plays an important role in anthocyanin biosynthesis expressed in the C locus and contributes yellow color scale by chalcone derivatives and the DFR gene expressed in the R locus associated with red color scale (Kim et al., 2004). Recently it has been also observed that the MYB1 transcription factor positively regulates anthocyanin biosynthesis (Li et al., 2022), however, the relationship between MYB and C locus remains elusive. In fact, the higher levels of proteins, lipids, flavonoids, pyruvic acid, phenolic compounds, and ascorbic acid (AsA) in red-scale onions provide additional protection against infectious diseases like rotting that ultimately contribute to the overall storage quality and food security(Abubakar et al., 2019; Pérez-Gregorio et al., 2010). In fact, the presence of multiple dry layers of skin surrounding the onion is associated with a multifaceted influence on diminishing the risk of microbial growth, mitigating the susceptibility to rotting, and restraining the respiration rate at storage (Tripathi and Lawande, 2019). The lowered respiration rate in turn fostered an extended storage life by means of effectively curtailing the metabolic activity like increased antioxidant activities of bulbs that are particularly pronounced in light red onions (Gorrepati et al., 2018; Singh et al., 2018). Moreover, choosing of smaller to medium-sized bulbs also offers additional advantages of minimal loss in quality during storage. Interestingly, it has been observed that among the onion cultivars, short-day varieties ensure better quality in long-term storage compared to long-day varieties (Gubb and MacTavish, 2002).

Furthermore, the pungency level and flavor of onions exhibit a profound interrelation with their storage potential. The characteristic pungent taste and aromatic profile exhibited by onions primarily owe their origin to the presence of sulfur compounds, most notably 1-propenyl- and methyl-l-cysteine sulfoxides. During the storage, a complex series of biochemical reactions occur that involves hydrolysis of γ-glutamyl peptides, leading to the increase of flavor precursors and their pungency (Cramer et al., 2021). Therefore, onions with elevated pungency levels by owing higher concentrations of sulfur compounds exhibit not only an amplified capacity to withstand storage challenges but also render them more adept at maintaining their sensory attributes and prolonging storage (Thangasamy et al., 2013). Interestingly, it has been observed that the gibberellin (GA) hormone plays an important role in maintaining the pungency level in long-term storage indicating their intricate mechanisms (Wakchaure et al., 2023). Similarly, the dormancy of onions is controlled by a combination of stimulating hormones such as gibberellin and cytokinin, as well as the inhibitory hormone abscisic acid (Sharma et al., 2016a). Auxin levels are critical in the dormancy break, and the process is regulated by a balance of promoters and inhibitors (Komochi, 2018). The intricate process of dormancy induction is regulated by the involvement of abscisic acid (ABA), which effectively inhibits sprouting. Although the different hormonal pathways are established as the most enriched regulators of dormancy, the molecular mechanisms are still in infancy. However, recently, for the first time, it has been explored that *WARKY-32* transcription factor (TF) as a candidate gene regulated onion bulb-dormancy release (Puccio et al., 2022). Additionally, gene ontology (GO) and network analysis revealed that *WARKY-32* is strongly correlated with the expression of genes involved in cell wall modification and division through gibberellin and auxin homeostasis, suggesting its pivotal role in dormancy break (Puccio et al., 2022). On the contrary, when exposed to higher levels of endogenous ethylene, onions suffer from a multitude of adverse effects during storage, including accelerated aging, sprouting, softening, loss of firmness, yellowing, breakdown of cell walls, degradation of pigments, and the development of undesirable off-flavors and odors (Chope et al., 2012; Sharma et al., 2016a). Moreover, ethylene is linked to sprouting and the termination of early dormancy and wounds during handling enhanced ethylene production results onions become more susceptible to sprouting and decay by bacteria and fungus.

Interestingly, such an encompassed diverse range of bioactive compounds plays a pivotal role in preserving the post-harvest storage quality of bulbs that largely depends on the nutrient absorption potential of crops regulated by their genetic traits (Nabi et al., 2010; Woldetsadik and Workneh, 2010). For example, the genetic potentiality of the interaction of onion with soil humic substance and mycorrhiza has a substantial impact on the postharvest quality and shelf-life due to their strong effects on soluble sugar and protein accumulation (Golubkina and Caruso, 2020). Generally, high sugar or carbohydrate deposition in onions due to excess nitrogen accumulation contributed to quicker moisture loss through respiration, resulting in bulbs becoming more vulnerable to decay and spoilage in storage (Barrales-Heredia et al., 2023; Etana et al., 2019). In contrast, onions with higher sulfur content possess improved storage capabilities due to the natural defense properties of sulfur compounds (Liu et al., 2022; Petropoulos et al., 2017). Similarly, the optimization of potassium levels has a positive impact on onion storage potential as it possesses multifaceted effects, including the maintenance of bulb firmness, the enhancement of disease resistance, and the preservation of flavor attributes, all of which contribute to the improved postharvest longevity and quality of the bulbs (Kumara et al., 2018). Therefore, the combination of these multifaceted genetic traits ultimately determines the onion bulbs' ability to stay fresh and usable at long-term storage.

4.2. Cultural factor

Cultural factors like fertilization, irrigation, harvesting time and techniques, curing procedure and duration, storage environment and packaging strategy entirely influence the quality of stored onion. Inappropriate fertilization and irrigation techniques could make the bulbs more susceptible to rotting while in storage. For example, it has been observed that overhead irrigation intensifies the chances of over 32 % of disease occurrence compared to furrow or drip irrigation (Tripathi et al., 2017). Again, excessive surface irrigation or irrigation prior to harvest has a strong negative effect on storage due to reduced dry matter accumulation, delayed bulb maturation and hardening of outer scales that ultimately influenced the post-harvest rotting and sprouting (Pejić et al., 2011). In contrast, extreme water-deficient conditions could lower the total yield by producing small-sized bulbs (Ortola and Knox, 2015). Similarly, immature or over-mature harvesting of bulbs and abrasive curing strategies could influence the early spoilage of onions in storage and affect the overall bulb quality (Tripathi and Lawande, 2019). In addition, the storage environment like temperature, humidity, and ventilation plays a pivotal role in determining the longevity of bulbs, and nonstrategic packaging further hampers their shelf life. Interestingly, it has been observed that optimum intra-row spacing not only influenced to increase the bulb size, yield, and quality also influenced the postharvest shelf life of onion by affecting the dry matter accumulation (Kahsay et al., 2013).

Besides, the storability of onion bulbs is largely hampered by inappropriate fertilization. For example, evidence showed that an excess amount of nitrogenous fertilizer not only delayed the maturity of bulbs but also enhanced the occurrence of thick-necked or bolted open-necked bulbs that indorsed pathogen profundity at storage (Khokhar, 2017). Again, the potassium-deficient bulbs showed very low potential to mitigate the occurrence of sprouting at storage (Thangasamy, 2016). Similarly, the lack of calcium-based nutrients not only weakened the cell wall but also heightened the water loss and allowed pathogens to invade more easily, results undermining the bulb's ability to defend (Shehata et al., 2017). Similarly, the immature or over-mature harvesting and rough handling of bulbs increased the rotting and sprouting percentage of onion and affected its marketability. Generally, excessive drying results in sunburn, sloughing, and shrinkage of onions while, a poor curing process leads to mold development in stored onions (Gorreapti et al., 2017). Equally, storage conditions like temperature, humidity, ventilation and darkness affect sprouting, decay, and texture which play a crucial role in maintaining the quality and shelf-life of onions. For example, extended humidity above 70 % along with elevated temperature (30 °C) in storage structure promotes diseases, while lower temperatures below 10 °C and humidity encourage physiological (weight loss) and sprouting losses, thus reducing the storability and overall quality of post-harvest onions (Petropoulos et al., 2017).

5. Advances in the regulation of postharvest deterioration of onions

Every year roughly forty percent of onions deteriorate only at the post-harvest stage attributed number of abiotic or biotic factors. In fact, at postharvest from dormancy to the sprouting stage onion bulb goes through a range of physiological changes that sound for the precise regulatory techniques (Pagano et al., 2023). Again, the intricate relationship of multiple pre-harvest conditions also largely attributed to the losses. In a sense, postharvest treatment failed to uphold the quality of onions that are already diseased, pest-infested, or of poor quality. In terms of postharvest management, developed countries exhibit more efficient practices, leading to lower losses (15 % −20 %) compared to developing countries that experience higher losses (Elik et al., 2019). However, by embracing modern technological practices both at the preharvest and postharvest stage such losses of onion are possible to significantly minimize. There are several ways in which technologies can play crucial roles that are discussed in the forthcoming paragraph.

5.1. Pre-harvest practices

Pre-harvest practices refer to the series of activities and management practices that are performed by the grower before harvesting and play a crucial role in the overall success of crops. For onion, pre-harvest practices optimized not only the yield and quality but also improved the storability of bulbs. The state-of-the-art scenarios of scientific and technological advances in pre-harvest practice include selection of verities, seed pre-treatment, irrigation, balanced fertilization, pre-harvest chemical treatment, harvest method and maturity that largely contributed to minimizing the postharvest losses of onion. Here, each of the advanced approaches is briefly discussed and the relative effects of chemical pretreatment in minimizing the postharvest losses are listed in Table 2.

5.1.1. Variety selection

The varietal difference of onion not only affects test and quality but also upholds the unique potential of prolonged storage of bulbs. For instance, it has been postulated that light red to red colored onion varieties showed better storage potential in comparison to white color varieties (Singh et al., 2011). Onion scale color is associated with the presence of pigments that function as anti-oxidants thereby combating postharvest diseases. Similarly, small to medium-sized onion varieties generally have a thinner neck, which creates a smaller entry point for pathogens, resulting in the ultimately reduced rate of rotting of bulbs in storage (Tekalign et al., 2012). Generally, short-day cultivar produces this small to medium-sized bulb might be due to the low accumulation of solid matter (Zavadska et al., 2021). Again, the higher number of external scales on the onion bulb led to better storage performance, as such scales act as protective layers of bulbs (Sekara et al., 2017). It has also been observed that genetically modified NIKSP-86 onion variety with reduced epicuticular wax contents play a crucial role in reducing the virus transmission in the field through insects, which in turn enhances the storage life of onions by minimizing the occurrence of rot (Cramer et al., 2021).

5.1.2. Seed pre-treatment

Seed treatment with fungicides before sowing greatly decreases rotting loss of onion in storage. For example, the application of benomyl at a rate of 2 g Kg^{-1} of seeds, as well as Bavistin, showed reduced neck rot diseases in stored onions (Tripathi and Lawande, 2019). Similarly, Folicur, Seed plus, Penncozeb, Mirage and Ridomil Gold are also used for seed treatment. Mirage and Seed plus were able to reduce the disease

Table 2
Pre-harvest chemical practices for the regulation of post-harvest storage quality of onion.

Pre-harvest Treatments	Effects	References
Maleic hydrazide	-Limits onion sprouting and rotting during long-term storage -Reduces bulb early shrinking	(Gomathi and Irene, 2020; Raut et al., 2017)
Carbamate and isopropyl N-phenyl (CIP)	-Prevents sprouting. -Reduces respiration rate	(Petropoulos et al., 2017)
Ethephon	-Reduces sprouting by influencing the concentration of ethylene in the cell. -Enhances the content of total phenolics, flavonoids, and pyruvate in the bulbs.	(Muhie, 2022; Rani, 2016)
Chlormequat (2-chloroethyl trimethyl ammonium chloride) 2500 ppm	-Reduces rotting percentage in storage compared to the untreated control group. -Acts as an anti-gibberellin agent, helping maintain onion bulb quality by inhibiting sprouting and reducing moisture and weight loss.	(Anbukkarasi et al., 2013; Vethamoni and Gomathi, 2018)
Mepiquat chloride (500 ppm)	-Lowers total storage loss percentage compared to untreated blub.	(Vethamoni and Gomathi, 2018)
Salicylic Acid (250 mg per liter)	-Increases onion bulb yield -Reduces field losses from diseases and pests while minimizing weight loss, rotting, and sprouting during storage.	(Bhasker et al., 2020)
Mancozeb (0.25 %)	-Increases onion yield and prevent diseases in storage conditions, significantly reducing onion fungal diseases.	(Ekabote et al., 2021)
Streptocyclin (0.02 percent)	-Delays physiological weight loss, rotting during storage. -Additionally, when used in conjunction with either cycocel or carbendazim, streptocycline helps to minimize post-harvest losses.	(Sharma and Chauhan, 2021)
Silver nanoparticles (AgNPs) @ 20 ppm	-Exhibits superior outcomes in terms of yield, yield components, and quality characteristics of onion.. -Possesses antifungal properties against different fungal species, including *Aspergillus flavus* and *Aspergillus niger*. -Inhibits fungal growth, spore formation, and hyphal development in the field as well as in storage.	(Das and Deb, 2018; Fouda et al., 2020)
Copper nanoparticles (CuNPs)	-Improves various growth parameters, such as plant height, leaf count, fresh and dry weight, yield, and bulb quality. -Enhances the accumulation of macro- and micro-nutrients, total soluble solids, phytochemical compounds, vitamins, and amino acids. -Exihibts antifungal activity against various plant pathogenic fungi, including *Fusarium oxysporium* and *Botrytis cinera*.	(Mottaleb et al., 2021; Shukla et al., 2019)
Zinc nanoparticles (ZnONPs) @250 ppm	-Effectively inhibits bioactivity of pathogen and enhances seed quality, providing protection against Purple Blotch disease.	(Chaithanya et al., 2023)

incidence by 40 % and 43 %, respectively (Sintayehu et al., 2011). Recently it has been reviewed that seed priming allowed the resumption of pre-germinating metabolic activity and boosted key molecular events like DNA repair and hormonal regulation that bring the onion seeds into the advance physiological stage of germination (Pagano et al., 2023). However, the mechanisms underlining the seed priming to reduce disease incidence and prolog storability still remain unclear which demands more intensive research.

5.1.3. Balanced fertilization

Onions are the heavy feeder of nitrogen, phosphorus and potassium along with other micronutrients (Amare, 2020). Hence, balanced fertilizer is essential for the successful cultivation of onions. However, the requirement for nutrients changed with the plant's physiological stages. For instance, higher amounts of nitrogen are required in early growth stages while, the late application especially prior to harvest not only delays the maturation of bulbs but also affects the storage potential (Brewster, 2018). Similarly, potassium plays a vital role in enhancing the quality and storage potential of onions by promoting the total soluble content (TSS) content and reducing the moisture loss in storage. Moreover, an adequate amount (60–70 kg ha^{-1}) of potassium leads to around 32 % decreased physiological weight loss with reduced sprouting percentage (Nabi et al., 2010). Additionally, calcium fertilization positively impacts onion bulb storability not only by strengthening the cell walls but also by forming a protective barrier against pathogens and water loss (Shehata et al., 2017). It also hinders pathogen infection by inhibiting polygalactorunase activities through which pathogens infect at the cellular level (Khalifa et al., 2012). Alike sulfur-based fertilizer is essential for improving pyruvic acid content in onions, while excessive application over 45 kg ha^{-1} leads to an increased sulfoxides content in plant cells results decreased storability (Sharma et al., 2016b). However, the integrated application of chemical fertilizer with organic manures could upsurge both the yield and prolong the storability of onions by improving the dry matter accumulation (Bettoni et al., 2014; Gnanasundari et al., 2022). For example, the integrated application of organic fertilizer (20 Mg FYM ha^{-1}) with inorganic fertilizers (69 Kg N, and 92 Kg P_2O5) not only increased the productivity of onions but also displayed enhanced storability with minimum rotting, and sprouting (Tekeste et al., 2017).

5.1.4. Adequate irrigation

In high yield farming irrigation is becoming a commonplace of discussion and the right time and method of irrigation have great consequences on onion quality and storability. For example, early-stage regular interval basis irrigation not only fulfills the moisture requirement but also improves the bulb development by increasing total soluble content through optimum nutrient uptake (Tripathi and Lawande, 2019). Again, the cut-off irrigation technique is found to be an effective intervention approach upholding the post-harvest quality of onion in addition to increased yield (Geries et al., 2021). Similarly, it has been observed that drip irrigation reduces around 30 % storage losses in comparison to surface irrigation and stopping irrigation 2–3 weeks before harvesting at 5–10 % top-down condition potentially prevents rotting and sprouting of bulbs in storage (Belo et al., 2023; Sharma, 2023). Hence, sounds irrigation as an indicator has meaningful impacts on both the qualitative productivity and prolong storability of onion

5.1.5. Chemical pre-treatments

Chemical pre-treatment refers to the application of various chemicals to onion bulbs to enhance the yield, shelf-life and overall post-harvest quality including inhibition of microbial growth, sprouting and physiological decay. In recent years, several growth substances and chemicals, like abscisic acid, gibberellin, auxin, cytokinin, bavistin, dithane-M-45, streptocyclin, procymidone, maleic hydrazide, ethrel, cycocel, carbendazim, aureofungin have gained significant recognition as pre-harvest foliar applications in agriculture to minimizing the post-harvest losses as shown in Table 2. For example, the pre-harvest spray of maleic hydrazide (2000 ppm) along with carbendazim (1000 ppm) extended the shelf-life of onion up to six months by suppressing the pathogen infestation might be due to their stimulatory effects on TSS accumulation (Raut et al., 2017). Similarly, pre-harvest foliar application of maleic hydrazide, cycocel and salicylic acid at 1 mML^{-1} concentrations was found to be suppressed sprouting and rotting loss at storage (Tripathi and Lawande, 2019). Again, foliar application of hormones like gibberellic acid was found to improve both the chlorophyll content and photosynthetic performance in onions which, in turn, contributed to better bulb quality and extended storage life (Rostami and Mohammadi, 2022). Furthermore, in recent year's application of nanomaterials as a novel approach has an extra attention to minimize storage losses as well as improving the yield quality and quantity. For example, it has been postulated that the application of silver nanoparticles (AgNPs) at 150 ppm concentration successfully reduced the diseases caused by Aspergillus (Rostami and Mohammadi, 2022). Similarly, copper nanoparticles (CuNPs) were found to elevate the storability of onions by impeding the metabolic activities of Fusarium species (Mottaleb et al., 2021). However, the effectiveness of chemical pre-treatment largely varied with several factors including variety, storage condition and storage durability (Gunaratna et al., 2023).

5.1.6. Pre-harvest microbial-inoculation

To keep the onion fresh with the assurance of quality during storage is vital to ensure the year-round supply for consumption. In accordance with the other pre-harvest practices, biological control emerged as the most suitable alternative to control the soil-born phytopathogens of onions like *Fusarium* species and *Pythium* species (Gunaratna et al., 2023). Generally, biological control involves harnessing the benefits of beneficial microbes from their genes or metabolites to suppress or mitigate the negative effects of harmful pathogens. For example, it has been explored that *Trichoderma* species has a strong antagonistic effect on the growth of *Fusarium* species at in vitro conditions might be due to the enhanced synthesis of chitinase and $\beta-1$, 3-glucanase (Kumar and Iqbal, 2020). However, very recently explored that field treatment with *Trichoderma* species (*T. asperellum* and *T. virens*) showed no significant effect in reducing the post-harvested loss of onion associated with mold and rot (Gunaratna et al., 2023). These might be due to the species divergent as previously reported that among the seven tested species of *Trichoderma* showed the lowest 28.8 % antagonistic effect for *T. koningii* while, *T. harziianum* showed 81.1 % efficiency in controlling the *Fusarium* rot (Cherkupally et al., 2017). Moreover, as an integrated management strategies climatic conditions and soil health with onion verities should be taken into consideration during microbial treatment to minimize the post-harvest loses.

5.1.7. Harvesting time and methods

Onions are typically ready for harvest at 70 to 90 days after transplanting when the leafy green tops begin to turn yellow and eventually fall off above (about 37.5 mm or 50 mm above) the top of the bulb. Moreover, seed development and vigor are largely influenced by the harvesting time and height of harvest. For example, recently, it has been postulated that onion at 86–88 days with a 20 cm neck cut showed the best seed vigor due to enhanced achieved of physiological and biochemical traits (Sheykhbaglou et al., 2023). Generally, mature onions have firm and well-formed bulbs with tightly closed necks and protective dry scales containing pectin uronic acid that ultimately contribute to better storability (Coolong et al., 2008). Additionally, onion lifting at 80 % leaf fall increased up to 45 % quercetin content compared to earlier lifting which led to enhanced self-life and quality (Mogren et al., 2007). Additionally, proper harvesting techniques are crucial to minimize the risk of damage by disease in prolonging the storability of onion. For instance, among the differential methods of harvesting hand plucking and manual collection have shown 27 % less decay loss compared to digger and mechanical collection (Kwon and

Lee, 2023). Hence, employing appropriate harvesting methods with perfect timing is essential to ensure the prolong storability of onions.

5.2. Post-harvest practices

Effective pre-harvest practices contributed to a more efficient and successful agricultural production cycle. Whereas, proper handling, curing, storage conditions and efficient disease management are the key post-harvest practices that strongly impact the quality and shelf-life of harvested onions. Therefore, each of the advanced post-harvest practices is briefly discussed underneath with their employed roles to enhance the quality of harvested onions restricted by their shelf-life.

5.2.1. Proper handling and curing of harvested onion

Proper handling and curing of harvested onions are crucial to ensure the quality, flavor and prevent spoilage for prolong shelf-life. Improper handling during or after harvest may cause surface or internal mechanical injuries that severely affect the overall quality and storability of bulbs by promoting respiration and disease infestations (Petropoulos et al., 2017). Interestingly, such a post-harvest loss is very low in the case of well-trained educated farmers due to their meaningful decision and accurate handling (Falola et al., 2023). Similarly, curing is an essential post-harvest practice involved in allowing the harvested onions to dry in a well-ventilated area. Generally, curing helps to toughen the outer scales, minimize skin cracks, and promote narrower necks, thereby preventing pathogen infections (Gorreapti et al., 2017). This can be accomplished through natural methods like sun curing or shade curing, as well as artificially using forced warm air. However, among the curing methods shade curing results in lower physiological weight loss of onion during storage. In contrast, sun curing with foliage aids in translocating the growth-inhibiting hormones from leaves to bulbs, more accumulation of solid materials and increased firmness of outer scales that ultimately extend the dormancy period of bulbs (Petropoulos et al., 2017). Furthermore, 8–10 days long curing s not only improves the quality but also causes higher numbers of outer scale and color depth, results reduce the rate of sprouting, rotting and physiological weight loss (Muhie, 2022).

5.2.2. Chemical treatments

Several post-harvest chemical treatments like fumigation with sulfur, spraying or dipping on fungicides and ozonation are common to reduce the storage losses of onions as well as to maintain the quality as shown in Table 3. Generally, sulfur fumigation is employed to prevent mold during storage. Literature surfing explored that about four-hour fumigation with sulfur dioxide greatly improves the storability of onion by hampering the activities of *Aspergillus niger* (Sharma, 2023). Similarly, the thymol fumigation reduced about 96 % severity of fungal diseases in harvested onion by inhibiting the chitin or glucagon synthesis in fungal cell walls (Ji et al., 2018). Moreover, the phenolic compounds of thymol are able to reduce the respiration rates and ethylene production of onions. Although there is a conflict about ethylene and how it affects onion storage life recently it postludes that ethylene pretreatment has great potential to suppress the sprout at storage even though higher compare to commercial malic hydrazine (Forney et al., 2022). Moreover, the dipping of harvested onion in 0.1 % carbendazim solution before storage showed a significant reduction of rotting loss might be due to the stimulation of cellular malfunction of pathogens (Verma and Srivastava, 2018). Again, a number of studies explored that exogenous ozone acts as a potent oxidizer and antimicrobial agent preventing the rotting loss of stored onion. For example, ozone treatment at 14–24 ppm concentration was found to inhibit the mycelial growth and conidial germination of most of the *Alternaria, Fusarium and Botrytis* species in harvested onion at storage (Savitha et al., 2022). Moreover, ozone exposure delayed color changes and softening of onion bulbs during storage thereby increasing marketability (Lim et al., 2021).

Table 3
Advanced postharvest chemical practices for the regulation of storage loss and quality of onion.

Post-harvest treatments	Effects	References
0.25 % mancozeb or 0.1 % carbendazim or benomyl	-Reduces storage losses -Reduces black mold and blue mold infestations	(Kumar and Iqbal, 2020; Kumar et al., 2015)
Falisolan (carbendazim 60 % or bronopol 6 %)	-Reduces storage diseases due to *Aspergillus species, Fusarium* species and *Botrytis* species	(Kumar et al., 2015)
Fumigation with sulphur dust, bavistin, dithane M-45 and streptomycin	-Minimizes the percentage of rotting, -Reduces the disease infection and maintain the quality of onions during storage	(Anbukkarasi et al., 2013; Sharma, 2023)
Onion bulb immersing in acetic acid at higher concentration (0.4 %)	-Distinctly reduced the *Aspergillus niger* incidence	(Sharma, 2023)
Penicillium roqueforti and *P. viridicatum*	-Repressed the growth of Aspergillus niger by 66 % and 60 %	(Khokhar et al., 2012; Zhao et al., 2021b)
silver nanoparticles (AgNps) 150 ppm	-Impedes the growth and development of mycelia of *Aspergillus niger* and *Sclerotium cepivorum*, aflatoxin production of *A. flavus.* -Diminish spores number and cause abnormality	(Darwesh and Elshahawy, 2021)
Copper nanoparticles (CuNPs)	-Show antifungal activity against *Fusarium oxysporium, Botrytis cinera* and *Alternaria alternata*,	(Ouda, 2014)
Zinc oxide nanoparticles (ZnO NPs) (12 mg l-1)	-Exihibit antifungal activity against *Fusarium oxysporum* and *Penicillium expansum, Aspergillus niger, Penicillium notatum, Aspergillus flavus* and *Aspergillus fumigatus* etc.	(Yehia and Ahmed, 2013)
Sulphur nanoparticles (SNPs) (50–80 nm particle size), Nano-MgO,	-Hamper the spore germination of *Botrytis cinera* and *Penicillium expansum*, antifungal activity against *Aspergillus niger*	(Sachdev et al., 2016; Yehia and Ahmed, 2013)
Bulbs with 2.5 cm neck cut	-Reduce decay loss (6.21 %), physiological loss (15.17 %) and total loss (6.21 %), -Enhance dormancy and extend shelf life by limiting the access of disease	(Anbukkarasi et al., 2013)
Bulb curing (6–8 days)	-Reduced rotting and sprouting in bulbs - Decline respiration rate - Shade curing shows more effectiveness than sun curing - Protect the bulbs from microbial invasion and pathogen infection - Artificial curing (30 °C temperature and 98 % relative humidity) for up to 9 days improved bulb quality	(Eshel et al., 2014; Gorreapti et al., 2017)
Dithane M-45 0.2 % (spray)	-Lowers total losses	(Anbukkarasi et al., 2013)
Gaseous ozone (1.27 ppm)	-Reduces aerobic bacteria count (*Rahnella aquatilis*) and fungi (yeast and mold) - Improves shelf life by slowing down rotten rate and color change	(De Santis et al., 2021)
Exogenous ethylene	- Delays dormancy break by increasing the concentration of abscisic acid (ABA) and phaseic acid (PA) -	(Alamar et al., 2020)
Packaging techniques (wooden crates, jute bags, nylon net bags)	-Retard bulb rot incidence and reduced storage losses because of good ventilation	(Tripathi and Lawande, 2019)
Isopropyl N-Chlophenyl Carbamate (CPIC), maleic hydrazide (MH)	-Sprout inhibition	(Petropoulos et al., 2017)

5.2.3. *Irradiation*

As a credible integrative approach, radiation by gamma sources has been established to be a beneficial post-harvest strategy to extend the self- life and keep the quality of harvested onion through efficiently addressing issues like sprouting, rotting and diseases (Khade et al., 2023). The working principle of gamma irradiation on crops involves exposing the bulbs by ionizing gamma rays, derived from sources like cobalt-60 or cesium-137 (Venugopalan and Suprasanna, 2022). Interestingly such an exposure to the onion leads sudden increase in sugar content during the dormancy period, which inhibits the sprouting of bulbs, as plant cells require higher concentrations of sugar for sprouting (Avdyukhina et al., 2018). For example, the application of 120 Gy of irradiation with sodium benzoate notably extends the shelf life of onion by improving radical scavenging activity, and polyphenol content (Sharma et al., 2020). Additionally, it is beneficial for minimizing microbial infestation with increased quality indicators like pyruvic acid, ascorbic acid, phenolic acid, flavonoids and anthocyanin content with texture and color retention thereby improving overall quality and marketability (Kavita et al., 2024). Therefore, the careful regulations of gamma radiation in post-harvest treatments could play a crucial role in reducing storage losses, enhancing food quality and securing safety of the consumed agricultural products like onions.

5.2.4. *Modified storage environment*

To retain the freshness of harvested onion at prolog storage, minimization of biochemical changes, enzymatic activity and disease severity thought to be essential in addition to several pre-harvest agronomic practices (Gouda et al., 2023; Kargwal et al., 2020). Interestingly, proper storage conditions minimize chemical changes resulting in reduced physiological weight loss, rotting and sprouting and extended shelf-life of onions. Generally, onion could be stored either at medium temperature (25–30 °C) or cold temperature (0–2 °C) with 65–70 % relative humidity. Although cold temperature minimizes the storage losses about 4.1-fold compared to ambient storage, it involves higher costs (Tripathi and Lawande, 2019). Many researchers also showed that a controlled storage environment involves the hormonal regulation of stored bulbs. For example, a storage atmosphere with 1 % O2 and 99 % N_2, at 5 °C compassion exhibited extended storability of onion by lowering the concentration of abscisic acid, which in turn slower the sprouting rate (Chope et al., 2012). Similarly, the forced ventilated storage structure also enhances onion storage quality by stimulating the activities of pyruvic acid and TSS (Petropoulos et al., 2017). Interestingly, very recently it has been explored that a 15–20 % increase in atmospheric CO_2 significantly reduces the postharvest weight of loss of onion at room temperature conditions by promoting phytochemical synthesis which results in enhanced shelf-life of onion bulbs at storage (Gouda et al., 2023). Therefore, proper post-harvest practices and storage conditions are not only essential to enhance the storability of onions by reducing loss but also essential for providing high quality onions to consumers.

5.2.5. *Post-harvest biological practices*

In recent years, the practices of microbial treatment to inhibit the growth of targeted microorganisms responsible for post-harvest deterioration have drawn extra attention. Several microbial antagonists were identified and artificially introduced to a variety of agricultural commodities including onion to preserve the post-harvest quality. Although there is little information about the mechanism, postharvest application of microbial antagonists was found better and more useful compared to pre-harvest application (Sakib et al., 2020). For instance, post-harvest application of *Brevebacillus fortis* successfully controls the *Fusarium* rot disease in onion by inhibiting conidial growth (Johnson et al., 2020). Moreover, they observed that *B. fortis* secrets a cationic extracellular peptide edeine compound that has strong antifungal priorities, consequently reducing about 67–91 % conidial growth only at eleven hours of postharvest application. Additionally, *Pseudomonas, Bacillus* and *Tichoderma* have also been reported as successful growth inhibitors of the Fusarium rot in harvested onion (Savitha et al., 2022). Hence, as a component of integrated management practices of postharvest deterioration (IPPPD), postharvest application of microbial antagonists could be a better, practical and useful alternative to control the diseases of harvested onions.

6. Conclusions and future perspectives

The inherent perishability of onions leads to their limited shelf life, which is largely attributed to the diverse pre-harvest and postharvest operational approaches including variety, intercultural practices, maturity, harvesting time, handling and storage environment. Most surprisingly, about 30–40 % of onions never reach the consumer levels from the point of harvest. This postharvest loss of onions is primarily accredited by physiological weight loss (20–30 %), rotting (12–15 %) and sprouting (5–8 %) which is aggravated by poor storage environments. Again, the physiological loss of harvested onion could potentially be more substantial in tropical regions and /or during the dry months when temperatures remain high with low humidity. In contrast, rotting losses with diseases like rot are most widespread in high humid conditions, while sprouting begins at below 20 °C after dormancy break. Moreover, onion storability is critically influenced by genetic traits like dry matter accumulation, nutritional composition, scale color, outer skin, dormancy period, bulb size and respiration rate that largely vary from cultivar to cultivar. Besides, intercultural approaches like irrigation, fertilization, harvesting and handling, and proper curing entirely influenced the overall quality and self-life of harvested onion. Therefore, prolonged storability of onions is a complicated issue that requires a precise regulatory approach or technique to avoid the robust effect on their quality and marketability. However, technological advances both at pre and postharvest stages play a crucial role in minimizing the losses of harvested onion while upholding the quality. As a component of integrated management practices of post-harvest deterioration (IMPPD) genetically modified varieties having enhanced bulb quality with increased shelf-life by regulating hormonal activities of bulbs have an extra attention in recent years (Fig. 6). Similarly, seed pretreatment with growth regulators such as cycocel, ethrel, ethylene compounds, carbendazim and fungicides prior to harvest was found to enhance the postharvest shelf life of onions. Additionally, chlormequat also called cycocel observed to initiate an anti-gibberellin agent that inhibits sprouting and moisture loss resulting in improved storability of harvested onion (Vethamoni and Gomathi, 2018).

In contrast, an improved storage environment either medium temperature (25–30 °C) or cold temperature (0–2 °C) with 65–70 % relative humidity and 15–20 % elevated atmospheric CO_2 has been proven as an efficient approach to minimize the losses (Gouda et al., 2023). Similarly, radiation by gamma sources and pre and/or postharvest microbial inoculation has been established as a credible integrative approach in recent years to extend the self-life and keep quality by addressing the issues like sprouting, rotting and diseases. Moreover, as an alternative to synthetic fungicides the application of diverse types of nanoparticles is being explored as a novel and promising strategy to retard the decay of onions. However, the underlying mechanisms and their genetic evidence of how to postpone postharvest deterioration remain elusive. Hence, further investigation is needed precisely focusing on the aspects of nutrient management, hormonal regulation, frequency of nanoparticle application and their toxicity level, and pre and post-harvest biological practices that could make it possible to minimize the post-harvest losses while ensuring extended onion storage life and quality.

CRediT authorship contribution statement

Tonima Islam Suravi: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Md. Kamrul Hasan:** Writing – review & editing, Writing – original draft, Supervision, Investigation,

Funding acquisition, Formal analysis, Conceptualization. **Israt Jahan:** Writing – original draft, Investigation, Formal analysis. **Jannat Shopan:** Writing – review & editing. **Munmun Saha:** Writing – review & editing. **Biwsojit Debnath:** Writing – review & editing. **Golam Jalal Ahammed:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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