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Assessment of physiological stress on plants grown in soil contaminated with microplastics

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The current study was conducted with the aim to analyze the effect of microplastics on vegetable plants i.e., Spring Onion and Okra. Three types of microplastics sources were used i.e., Transparent plastic Bottles (Polyethylene terephthalate) (PET); transparent plastic bags (High-density polyethylene) (HDPE) and Polyester fiber (100% Polyester) (PES). Each type of plastic was mechanically/manually shredded to an approximate size of 4 mm and subsequently incorporated into soil at a concentration of 1% w/w. Control groups without microplastic addition were also established for comparison. Germinated seeds of spring onion and okra were sown in each experimental setup and plants were monitored and analyzed for morphological characteristics, oxidative stress indicators, and pigment content, including chlorophyll and carotenoids. The findings revealed that microplastic exposure had a statistically significant effect on plant health and development. Among the three microplastic types, PET induced the least detrimental effects, while HDPE and PES exhibited more pronounced impacts. All microplastic treatments led to a decrease in chlorophyll and carotenoid levels, alongside an increase in reactive oxygen species (ROS) production and lipid peroxidation, indicative of heightened oxidative stress. Additionally, reductions in key morphological parameters such as plant height, leaf number, and biomass were observed. Results of the study underscores the potential ecological risks posed by microplastic contamination in soil, highlighting its capacity to adversely affect plant physiology and development. Therefore, emphasizes the urgent need for strategies to mitigate microplastic pollution in terrestrial ecosystems.

Keywords Carotenoid, Chlorophyll, High-density polyethylene, Microplastics, Polyethylene terephthalate, Polyester fiber, Oxidative stress

The widespread use of plastic products has increased the possibilities of poor management and disposal practices, which in turn have triggered a boost in terrestrial microplastic contamination. According to data from Plastics Europe the worldwide production of plastic reached approximately 335 million tons by 2016. This marked an average annual growth rate of 8.6% since the 1950s, equivalent to about 1.7 million tons annually (Plastics[1]). Microplastics can exist within the environment in two forms: as deliberately produced microplastics (referred to as primary microplastics) or as a byproduct of the ongoing degradation of plastic debris, resulting in the generation of increasingly smaller plastic fragments (referred to as secondary microplastics)[2].

Synthetic polymers, such as polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS), and polyurethane (PUR), are produced using materials like coal, oil, and natural gas. These synthetic polymers are commonly recognized as plastics[3]. While plastic pollution in marine and freshwater ecosystems has garnered substantial attention, its impact on the soil ecosystem has received comparatively less consideration[4].

Microplastics in soil originate from both anthropogenic and natural sources. Anthropogenic sources stem from human activities, industry, and agriculture. These sources encompass a range of contributors such as agricultural plastic film, the use of wastewater for irrigation, the incorporation of soil amendments like organic fertilizers and sewage sludge, surface runoff, and indiscriminate littering[5]. In 2017, the usage of plastic mulching film for agricultural purposes in China surpassed 1.47 million tons, leading to the significant buildup of microplastics

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within agricultural soils[6,7]. Plastic mulch films are extensively employed in agriculture and are recognized as a significant origin of plastic remnants discovered within soil. The utilization of low-density polyethylene (LDPE) for mulching purposes is prevalent, contributing to the presence of both visible and minuscule plastic fragments in agricultural soil[8].

Effects of microplastics in soil have been either devastating or even beneficial to the rhizosphere functions such as plant health and microbial activity. Some studies also depict beneficial interactions between symbiotic fungi and plants after introduction of plastics in the soil[9]. Microplastics primarily infiltrate soil through mechanisms like mulch degradation, organic fertilizer application, and plastic landfill deposition, resulting in discernible disparities in their spatial distribution within soil systems. Various abiotic and biotic processes, notably leaching and bioturbation, expedite the lateral and vertical movement of microplastics within soil, effectively broadening the scope of their contamination. The evolution of microplastics in soil is chiefly propelled by mechanical abrasion, oxidation processes, and biological factors. These forces collectively drive the physical, chemical, and biological alterations experienced by microplastics in soil environments. Furthermore, the presence of microplastics can influence soil characteristics, nutrient cycling processes, and the microbial community, with outcomes contingent on factors such as microplastic shape, polymer composition, size, and concentration[10].

Vascular plants have the capability to serve as repositories for microplastics and nanoplastics due to their capacity to absorb these synthetic materials onto their surfaces. Furthermore, plants can even uptake nanoplastics into their internal structures. The presence of plastics whether on plant surfaces or within their internal compartments, can induce a range of phytotoxic consequences. These effects encompass disruptions to growth, photosynthesis, and oxidative equilibrium[11]. The deleterious impact of microplastics on the terrestrial ecosystem induces alterations in both physicochemical and biological attributes of soil. This, in turn, brings about shifts in nutrient cycling and subsequently poses potential climatic risks in the future[12].

Despite being inert nature of microplastics, they can release toxic unreacted and distinct monomers and oligomers which adhere significant damaging properties[13,14]. PET microplastics release low-molecular oligomers (< 1 kDa) and common additives like phthalates e.g., bis(2-ethylhexyl) phthalate, benzyl butyl phthalate and acetaldehyde during degradation[15]. Antimony, a key component used in the production of PET, can leach into beverages stored in PET containers. Even trace amounts of antimony may become harmful, especially when the containers are exposed to heat or used for long-term storage[16]. In barley seeds, aqueous extracts from PET microplastics even those measuring 2–3 mm caused chromosomal abnormalities and suppressed growth. Among the polymers tested, PET exhibited the highest level of genotoxicity[17]. Like PET and PP, HDPE releases extractable organic additives, as demonstrated in experiments using ethanol, where the removal of these additives eliminated the observed toxicity. Toxic effects in nematodes exposed to HDPE leachates persisted until polar solvents extracted the additives, indicating that chemical migration was the primary cause of harm. Although specific studies on PES leaching are limited, its chemical similarity to PET suggests it may release comparable small molecules, such as oligomers or additives[18].

Microplastics pose serious environmental threats, so it is essential to understand where they come from and how they get into nature. One major but under-researched pathway is landfill leachate especially when it comes to very small particles under 50 μm, which may be even more harmful. The study examined microplastics present in landfill leachate and in leachate treatment processes, with detection capabilities down to 10 μm. Findings revealed that untreated leachate contained an average of 235.4 ± 17.1 particles per liter and 11.4 ± 0.8 μg per liter by mass. Notably, more than half of the particles were smaller than 50 μm. In total, 27 types of polymers were identified, with polyethylene and polypropylene being the most prevalent in the untreated samples. Microplastics tend to remain suspended in liquid, making them difficult to remove via traditional sedimentation methods due to their low density and irregular shapes. However, membrane filtration treatments proved highly effective, reducing microplastic concentrations to just 0.14% by particle count and 0.01% by mass. Despite this, the average density of particles increased after treatment, and some microplastics, including those possibly originating from the membranes themselves, were still detected in the effluent. The high concentration of microplastics in the sludge dewatering liquor was estimated. This stream contained approximately 3.6 times more microplastic particles than the dewatered sludge itself. The results of the study suggested the promising strategy which focuses on removing microplastics from the sludge dewatering liquid before it is recirculated, thereby controlling the release of pollutants into the environment[19].

Beyond environmental repercussions, microplastics also significantly impact human health, as their presence in the human body has been verified. The pathways through which microplastics enter the human body lead to various health conditions, reflecting the diverse means of entry. Moreover, microplastics interfere with the human endocrine system, inducing adverse effects. At the ecosystem level, the ramifications of microplastics are interconnected, capable of disturbing essential ecological processes and systems[20].

The present study is aimed to investigate the effects of microplastics (MPs) derived from three different plastic sources: polyethylene terephthalate (PET), high-density polyethylene (HDPE), and polyester (PES) on the physiological and biochemical responses of vegetable plants. Specifically, the study focused on evaluating changes in morphological growth parameters, chlorophyll and carotenoid content, and oxidative stress markers compared to a control group. The objective was to assess and compare the extent of phytotoxic effects induced by different MP types under controlled soil conditions.

Materials and Methods

Microplastic sources

Three different types of microplastics (MPs) were used in this study: (1) Polyethylene terephthalate (PET): Transparent plastic water bottles (newly used) (2) High-density polyethylene (HDPE): Transparent plastic bags (new, unused) (3) Polyester (PES) fibers: 100% polyester fibers purchased new from the local market (Fig. 1). The MPs derived from transparent plastic Bottles (PET) and plastic bags (HDPE) were mechanically shredded

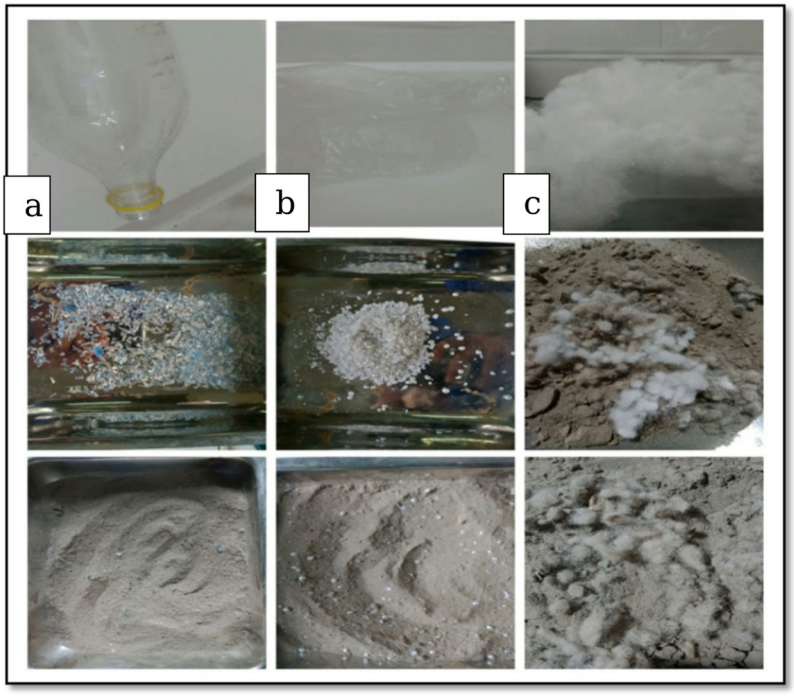

Fig. 1. Microplastics sources (**a**) Plastic Bottles (PET) (Polyethylene terephthalate) (**b**) Transparent plastic bags (HDPE) (High-density polyethylene) (**c**) Polyester fiber (PES) (100% Polyester).

to the size of 4 mm and further sieved through 5 mm in order to maintain a uniform spherical shape whereas MPs derived from polyester (PES) fiber were manually cut to the size of 4 mm and finally sieved through mesh size of 5 mm. Zhang et al.[21] performed the FTIR analysis to confirm the structural properties of polystyrene microplastics (PS-MPs), revealing characteristic peaks at 2916.087 cm^{-1}, 1471.544 cm^{-1}, and 717.450 cm^{-1}, corresponding to -CH_2- antisymmetric stretching, variable-angle vibration, and in-plane swing, respectively. The spectral data closely matched that of standard PS-MPs, validating their identity and consistency. The data also determined that PS-MPs particle size was 200 nm and were negatively charged. Although in the present study different polymer types (PET, HDPE, and PES) were used but the cited study provides supportive evidence regarding the utility of FTIR in confirming microplastic identity.

Plants selection

Two different plants were selected for the study i.e., Spring Onion *(Allium fistulosum)* and Okra *(Abelmoschus esculentus)*. Selected plants seeds were purchased from a local farm near countryside. The seeds of nearly equal size and weight were selected and then washed with distilled water several times. After washing, seeds were transferred on the Whatman No.1 filter paper for germination in sterile Petri dish and incubated at 21 ℃ for almost 10–16 days and allowed to reach to height of about 3 cm.

Exposure treatment

Total 75 kg of garden soil was collected from local nursery in Lahore, Pakistan to avoid presence of toxic pollutants in soil. In Laboratory the collected soil was spread in clean stainless-steel trays and were left to dry naturally in air for 24 h in clean environment. After drying, the soil was sieved through 5 mm sieve to ensure the removal of any large roots and gravel. The clay pots of two different sizes were selected. For the plantation of Spring onion 12 pots with the height of 15 cm and diameter of 13.9 cm were used and for the plantation of Okra 12 pots with height of 33 cm and diameter of 26 cm were used. The three different experimental setups were built for each plant. For each setup soil was thoroughly mixed with microplastic in ratio of (1% w/w) for each type of microplastic along with control samples (without any microplastics).

The use of only one concentration ratio 1% (w/w) of microplastics was selected in this study for all treatments. This decision was based on the need to manage experimental complexity, as three different types of microplastic sources (PET, HDPE, and PES) were tested on two plant species: Spring onion and Okra. Including multiple concentrations for each treatment would have substantially increased the number of experimental units, increasing the risk of handling errors. Furthermore, the selected concentration is environmentally relevant as documented in published scientific studies[22–24]. Previous studies have reported that microplastic contamination in soils can reach levels as high as 7% (w/w) in highly polluted environments[25]. Therefore, the 1% (w/w) concentration used in this study falls within a realistic range of environmental exposure and is supported by previous work[26], which also employed similar concentrations to assess plant responses to microplastics.

For Spring onion, a total of three experimental pots (one for each type of microplastic) and one control pot were prepared. Each pot was filled with 900 g of soil, thoroughly mixed with 9 g of microplastics, corresponding

to a 1% (w/w) concentration. For Okra, a total of three experimental pots (one for each type of microplastic) and one control pot were prepared. Each experimental pot contained 2000 g of soil thoroughly mixed with 20 g of microplastics, maintaining a 1% (w/w) concentration. The mixing of microplastic with soil was done in stainless steel trays for 10 min with constant stirring with metal spoons. The control soil was also shaken without microplastic in order to achieve equivalency. After the thorough mixing of soil and microplastics the mixtures were transferred into clay pots and kept in greenhouse for about 1.5 months before sowing seeds. This incubation period of 1.5 months allowed the interaction between soil and microplastics to ensure the possibility of microplastics leaching into soil. Research was conducted by[27] with aim to analyse the leaching of microplastics from various types of plastic waste and to investigate the influence of pH on the disintegration process. Seven types of plastic were studied such as Polyethylene Terephthalate (PET), High-Density Polyethylene (HDPE), Polyvinyl Chloride (PVC), Low-Density Polyethylene (LDPE), Polypropylene (PP), Polystyrene (PS), and Polycarbonate (PC). Characterization of microplastic particles in the leachate, using Scanning Electron Microscopy with Energy Dispersive X-ray (SEM–EDX), Fourier Transform Infrared Spectroscopy (FT-IR), and a particle size analyzer, showed that leachates from PET, LDPE, PS, and PP contained fibers along with other particles. It was assessed that among all samples, PC leachate had the highest concentration of microplastic particles (19,868 items/L), while PET had the lowest (4,099 items/L). In terms of mass concentration, PC also had the highest (184.1 mg/L), whereas PS had the lowest (43.1 mg/L). SEM analysis confirmed the presence of fibers in the leachates of PET and LDPE. EDX analysis identified elements consistent with the types of plastic used, while FT-IR analysis yielded inconclusive results for some materials. Most microplastic particles detected were smaller than 500 nm. It was confirmed that microplastic particles can disintegrate from various plastic materials under appropriate environmental conditions. Plastic materials were found to be more susceptible to degradation under acidic and basic conditions compared to neutral pH.

During this period of incubation each pot was watered with spray bottle (30 mL) after two days to keep the soil moisture content high. At the completion of incubation period the seedlings from filter paper were transferred to their respective pots. Three seedlings of each plant were sown in their respective pot at the depth of 2 cm. Each pot of Spring onion was watered 60 mL and each pot of Okra was watered 120 mL, after every two days. The setup took place in controlled conditions with cover on top provided with shade cloth. The experimental designs and methods used for the application of different treatments by microplastics closely followed conventional and generally applicable methods[23].

Measuring soil physical parameters

The pH of soil usually ranges from 3 to 9. Soil with different pH as mentioned below may be categorized as: Strongly acidic (pH < 5.0), moderately to slightly acidic (5.0–6.5), Neutral (6.5 -7.5), moderately basic (7.5 -8.5), and strongly basic (> 8.5)[28]. The pH and electrical conductivity (EC) of soil was measured using the method proposed by Nemer et al.[29]. According to this method the pH and EC was determined in 1:1 (Soil: Water) suspension, for this suspension 10 g of soil was weighed and added in 100 mL water after that mixing of the contents was done by magnetic stirring for about 30 min. The solution was filtered using Whatman filter paper. The liquid filtrate was then used to measure the pH and EC of Soil[30]. The pH and EC meter was used to measure portable meters and three times readings were taken to calculate mean.

The organic matter content of soil was determined by the method of Nemer at el.[29]. In this experiment before soil being processed, the soil was allowed to pass through the sieve of 2 mm to make sure that soil was completely homogenized. After passing through sieve 10 g of air- dried soil was placed in porcelain crucible with the lid on and dried at 105 °C for 30 min. After 30 min the crucible was weighted (w_1). Next, the crucible was placed in muffle furnace for 24 h at 500 °C. After 24 h the crucible was removed from furnace and weighted (w_2). The organic matter content of soil was measured using the formula mentioned below:

$$\text{Organic Matter} = \text{OM}\,(\%) = \frac{\text{W1} - \text{W2}\ \times 100}{\text{W1}}$$

Here W_1 = weight of crucible after 105 °C, W_2 = weight of crucible after 500°°C.

Plants morphological measurements

Plants are typically divided into two basic parts: the root and the shoot. The shoot length of plant was measured from the base (surface) of plant to its elevation in cm using measuring tape. After measuring shoot length, the shoot of each plant was carefully removed from soil surface using sharp blade. The shoot was immediately weighed on electronic balance to measure the fresh weight. The measurement of shoot dry weight was carried out by placing the shoots in oven for drying at 60 °C for 48 h till no moisture left. After the shoots dried, it was removed from the oven and weighted on electronic balance.

Roots were removed from the soil using shovel; the root length was measured from the start of root to its longest length in cm using measuring scale. Roots were thoroughly washed with water to remove any particle and then dried up using a tissue paper. After roots were air dried, they were immediately weighed on electronic balance to measure roots fresh weight. The measurement of root dry weight was done by placing the roots in the oven at 60 °C for drying for 48 h in order to remove moisture. After the roots dried, they were removed from the oven and weighed on electronic balance.

Measuring oxidative stress status

Lipid peroxidation

Lipid peroxidation (LPO) was measured using the method proposed by[31,32] to determine the amount of malondialdehyde (MDA), an end-product of decomposing lipid and unstable peroxides. Plant leaves were

thoroughly washed with distilled water and left for few minutes to dry naturally. Leaves upto 2 g were weighed and homogenized in pestle and mortar using 10 mL of cold acetone and then filtered through Whatman filter paper. The filtrate was brought up to 15 mL by adding distilled water. An aqueous solution of Trichloroacetic acid (TCA) (20%) was made by adding 20 g of TCA into 80 mL distilled water and aqueous solution of Tertiary Butyl Alcohol (TBA) (0.67%) was made by adding 0.67 g of TBA into 99.33 ml of water. 2 mL of TCA (20%) and 1 mL of TBA (0.67%) was added in 2 mL of plant extract. This mixture was boiled for 15 min in water bath and later cooled under tap water. The mixture after boiling was centrifuged at 15,000 rpm for 10 min. The supernatant was added into measuring cylinder and the volume of supernatant was brought up to 10 mL by adding distilled water. The absorbance of supernatant was recorded at 532 nm and 600 nm. 2 mL of distilled water was used instead of plant extract and treated with the same procedure to be used as blank. The given formula was used to measure the MDA equivalent:

$$\text{MDA equivalent} = \frac{[(A532 - A600)] \times 10^6}{157000}$$

Due to the unavailability of specific reagents and technical limitations, enzymatic assays for antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) could not be performed in the current study. To provide methodological context and support future research a relevant study by Zhang et al.[21], is cited. Study was conducted by[21] to measure the antioxidant enzyme activities in roots of rice seeds. Superoxide dismutase (SOD) activity was measured using the NBT (nitroblue tetrazolium) reduction method[33–35], CAT activity using hydrogen peroxide decomposition[35,36], and POD activity via the guaiacol oxidation method[37].

Chlorophyll and carotenoid content

The measurement of chlorophyll and carotenoid content was carried out by using the method of[38]. Measurements were expressed as total Chlorophyll per m^2 of leaf material (mg m^{-2})[39].

The samples were transported to the laboratory in plastic bags. The leaves were thoroughly washed with distilled water to remove any impurity and left for air drying for 15 min. 2 g of fresh leaves were taken and homogenized in pestle and mortar with 50 mL of 80% acetone (80 mL acetone with 20 mL water). The mixture was then centrifuged at 5000 rpm for 5 min. The volume of supernatant was adjusted up to 100 mL by adding acetone. The absorbance was recorded at 480 nm, 510 nm, 645 nm and 663 nm. The absorbance was taken against the solvent (80% acetone) as blank. The chlorophyll and carotenoid content were measured using the formula as given below:

Chlorophyll *a*: $[12.7(A_{663}) - 2.69(A_{645})] \times V/1000^* W$
Chlorophyll *b*: $[22.9(A_{645}) - 4.68(A_{663})] \times V/1000^* W$
Total Chlorophyll (a + b): $[20.21(A_{645}) + 8.02(A_{663})] \times V/1000^* W$
Carotenoids: $[7.6(A_{480}) - 1.49(A_{510})] \times V/1000^* W$

Statistical analysis

Statistical analysis was performed by using the GraphPad Prism version 8.0. Data was expressed as Mean ± and standard deviation (SD) based on selected replicates. Differences among different microplastic groups were analyzed using one way analysis of variance (ANOVA) where significant difference was revealed at $p < 0.05$. Tukey's multiple comparisons post hoc test was used to determine pairwise group differences.

Results and discussion

Soil physical parameters

The pH of soil before treatment with three different sources of plastics was 7.8. Soils with three different microplastic treatments were measured after 3 and half months. No significant difference in soil pH was observed in all three experimental set ups except soil treated with 1% HDPE which had shown slight decline in pH (6.9). Similarly, no significant difference in EC (200 µ S/cm before treatment) was observed with only slight difference of 109 µ S/cm in soil treated with 1% HDPE. The organic matter content in soil before treatment was 4.29% and was observed to decline in control 3.05% and soils treated with 1% PET (2.90%), HDPE (1.14%) & PES (1.22%) (Table 1). The reduction in organic matter in the control soil (from 4.29% to 3.05%) was noted during the experimental period. The decrease may be attributed to natural decomposition processes during the incubation period since all soil setups including controls underwent the same pre-incubation conditions. A study has reported that plastic films (2, 5, and 10 mm size fragments, added at 0.5% and 1.0%) have been shown to create channels for water movement, leading to increased water evaporation[40]. This could lead to soil drying, with potentially negative consequences for plant performance.

		Soil samples				
			After treatment			
	Soil analysis	Before treatment	Control	PET	HDPE	PES
1	pH	7.78	7.90	7.78	6.89	7.81
2	Electrical Conductivity (µ S/cm)	200	200	203	109	200
3	Organic Matter Content (%)	4.29	3.05	2.90	1.138	1.216

Table 1. Table indicates the parameter of soil before treatment and after treatment with microplastics.

Plants morphological measurements

Shoot length of spring onion and okra

The results showed the reduction in shoot length of Spring onion plants grown with 1% PES as compared to control. The average length of plant grown in 1% PES (57 cm ± 1) was lower than other treatments when compared with control (61.33 cm ± 1.52) (Fig. 2). Statistical analysis showed significant ($p = 0.0167$) reduction in shoot length was noticed. Minor changes were observed in shoot length of plants grown in 1% HDPE and 1% PET.

The shoot length of Okra did not show huge difference in comparison with control (90.93 cm ± 0.89). They seemed more resistant to microplastics. Little changes were observed in shoot length of Okra grown in soil with 1% PES (87.22 cm ± 1.65) shown in (Fig. 2). There was no significant difference between control group and different plastics. However, HDPE and PES showed significant (0.045) difference. Lozano and Rillig[41] highlighted that microfibers can influence plant community composition and species dominance, suggesting that plant species exhibit distinct physiological responses when exposed to the same type of microplastics. Other research has documented a range of impacts, such as reduced biomass in shoots and roots, lower seed germination rates, and evidence of both genotoxicity and oxidative stress[22,23,42,43]. Interestingly, the nature and extent of these effects vary depending on the plant species and the type of plastic involved, with some studies even reporting enhanced shoot and root growth under certain conditions[44]. *Cucurbita pepo L.* was used in a most recent study as a model crop, in searching for the toxicological effects of four microplastics: polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) all frequently occurring in polluted soils. All four plastic types negatively influenced plant development, hence having a significant effect on the shoot growth. Out of all tested materials, PVC was the most toxic, while the least toxic was PE[45] this finding also corresponds to that observed in other crop species[24,46].

Root length of spring onion and okra

The root length of Spring onion plant grown in soil treated with 1% PES was reduced having the average of (4.43 cm ± 0.37) indicating significant ($p = 0.035$) reduction in comparison with the control (5.46 cm ± 0.54) while other treatments showed slight effect in root length in comparison with control (Fig. 3). There was a noteworthy reduction in root length of Okra under the 1% PES as compared to control (15.89 cm ± 0.09) while other root lengths showed no effect. There was significant ($p < 0.0001$) reduction in root length. The average length of plant grown in 1% PES (10.48 cm ± 1.53) was shown in (Fig. 3).

As discovered in the study by Urbina et al.[47], exposure of maize to an extremely low concentration (0.0125 mg/L) of polyethylene microplastics did not significantly affect biomass, stem-to-root ratio, or height growth rate. In contrast, for *Triticum aestivum* (wheat) exposed to plastic films and *Lolium perenne* (grass) exposed to microplastic fibers, the results were opposite and the biomass dropped[8,22]. Fiber exposure resulted in increased biomass in *Allium fistulosum* (a crop species), indicating a species-specific response[42]. Also, plants have shown differences in the expression of root morphological responses as seen in differences in root length between forbs such as *Plantago lanceolata* and *Allium fistulosum* upon microplastic exposure[42,48]. These responses in plants differ among plant species indicating that microplastics in soil may be exerting different influences on different plants, thereby affecting overall plant productivity and community composition[44]. Thus, some species may be able to exploit the altered soil condition caused by microplastics better than others, granting them a competitive edge in plant communities.

Shoot fresh weight of spring onion and okra

The reduction in the Spring onion fresh weight of shoot was observed in comparison with control. The plants grown with 1% PES (15.73 g ± 0.28) had lowest average weight as compared to average of control (18.22 g ± 0.63) (Fig. 4). The other treatment showed a slight change in shoot fresh weight when compared with control. Statistical analysis performed also supported significant (p = 0.003) reduction in fresh weight of spring onion.

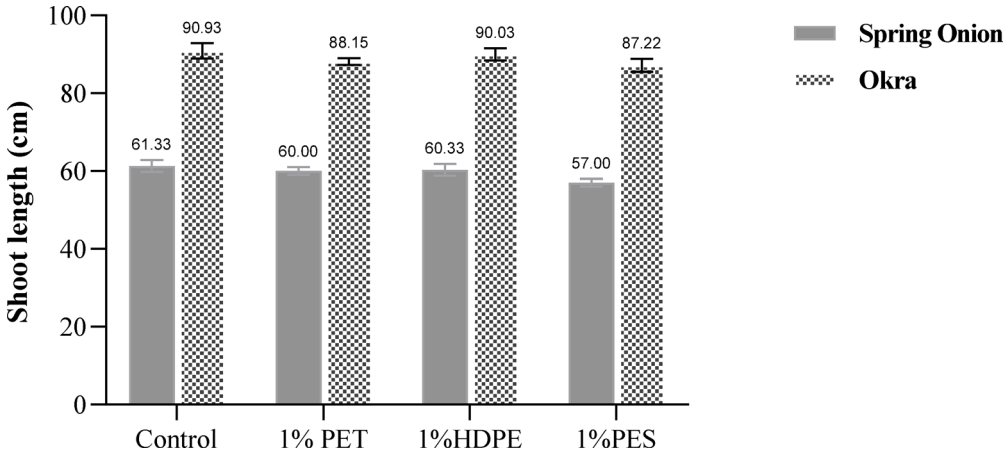

Fig. 2. Mean shoot length (cm) of plants (Spring onion, Okra) exposed with different microplastics.

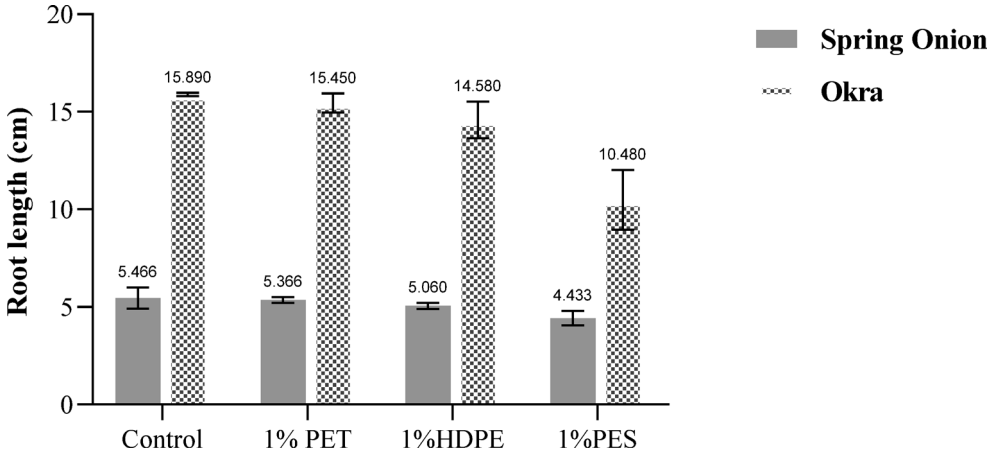

Fig. 3. Mean root length (cm) of plants (Spring onion, Okra) exposed with different microplastics.

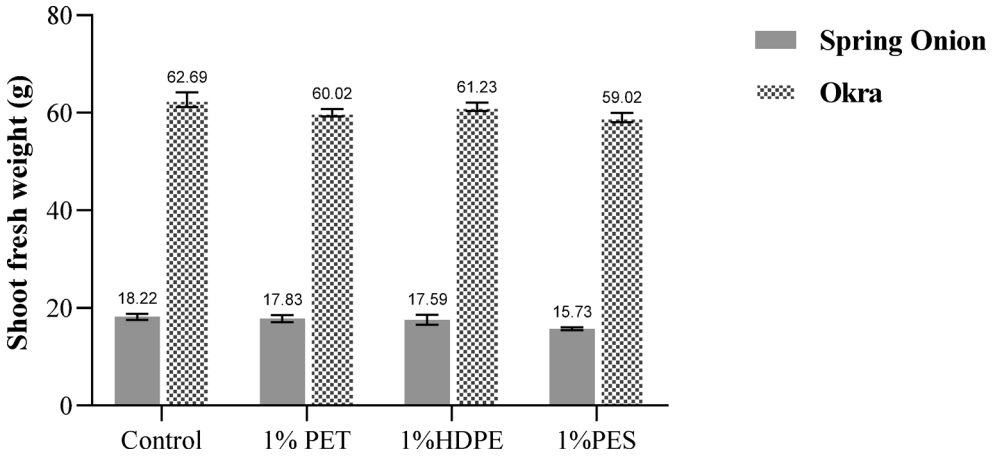

Fig. 4. Mean shoot fresh weight (g) of plants (Spring Onion, Okra) exposed with different microplastics.

The shoot fresh weight of all the treated Okra plants were almost same with the average weight of control (62.69 g ± 1.5). The plants grown in the soil with the 1% PES showed a little reduction in fresh weight of shoot with microplastic 1% PES (59.02 g ± 0.92) (Fig. 4). Statistical analysis showed p = 0.03 showing reduction in fresh weight of okra shoot.

Shoot dry weight of spring onion and okra

The dry weight of Spring onion shoot was decreased clearly in the presence of 1% PES. The lowest weight of shoot was observed in 1% PES (5.89 g ± 0.89) as compared to average weight of control (8.21 g ± 0.28) other treatments showed no change compared to the control (Fig. 5). Exposure of 1%PES has significantly (p = 0.005) affected the spring onion dry weight.

The results of Okra plant shoot dry weight showed a slight difference in weight of every treatment. The plant treated with 1% PES (22.7 g ± 1.0) showed an average reduction in weight as compared to control (25.24 g ± 1.8) in (Fig. 5). Analysis of variance also showed significant decrease (p = 0.041) in shoot dry weight.

Root fresh weight of spring onion and okra

The fresh weight of Spring onion root (bulb and roots) was decreased in plants treated with 1% of PES (17.62 g ± 1.19). The other treatments plant of HDPE and PET average were near average of control (21.1 g ± 1.57) (Fig. 6). Post hoc test showed significant (p 0.033) decline in root fresh weight. The fresh weight of Okra root was decreased in plants treated with 1% of PES (13.78 g ± 0.76). Fresh weight of Okra showed borderline significance ($p \sim 0.06$) when compared with control group. The other treatments plant of HDPE and PET showed average close to average of control (17.45 g ± 1.10) (Fig. 6).

Root dry weight of spring onion and okra

The average dry weight of Spring onion root (bulb and roots) was decreased in plants treated with 1% PES (8.53 g ± 0.47). But the dry biomass of other treated plants was remained close to the average of control

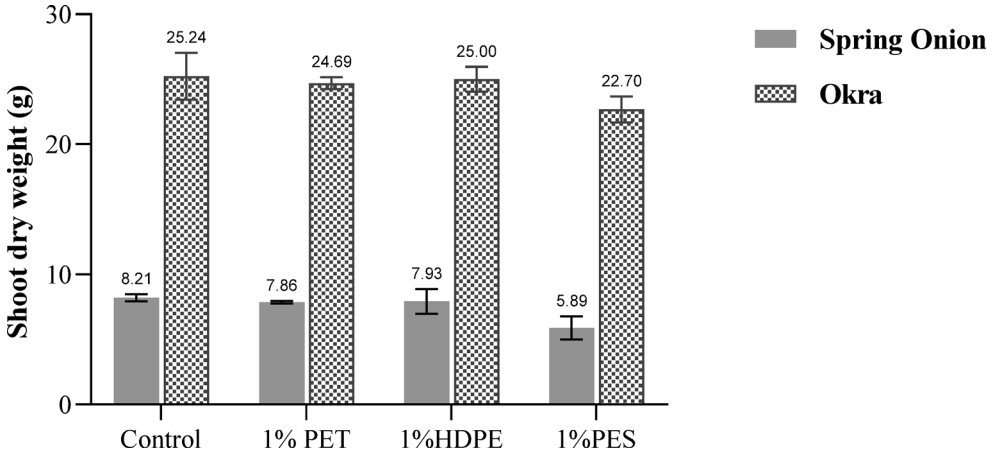

Fig. 5. Mean shoot dry weight (g) of plants (Spring onion, Okra) exposed with different microplastics.

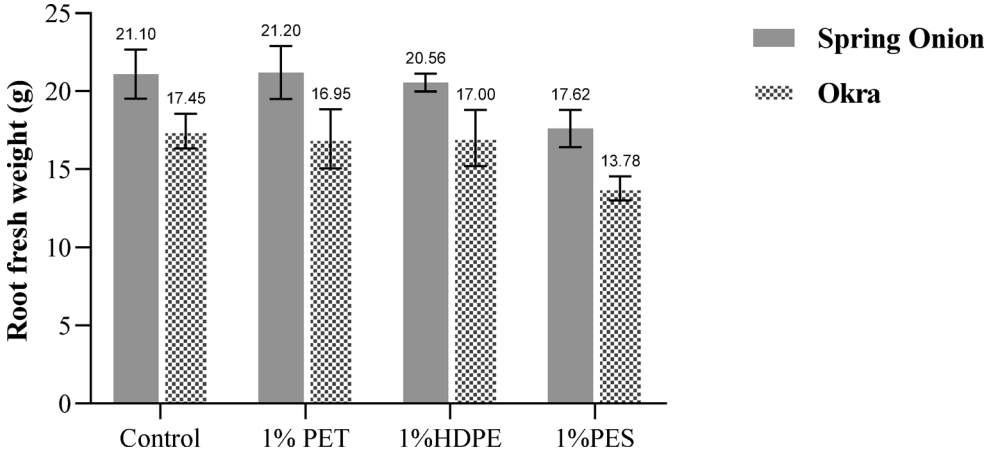

Fig. 6. Mean root fresh weight (g) of plants (Spring onion, Okra) exposed with different microplastics.

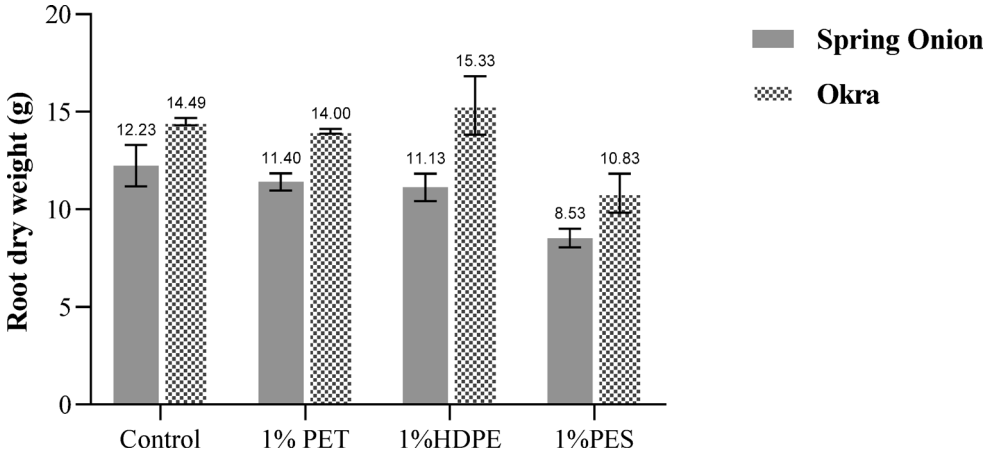

Fig. 7. Mean root dry weight (g) of plants (Spring onion, Okra) exposed with different microplastics.

(12.23 g ± 1.06) (Fig. 7). 1%PES interaction with spring onion showed significant reduction in root dry weight of spring onion.

There was no significant difference ($p > 0.05$) between dry weights of all treatment as they compared with plants. The Okra plant with lowest root dry weight was treated with 1% PES (10.83 g ± 1) compared to the

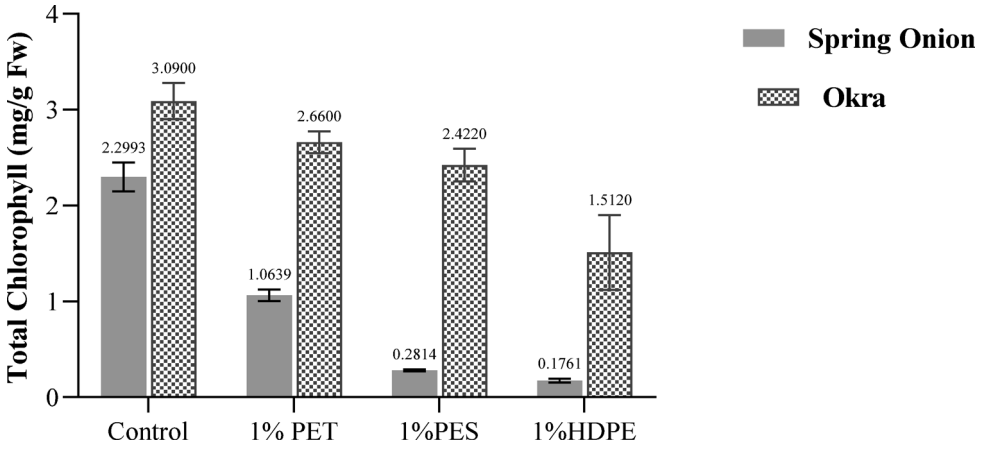

Fig. 8. Mean value of Total Chlorophyll (mg/g Fw) of plants (Spring Onion, Okra) exposed with different microplastics.

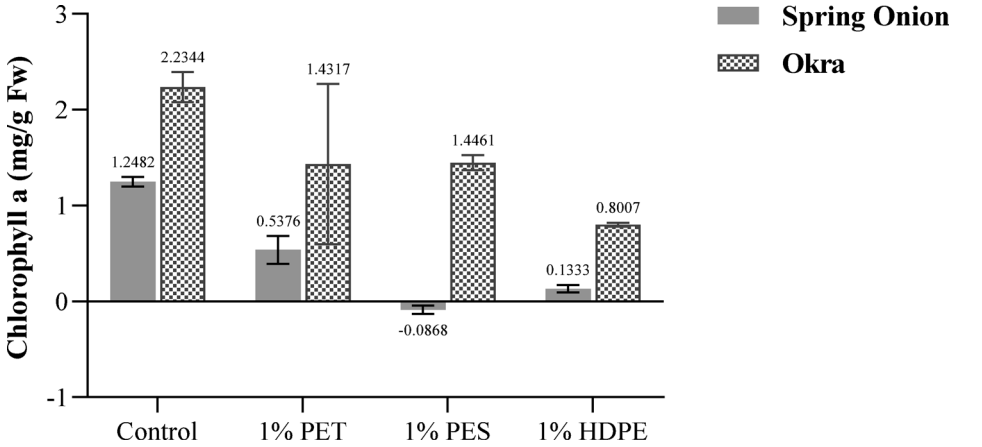

Fig. 9. Mean value of chlorophyll *a* (mg/g Fw) of plants (Spring Onion, Okra) exposed with different microplastics.

average of control group (14.49 g ± 0.19) whereas treatment with 1% HDPE the dry weight was higher than control and no significant difference was observed for treatment with 1% PET (Fig. 7).

Chlorophyll content of spring onion and okra

The total chlorophyll content in Spring onion plant was also affected by presence of microplastics in all treatments as compared to control (without microplastic). All the treatment showed different values of chlorophyll content; the lowest chlorophyll content was seemed to be in plant treated with 1% HDPE (0.1761 mg/g Fw ± 0.02) as compared to chlorophyll content of control (2.299 mg/g Fw ± 0.15) as shown in (Fig. 8).

The concentration of total chlorophyll in Okra plants at all treatments had shown different results. The plant with lowest total chlorophyll content was treated with 1% of HDPE (1.512 mg/g Fw ± 0.39). Statistical analysis also showed significant reduction i.e. $p < 0.001$ when exposed to 1%HDPE. Whereas, the control (3.090 mg/g Fw ± 0.18) had highest value of total chlorophyll content (Fig. 8).

The concentration of chlorophyll *a* in Spring onion plants was higher in the control group (1.2482 mg/g FW) compared to those exposed to various microplastic treatments. Notably, plants treated with 1% PES exhibited a negative chlorophyll *a* value (−0.0868 mg/g FW), indicating a potential degradation or interference in pigment measurement. In comparison, chlorophyll concentrations were lower in plants treated with 1% HDPE (0.1333 mg/g FW) and moderately higher in those treated with 1% PET (0.5376 mg/g FW) (Fig. 9). In case of all microplastic groups there was significant ($p = 0.000$) reduction in chlorophyll *a* in spring onion plant.

In Okra plants, chlorophyll *a* concentration was highest in the control group (2.2344 mg/g FW) compared to plants exposed to various microplastic treatments. The lowest chlorophyll *a* level was observed in the 1% HDPE treatment group (0.8007 mg/g FW). There was significant ($p = 0.027$) reduction in chlorophyll *a* of Okra plant when exposed to 1% HDPE. However, no statistically significant differences were found between the chlorophyll *a* concentration in plants treated with 1% PES and 1% PET (Fig. 9). The results clearly indicated that chlorophyll

a concentration in both spring onion and okra plants (Fig. 9) were consistent with the patterns observed in total chlorophyll content across all treatment groups (Fig. 8).

The concentration of chlorophyll *b* in Spring onion plants was significantly higher in the control group (1.0970 mg/g FW) compared to those subjected to various microplastic treatments. The lowest chlorophyll *b* content was recorded in plants treated with 1% HDPE (0.1727 mg/g FW). A similar trend was observed in Okra, where the control group exhibited the highest chlorophyll *b* concentration (1.1484 mg/g FW), while the 1% HDPE treatment resulted in the lowest value (0.5272 mg/g FW) (Fig. 10). There was significant decrease i.e. $p = 0.012$ in chlorophyll *b* when exposed to 1% HDPE. These chlorophyll *b* results were consistent with the patterns observed in total chlorophyll content in both plant species.

MP stress reduce photosynthesis in plants[49] as reported by several studies. In a study conducted by Lian et al.[50] it was observed that carotenoids and chlorophyll *a* and *b* were reduced by 12.5%, 9.1%, and 8.7% respectively, with the application of Polystyrene microplastics (PS MPs) in lettuce (*Lactuca sativa L.)* indicating that PS MP stress reduced shoot height, shoot dry weight, and leaf area. Similarly in some other plant species including, cabbage (*Brassica oleracea L.*), cucumber (*Cucumis sativus L.*), tomato, pakchoi (*Brassica rapa L.*), and lettuce there was negative correlation between application of Polyacrylonitrile microplastics (PAN MPs) and chlorophyll *a* and *b* contents in leaves, leading to reduce growth[51]. In another study Liu et al.[52] reported that there was significant reduction in photosynthesis due to reduce contents of carotenoids, chlorophyll *a* and *b* in a highly MP dose-dependent manner. The application of PVC and PE MPs in pumpkins in a dose-dependent manner caused reduction of photosynthesis rate and chlorophyll contents[45]. PE MP-induced reduction in photosynthesis was analyzed by[6,7] in lettuce associated with a reduction in chlorophyll contents, Rubisco activity and leaf gas exchange. It is also reported that MP stress in plants reduce N uptake and cause the disruption of chlorophyll contents, low carbon fixation rate and decline in iron (Fe) contents in the shoot and leaves when exposed with PVC and PP MPs[45]. Plants exposed to different MPs resulted in significantly high production of hydrogen peroxide (H_2O_2), a dominant (Reactive Oxygen Species) ROS leading to reduce photosynthesis[53]. MP stress induced by PS caused higher ROS production and oxidative damage to chloroplast structure and thylakoid membrane inhibiting photosynthesis[50].

Carotenoid content of spring onion and okra

The concentration of total carotenoids in Spring onion was found decreased when exposed with 1% HDPE and 1% PES. The lowest concentration of carotenoid content was detected in plants grown in 1% HDPE (0.105 mg/g Fw ± 0.20) whereas the concentration of carotenoids in control (1.085 mg/g Fw ± 0.06) plants was highest as shown in (Fig. 11). Variance test showed significant reduction i.e. $p = 0.0001$ and $p = 0.0008$ when spring onion came in contact with 1% HDPE and 1% PES respectively.

The highest concentration of total carotenoid content for Okra plant was found in control (2.457 mg/g Fw ± 0.08), whereas the lowest concentration of carotenoid content was found in plants grown in 1% HDPE (0.623 mg/g Fw) (Fig. 11). All groups of plastic significantly ($p < 0.001$) reduced the carotenoid content in Okra plants.

Lipid peroxidation of spring onion and okra

The MDA content under the control conditions (without any microplastic) was (1.26% ± 0.65) for Spring onion and there was a significant increase in MDA content of every other treatment (with microplastics) that indicates the oxidative stress in plants. The highest MDA content (2.89% ± 1.01) was found in plant treated with 1% of HDPE and lowest MDA content (1.68% ± 0.36) was found in plant treated with 1% PET in comparison with control (Fig. 12). For Okra plant all the treatment had different values of MDA content in comparison with control. The control group had MDA content (0.36% ± 0.21). The highest MDA content (3.04% ± 0.33) was present in plants grown in 1% HDPE whereas the lowest MDA content (0.98% ± 1) was found in 1% PET as

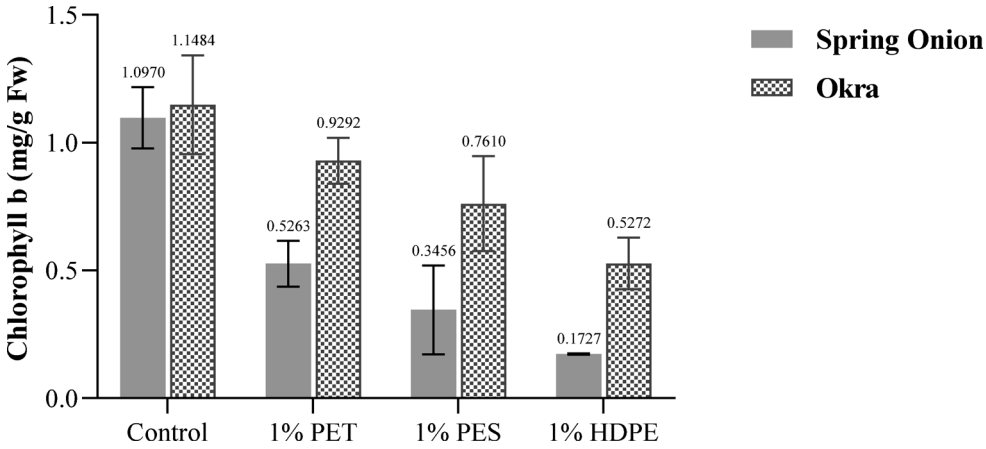

Fig. 10. Mean value of Chlorophyll *b* (mg/g Fw) of plants (Spring Onion, Okra) exposed with different microplastics.

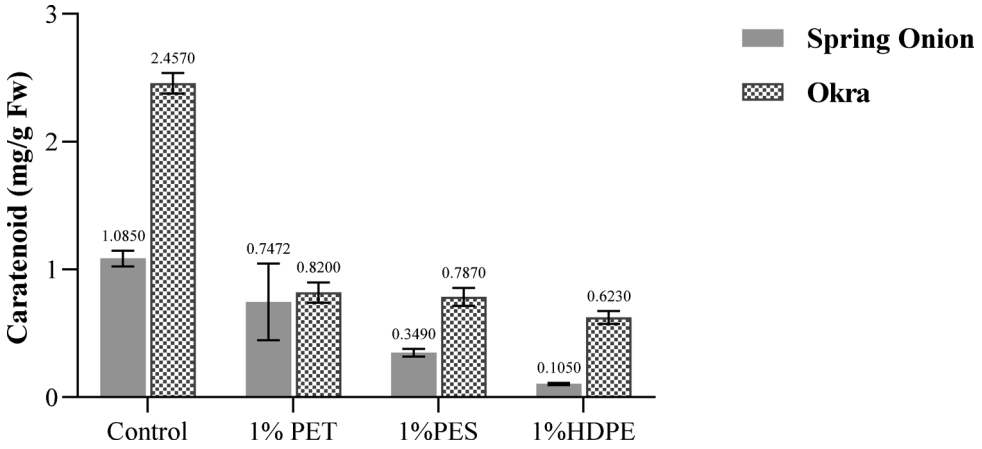

Fig. 11. Mean value of caratenoid (mg/g Fw) of plants (Spring onion, Okra) exposed with different microplastics.

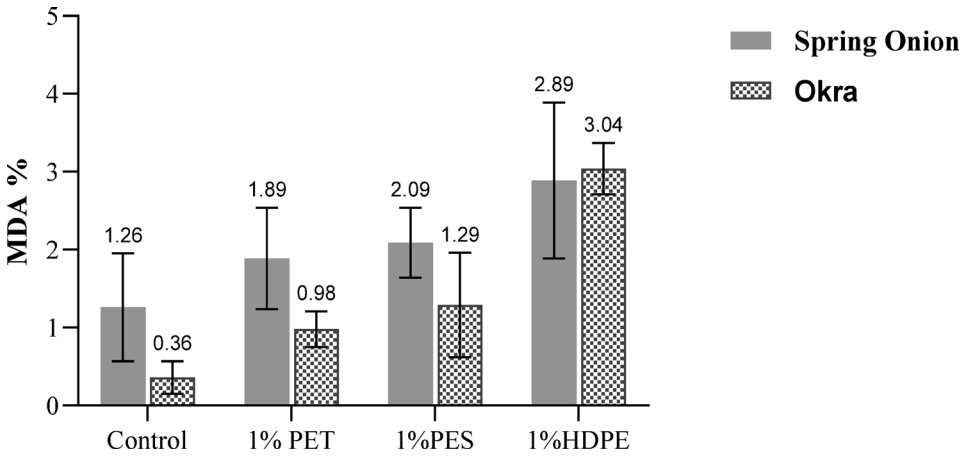

Fig. 12. Mean % value of MDA in plants (Spring onion, Okra) exposed with different microplastics.

shown in (Fig. 12). MDA was significantly increased ($p < 0.001$) in all case especially in case of HDPE that showed oxidative stress.

The physiological and biochemical responses of plants to stress induced by microplastic exposure are still not fully elucidated. Typically, phytotoxic effects are associated with the accumulation of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, and hydroxyl radicals[26]. An excessive buildup of ROS within plant cells can disrupt growth, impair photosynthesis, and interfere with various metabolic processes. Moreover, several studies have reported that abiotic stressors like heavy metals can hinder the synthesis of chlorophyll and carotenoids by inhibiting the activity of enzymes essential for chlorophyll biosynthesis[54–56]. In response to oxidative stress, plants activate an antioxidant defense system involving molecules such as glutathione (GSH) and ascorbic acid (AA) to detoxify ROS and protect cellular functions[26]. Several studies have reported that different antioxidant enzymes activity vary based on type of MP.[19] analyzed that under 1% Polyethylene microplastics (PE MP) stress SOD activity was relatively higher compared to 1% Biodegradable Plastic (BP MPs) stress whereas more CAT activity was promoted by BP MPs than PE MPs.[57] reported that in tomatoes PE and PS MP stress had caused strong reduction in antioxidants compared to Polypropylene microplastics (PP MP) stress showing that MPs with different polymer composition may have different effects on activation of antioxidants and plant growth.[58] found that many other factors such as particle size, polymer composition, surface functional group of different MPs may differently regulate the antioxidant activity by plants. The study also indicated that PP and Polyvinyl chloride (PVC MPs) caused neutral effects on antioxidants activity whereas PE MPs caused 31% positive effects, PS MPs caused 20% positive effects and Polycarbonate microplastics (PC MPs) caused 100% positive effects on the activities of different antioxidants. PVC and PP MPs are derived from plastic covers and their phototoxic effects may come from their leachates or toxic metals adsorbed on their surface leading to plant growth[59]. Studies have reported that plastic particles accumulate in pores of garden cress (*L. sativum*) seed coat and caused delayed water uptake and hence seed germination as observed in soybean (Glycine max) seeds[60]. In plants under normal growth conditions ROS content is stable while under stress plants accumulate high amounts of free radicals, O^{2-} and H_2O_2, key ROSs radicals[61]. [21] reported that with increase in

PS-MP concentration there was more ROS accumulation in root tip leading to altered root tip morphology in rice plants. Plants counter act the damage caused by ROS by regulating the activity of antioxidant enzymes[62]. The study was conducted to investigate the uptake of polystyrene MPs (PS-MPs, with a size of 200 nm) on rice plants. When compared with control the activity of SOD and CAT had shown low values at different PS-MPs concentrations with CAT activity significantly reduced at high concentrations of PS-MPs[21]. SOD is a metal containing enzyme and can dismutates harmful free oxygen radicals in plants by converting them into H_2O_2 which is finally converted into molecular oxygen and water by CAT to overcome oxidative stress in plants[63].

Conclusion

The present study evaluated the effects of microplastics (MPs) derived from three different plastic sources polyethylene terephthalate (PET), high-density polyethylene (HDPE), and polyester (PES) on two plant species: Spring onion (*Allium fistulosum*) and Okra (*Abelmoschus esculentus*). Results demonstrated that PES had the most pronounced negative effects on root and shoot length as well as biomass (fresh and dry weight), while HDPE significantly reduced chlorophyll and carotenoid content, accompanied by increased malondialdehyde (MDA) levels, indicating enhanced oxidative stress. These findings suggested that MP type plays a critical role in determining phytotoxicity, with HDPE and PES causing more severe adverse effects than PET. Furthermore, the data indicated that different plant species exhibit varying responses to MP stress. In the present study, Okra was less affected than Spring onion across several analyzed parameters. The study highlighted the ecological risks posed by certain types of MPs, particularly those originating from plastic bags and synthetic fibers. The current research focused on plant physiological responses emphasizing the importance of exploring potential adaptive mechanisms in future studies, including the role of microbial communities in degrading MPs and mitigating their impact. Further research should also examine the chemical composition of MP leachates and their interaction with plant systems across a wider range of species and environmental conditions.

Data availability

Data will be made available upon request. Readers may contact Dr. Sessay (muadic2016@gmail.com) or Dr. Zaira Ahmad (zaira_ahmad@hotmail.com) for access.

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Author contributions
All authors contributed to the study conception and design. Zaira Ahmad worked on researched problem, targeting and collection of data; Hina Chaudhry conceptualized and planned the study; Saima Atif and Dure Najaf did analysis; M.S Al-Buriahi and Fakhra Aslam worked on interpretation of results; Sumera Gull Bhatti and Norah Salem Alsaiari drafted the manuscript. All authors reviewed the results and approved draft.

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Declarations

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The authors declare no competing interests.

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