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RESEARCH ARTICLE

Micro-plastics and Organic Amendments: Combined Effect on Morpho-physiological Traits and Antioxidant Activity of Lettuce

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ABSTRACT

Soil health refers to its ability to function as a vital living system within ecosystems, sustaining plant and animal productivity while maintaining water and air quality. However, soil health is increasingly threatened by micro-plastic pollution, a human-made contaminant that accumulates in terrestrial ecosystems and affects soil quality. Micro-plastics, particularly polystyrene (PS), polyethylene (PE), and polyester (PET), may disrupt plant growth and counteract the beneficial effects of organic matter on soil aggregate stability. To investigate these effects, we conducted a soil incubation experiment on lettuce under a Randomized Complete Block Design (RCBD) with ten treatments involving micro-plastic fibers, either alone or in combination with organic matter sources such as compost, leaf litter, and wheat straw. Each treatment was replicated three times. The study analyzed changes in soil physicochemical properties, lettuce growth parameters (fresh and dry weight, root length), and soil enzyme activities including proline and catalase. Results revealed that micro-plastics alone significantly reduced soil aggregate stability, enzyme activities, and plant performance, particularly root dry and fresh weight. However, when micro-plastics were combined with organic matter, the adverse effects were minimized. Treatments with organic matter alone consistently improved lettuce growth, enzyme activity, and overall soil health compared to control. These findings highlight that while organic matter additions can partially mitigate the harmful effects of micro-plastics, high concentrations of PS, PE, and PET remain detrimental to soil quality, soil micro-biota, and plant growth. Understanding these interactions is critical to evaluating the long-term risks of micro-plastics and even biodegradable plastics in agricultural ecosystems.

Key words: Micro-plastics, PET, PS and PE, Organic Matter, Compost, Wheat straw and leaf litter.

INTRODUCTION

Soil health maintenance is key to agricultural sustainability that provides the most basic and diverse services to ecosystems. Reports indicate significant global impacts on soils, with approximately 25% heavily affected and 44% moderately affected primarily by pesticides, persistent organic pollutants, metals, and metalloids (Rai *et al.,* 2023). Plastic are polymers made up of hydrocarbons most of which are derived from fossil duels. The plastic polymers are broadly applied in food packaging, textiles, automobiles, medical devices and other consumer products. Plastics are divided into two major groups on the basis of their melting temperatures named as thermoplastics and thermosetting plastics. Thermoplastic making up the greatest portion of plastic waste include polypropylene (PP) (21%). low-density and linear low-density polyethylene (LDPE and LLDPE) (18%), polyvinyl chloride (PVC) (17%), and high-density polyethylene (HDPE) (15%). polystyrene (PS) and expandable PS (EPS) (8%), polyethylene terephthalate (PET) (7%. excluding PET fibers), and thermosetting plastic polyurethane (Rai *et al.,* 2023).

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Micro-plastics are small pieces of plastics which are less than five millimeters in length. Micro-plastics can also come from the various processes of fragmentation of large pieces of plastic debris in the environment or from the detachment of synthetic fibres from textiles during washing or use (Yang *et al.,* 2024).Polyethylene (PE), one of the plastics polymers, can be seen everywhere in the world as it possesses high-strength, flexibility and is resistant to chemical degradation (Ghatge *et al.,* 2020). Polystyrene is a tough plastic polymer that is commonly used for food containers, packaging materials, and hollow thermal insulators (Capricho *et al.,* 2022). Their inhabiting soils and water bodies can impact the composition of microorganisms, thus inhibiting the nitrogen and phosphorus supplies, which is yielded in fertility problems (Okeke *et al.,* 2022). Organic matter is a large factor in the state of the soil, influencing nutrient cycles, soil structure, and water retention. The presence of micro-plastics in soil can affect organic matter decomposition and microbial activity in several ways. The presence of micro-plastics is capable of altering the microbial structure and diversity within soil systems, and this may affect the processes and pathways of organic matter decomposition (Nishad *et al.,* 2020). Micro-plastics, in some studies, have shown the ability to provide microbial colonization surfaces, so microbial growth and activity may be affected (Niu *et al.,* 2020).The micro-plastics can stick to and absorb organic contaminants and fuels from the surrounding soil. This may disrupt nutrient cycling and lead to the reduced availability of nutrients to plants and microbes. This may have a feedback effect on earth fertility, which will stimulate or suppress the growth of vegetation (Dai *et al.,* 2021).The Lettuce plants have been demonstrated to be capable of taking up the micro-plastics from soils whereupon the micro-plastics accumulate in the plants such as leaves and roots. Micro-plastics can be found in plant tissues too and their presence can affect nutrient uptake, and water relations (which includes physiological processes) and can also have an effect on plant growth and productivity (Yu *et al.,* 2023).Generally, the rhizosphere, the soil zone affected by plant root exudates is the critical area for microbial activities and nutrient cycling. Micro-plastics in the rhizosphere can interfere with soil micro-biome and plant roots affecting changes in microbial community composition and function (Santini *et al.,* 2023). These interactions, in turn, can affect plant health, enrichment of the soil environment, and nutrient cycling. Organic matter addition to the soil that contributes to fertile soil and vigorous activity of soil microorganisms is a factor that will lead to enhanced growth and yield of plants. The findings of these studies have revealed that the enrichment of organic amendments can alleviate the damages to soil organic matter and plant yield caused by micro-plastics by bringing in the additional nutrients and activating the microbial decomposition processes (Aralappanavar *et al.,* 2024). This research seeks to fill the gaps in knowledge on the combined impact of multiple types of micro-plastics on soil rhizosphere processes by exploring the joint effects of multiple types of micro-plastics. Styrofoam, polyethene and polyester are kinds of micro-plastics that are very common in the environment today. Thunderstorms have their different types and their degradation rates which are different but the combination of them is possible in soil environments.

The synergetic impact of polystyrene, polyethene, and polyester micro-plastics on soil rhizosphere plant systems and microbial communities is significant (Zhang *et al.,* 2021). Micro-plastics in the rhizosphere could block root development, hinder nutritional uptake, and lower water adherence, all the way to the level trees grow and thrive. Tiny soil microbes such as bacteria, fungi, and archaea are indispensable parts of soil ecosystems, allowing nutrient cycling, organic matter decomposition, and removal of diseases. This study can collect scientific information on the impact of different micro-plastics on rhizospheric plant system in the soil and the soil microbial community, which can be used in policy-making and giving advises to halt micro-plastic emission, to develop and implement practices of sustainable waste management, and to rehabilitate degraded soil systems. Moreover, it may also provide useful information for these people to be used in environmental monitoring, conservation and land use planning.

MATERIALS AND METHODS

Study site

A pot experiment was carried out in the wire-net house of Department of Botany, The University of Punjab to analyze the effect of micro-plastics on Lettuce plant and soil micro-biota. Seeds of Lettuce (*Spinacia oleracea* L.) were purchased from Rachna Agri Business, Gujranwala. Seeds surface was cleaned and sterilized in order to proceed experiments. Seeds were soaked in dilution of 2% hydrogen peroxide (H_2O_2) for 10 to 15 minutes. Following sterilization, the seeds were air-dried for 2-3 hours at room temperature.

Micro-plastic Synthesis

Three kinds of polymers were selected to study the effect of micro-plastics. The three polymers are PE, PET and PS and the sources of these polymers were collected by manually cutting fabric's fibers, disposable plates and plastic cutlery spoons respectively 5mm in size. Primarily, rinsed the polymers with tap water to sterilize and after that air dried for ten minutes. Micro-plastic fibres at a concentration 0.3% w/w were mixed in 3 kg soil per pot.

Organic Matter

Dried and fallen of leaves were collected as a source of leaf litter was collected from plants that grown in our university. Wheat straw is an organic matter that is widely used in agriculture for soil improver and collected from Lettuce field. Compost caused improvement in soil and used as organic fertilizers for the plant's development and growth. Compost was prepared by mixing carbon rich materials, nitrogen rich materials, oxygen and moisture in form of animal manure, leaves, air and water respectively.

Soil and pots Preparation for plant growth

Lettuce plants are grown in the pots size of 23cm in length and 19 cm in width. A total of 3kg soil was filled in each pot. In order to stimulate the microbial activity 5.6g w/w of glucose was added with this soil for 14 days prior the plant growth. In the first category 3g of micro-plastic fibers (PE, PS and PET) was mixed in 3 kg of rhizospheric soil using a spatula, ensuring an even spread of fibers throughout the soil. This mixing process was thorough enough that no clumps of fibers were visible to the naked eye. Control was selected in each selected treatment without inoculation of organic matter and micro-plastics.

Experimental Design

To study the effect of micro-plastics on soil health and plant growth 10 different experimental treatments were designed (Liang et al., 2021).

Environmental Factors on Plant Growth

The growth of plant is affected by various environmental factors and season of germination. The seed were germinated in the dark at a room temperature of 24 C. Then seedlings were shifted in pots at environmental conditions: PPFD of 9000 micro mol (Photons) m-2s-1 at the top of canopy, photoperiod of 12 hours, temperature of 24 to 26 ℃, relative humidity of 55-66%. Plants were regularly watered and followed until the end of vegetative cycle.

Harvesting

Lettuce was harvested after 2 months of sowing. The height of plants was varying from 15 to 30 inches. Each treatment replicated as thrice. Plants were harvested from each replicates of all treatments after plant growth.

Physiological Parameters

Plant's growth is highly affected by various parameters including physiological parameters and many others. Plant fresh and dry weight, fresh and dry weight of roots, plant and root height. These parameters are affected by the presence of micro-plastics and absence of organic matters. That's why these following physiological parameters are analyzed.

Biochemical Parameters

Primary metabolic alterations induced by water deficit in plants, emphasizing their direct implications for plant development and productivity. Plants growth and yield could highly affect by decreasing level of biochemical parameters including chlorophyll, carotenoids pigments, proline, catalase and many others.

Photosynthetic Pigments

First of all, 30 test tubes were taken and wrapped with aluminum foil to avoid light. A leaf from each treatment was plucked and taken to the lab. Each treatment's leaf was rinsed with distilled water to avoid contamination. 0.5g leaf from each treatment was taken and cut into small pieces with the help of scissor. 10ml acetone as extraction solvent was used for each replicates of treatments. After that 0.5g leaf and 10ml acetone were taken into each test tube for each replicate. After that, photosynthetic pigments were recorded by using spectrophotometer.

Catalase (CAT) Determination

Catalases are essential antioxidant enzymes pivotal in enhancing plant oxidative stress tolerance through effective scavenging of elevated levels of stress-induced H2O2. For Catalase analysis, chemicals and apparatus H_2O_2, Na_2HPO_4, $NaH_2PO_4 .2H_2O$ and Scissors, pestle and mortar, measuring cylinder, beaker, test tubes, stand, electric balance, spectrophotometer, ice, tips, Eppendorf tubes, pH meter required respectively. The plant sample was cut and rinsed it with distilled water. Weighed about 0.5g sample and placed the sample in precooled mortar on ice. Added 2-3ml pre-cooled Phosphorus buffer solution. Homogenized the sample on ice and buffer solution was added up to 8ml. Centrifuged the sample at 8000-13000 rpm for 15 minutes at 4℃. Preserved the supernatant in another clean small tube at 4℃.

Statistical Analysis

Graphical and tabulated data was presented by using computer software MS EXCEL. Statistical analysis and ONE-WAY ANOVA were presented by using COSTAT software.

RESULTS

When Lettuce plants were grown under different types of micro-plastics (polystyrene, polyethylene, polyester), organic matter (leaf litter, compost, wheat straw) and combination of micro-plastics and organic matter (Polystyrene +polyethylene +polyester +compost, Polystyrene +polyethylene +polyester + leaf litter, Polystyrene +polyethylene +polyester + Wheat straw), following results were recorded.

Root Fresh and Dry Weight

Root fresh weight of Lettuce was measured by weigh balance and recorded the data. Results indicated overall reduction in root fresh weight of Lettuce in all the treatments of micro-plastics (treatment 2, treatment 3 and treatment 4) as compared to control as shown in fig 1. Treatment 4 showed maximum reduction in root fresh weight as compared to control.

Organic matter (treatment 1, treatment 5, and treatment 6) showed increase in plant dry weight as compared to control. Combinations of micro-plastic with organic matter (treatment 7, treatment 8 and treatment 9) showed better growth as compared to micro-plastic. Treatments has a significant (p≤0.001) difference in root fresh weight as shown in fig. 1.

Plant and root length

Full plant length was measured by the measuring tape and noted the results. Results indicated that micro-plastics treatments (treatment 2, treatment 3 and treatment 4) showed overall reduction as compared to control as shown in fig 1. Maximum reduction in plant length was showed under treatment 2. Root dry weight increase under Organic matter (treatment 1, treatment 5, and treatment 6). Combinations of micro-plastic with organic matter (treatment 7, treatment 8 and treatment 9) showed better growth as compared to micro-plastic treatments. Treatments have a significant (p≤0.05) difference in plant length as shown in fig 2.

Photosynthetic Pigments

Chlorophyll *a*

Photosynthetic pigments were measure by spectrophotometer and results were observed. Overall reduction in chlorophyll *a* was found in all the treatments of micro-plastics as compared to control as shown in fig 6. Chlorophyll *a* increase under Organic matter (treatment 1, treatment 5, and treatment 6). Maximum reduction was showed under treatment 3. Treatments has a non-significant difference in chlorophyll *a* content in Lettuce as shown in fig 6.

Chlorophyll *a/b*

Chlorophyll *a/b* ratio was measured and results were noted. The results showed that under treatment 1, treatment 5, treatment 6 chlorophyll *a/b* ratio increase as compared to control. However, Maximum reduction was showed under treatment 4 as shown in fig 3. Combinations of both micro-plastics and organic matter showed increase in chlorophyll *a/b* as compared to micro-plastics treatment. Treatments has significant (p≤0.01) differences in chlorophyll *a/b* in Lettuce as shown in fig 3.

Carotenoids

Results showed that overall reduction in carotenoids was observed as compared to control as shown in fig. Results showed reduction in total chlorophyll of Lettuce under micro-plastics treatments (treatment 2, treatment 3 and treatment 4) as compared to control as shown in fig. However, maximum reduction

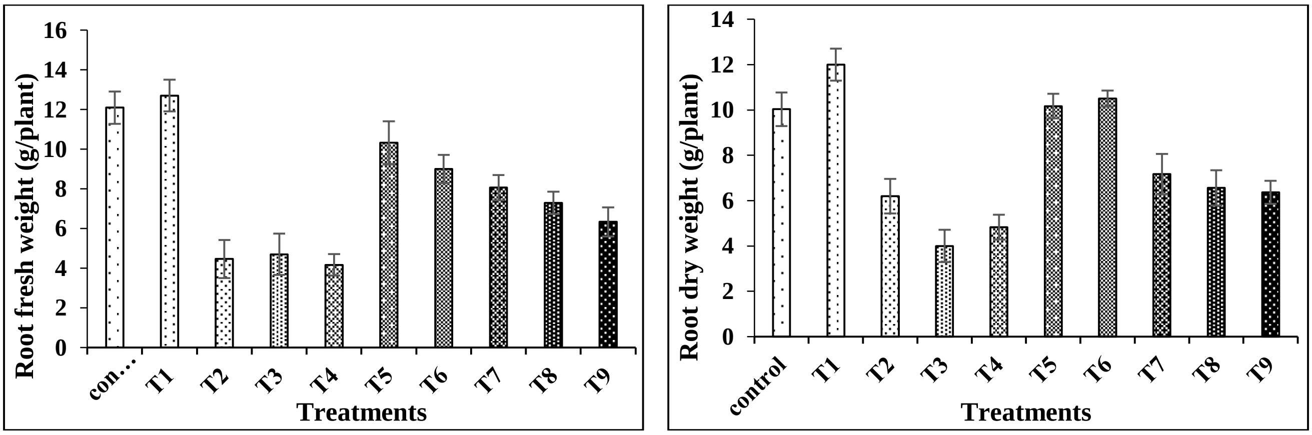

Fig. 1: Fresh and Dry weight of roots harvested from lettuce in various treatments.

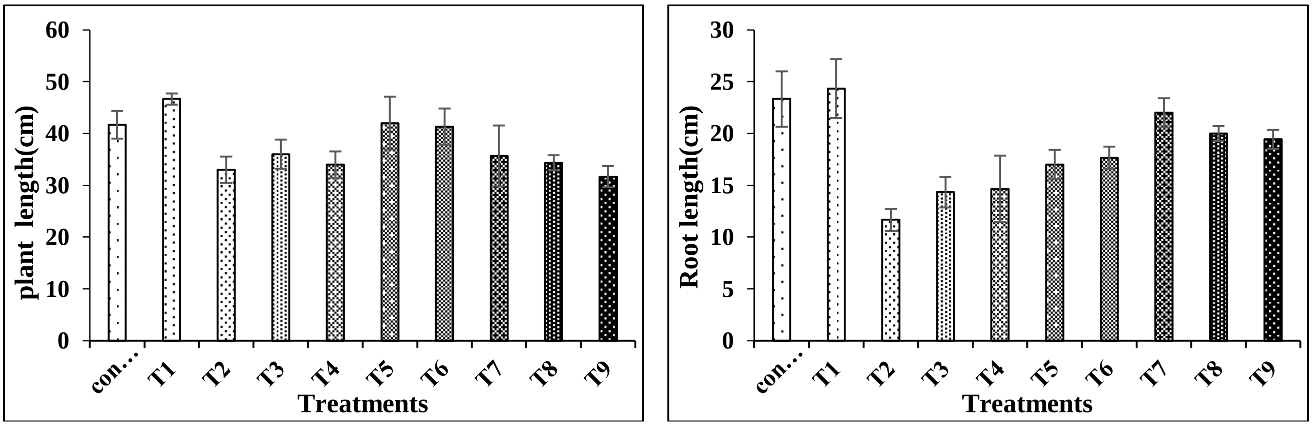

Fig. 2: Plant and Root length of lettuce in different treatments.

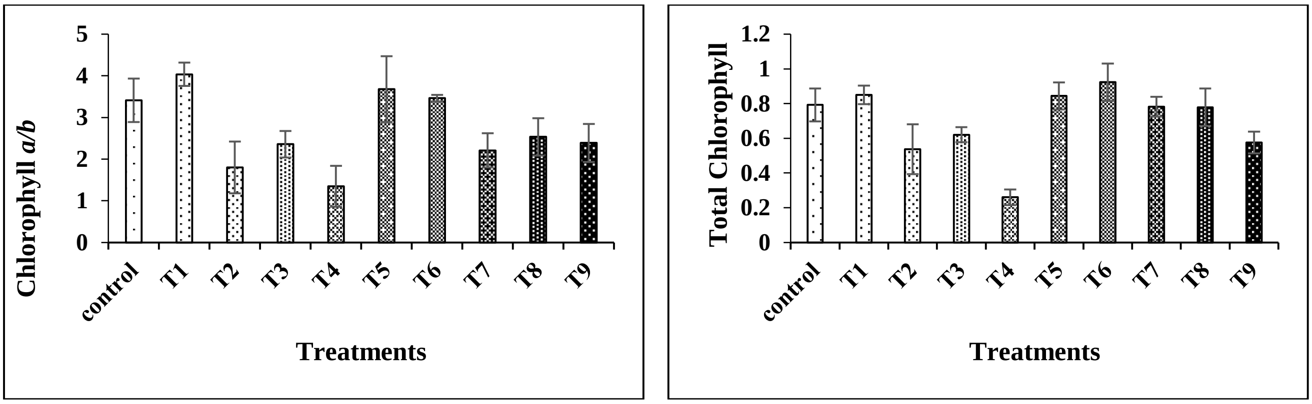

Fig. 3: Chlorophyll a/*b* and total chlorophyll of Lettuce in different treatments.

in carotenoids was observed under treatment 4. Treatments showed a significant (≤0.001) difference in carotenoids in Lettuce as shown in fig 4.

Catalase Activity

Catalase activity was measured by spectrophotometer and results were recorded. It was observed that catalase activity was reduced in all the treatments of micro-plastic (treatment 2, 3 and 4) as compared to control. Maximum reduction in catalase activity showed by treatment 4 (polystyrene micro-plastic). Organic matter showed increase in catalase activity as compared to control. Combination of both organic matter and micro-plastics showed increase in catalase activity as compared to micro-plastics treatments, while showed reduction as compared to organic matter treatments and control. Treatments has a significant (≤0.001) difference in catalase activity.

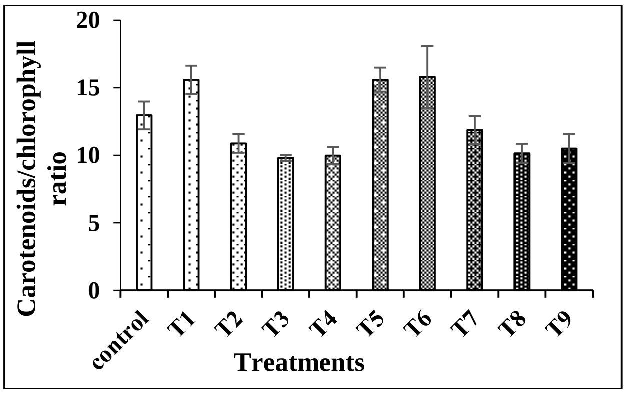

Fig. 4: Carotenoids/chlorophyll ratio in various treatments.

DISCUSSION

In latest studies, significant reduction was found in plant fresh weight of Lettuce grown under different types of micro-plastics (polystyrene, polyethylene, and polyester), combination micro-plastics and organic matter stress (Polystyrene +polyethylene +polyester +compost, Polystyrene +polyethylene + polyester +leaf litter, Polystyrene +polyethylene +polyester + Wheat straw) relative to control. When Chinese cabbage (*Brassica chinensis* L.) was exposed to high density polyethylene and general-purpose polystyrene micro-plastics ranging in sizes <25, 25–48, 48–150, and 150–850 µm at four applied amounts 2.5, 5, 10, and 20g/kg^{-1} micro-plastics of soil. Another study showed similar results, fresh weights of cucumber plants especially aboveground parts were significantly decreased after exposure to 300 nm polystyrene micro-plastics. Root fresh weight of Lettuce (*Lactuca sativa* L. var. capitata) was noted to decrease significantly by exposing them to polyethylene micro-plastics. Similarly, root fresh weight of strawberry plant was reduced by co-contamination of high density polyethylene micro-plastics along with cadmium(Zhang et al*.,* 2024). Recent analysis stated significant reduction in root length of lettuce in all treatments of micro-plastics. Lettuce plants were treated with polystyrene, polyethylene and poly-propylene micro-plastics. Micro-plastics treatment was observed to decrease tomato roots. Significant alleviation in root length of rice plants when upon contact with micro-plastics (Shi et al., 2022). New studies also found significant decrease in root length of Lettuce treated with micro-plastics at different levels, (polystyrene, polyethylene, polyester), organic matter (leaf litter, compost, Wheat straw) and combination of micro-plastics and organic matter (Polystyrene +polyethylene +polyester +compost, Polystyrene + polyethylene + polyester + leaf litter, Polystyrene + polyethylene + polyester + Wheat straw) (Shorobi et al., 2023). Significant decrease in light harvesting pigment *b* of Chlorella pyrenoidosa was found when exposed to micro-plastics poly-acrylonitrile polymers (Lin et al., 2020). Similar significant reduction is found in current research when Lettuce under micro-plastic treatments compost, polystyrene, Wheat straw, combination of polystyrene, polyethylene, polyester, leaf litter. Notable reduction in carotenoids was observed in Lettuce under micro-plastic different treatments micro-plastics (polystyrene, polyethylene, polyester), organic matter (leaf litter, compost, Wheat straw) and combination of micro-plastics and organic matter (Polystyrene +polyethylene +polyester +compost, Polystyrene

+polyethylene +polyester +leaf litter, Polystyrene +polyethylene +polyester + Wheat straw). Carotenoids were also significantly reduced in lettuce by treating with micro-plastics of polyvinyl chloride (Changmai et al., 2024).

Conclusions

Our experimental study on the effect of different types of micro-plastics and organic matters and their combinations on Lettuce observed that exposure of micro-plastics caused reduction in the full plant fresh and dry weight, root fresh and dry weight, full plant length and root length, photosynthetic pigments, proline content and catalase activity. Micro-plastics exposure caused more negative effect on Lettuce as compared to organic matter. But micro-plastics with organic matter caused less negative effect as compared to micro-plastics exposure. Lettuce plants showed maximum reduction in growth under micro-plastics exposure. Micro-plastics also negatively affected the soil properties and soil microbial activity. Organic matter showed increase in growth of Lettuce as compared to control.

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